Tu-Pos217

COMPARISON OF MOBILITY OF GLUCAGON RECEPTOR WITH OTHER PLASMA MEMBRANE COMPONENTS IN RAT HEPATOCYTE. Sharmila Shaila Gupte & Victoria Iwanij*, Dept. Biochem, USUHS, Bethesda, MD 20814 & *Dept. Genetics/Cell Biol, Univ. of Minn, St. Paul, MN 55108.

Lateral diffusion dependent interactions of the plasma membrane (PM) proteins are believed to be essential for signal transduction. To study the mechanism of transmembrane signalling, the lateral diffusion rates (D) of the glucagon receptor (gluc-R) and other PM components in the rat hepatocyte were measured using fluorescence recovery after photobleaching technique. The D of the phospholipid analogue DiI, and that of the hydrophobic probe DPH in PM were $2x10^{-8}$ cm^2/sec and $4x10^{-8}$ cm^2/sec, resp., and their recoveries (R) were 85% and 100%, resp.. The D of Na,K-ATPase, and gluc-R, labelled with anthroyl ouabain and mouse monoclonal anti gluc-R antibody to gluc-R, resp., were measured. Preliminary data indicate that the D of gluc-R is $4x10^{-10}$ cm^2/sec, and slower than that for Na,K-ATPase with 60% Rs for both proteins. These data suggest (i) the faster D of DPH is due to its location in the hydrophobic, fluid environment at the bilayer center, (ii) the lipid diffusion in rat hepatocyte is unrestricted, and (iii) the D and % recoveries of gluc-R are comparable to other PM proteins.

Tu-Pos218

SINGLE-CHANNEL AND FURA-2 ANALYSIS OF INTERNAL CA^{++} OSCILLATIONS INDUCED BY H_1 RECEPTOR STIMULATION IN HELA CELLS: THE ROLE OF H^+ IONS. R. Sauvé, M. Chahine, and A. Diarra. Dept. of Physiology, Univ. of Montreal, Canada H3C 3J7

Patch-clamp and Fura-2 experiments were undertaken in order to investigate the role of H^+ ions in the cyclic release of internal Ca^{++} following H_1 receptor stimulation in HeLa cells. The results obtained essentially indicate 1) that the $[Ca^{++}]_i$ oscillatory process is a biphasic phenomenon with an initial phase related to the release of Ca^{++} from internal stores coupled to a second phase requiring the presence of external Ca^{++}; 2) that an increase in internal pH (ΔpH 0.4) abolishes selectively the appearance of $[Ca^{++}]_i$ spikes without increasing the basal calcium level; 3) that the observed inhibitory effect of alkaline pH is independent of external Ca^{++} and 4) that Ca^{++} oscillations can always be initiated at alkaline pH during the initial phase. The periodic release of $[Ca^{++}]_i$ could also be abolished selectively using protonophores such as FCCP or H^+-ATPase inhibitors such as NBD-Cl. From these results it is concluded that the periodic release of Ca^{++} is coming from internal pools which are reloaded in Ca^{++} via a pH dependent mechanism.

Tu-Pos219

PERIPHERAL BENZODIAZEPINE RECEPTOR (pBr) OF RAT KIDNEY MITOCHONDRIA: WHAT IS THE FUNCTION? M.W.McEnery, A.M.Snowman and S.H. Snyder Dept. Neuroscience, Johns Hopkins Sch. of Med., Baltimore, MD 21205

The mitochondrial benzodiazepine receptor can be regarded as a high affinity intracellular receptor which binds the benzodiazepine Ro5-4864 and the isoquinoline carboxamides PK11195 and PK14105 with great specificity. This site is present in kidney mitochondria and accounts for 0.2% of the total mitochondrial protein; in contrast, this site is 50-fold less abundant in brain and liver mitochondria. We have purified this receptor complex from rat kidney and have determined its subunit composition (30-35kDa and 18 kDa) and native molecular weight (52kDa). The 18 kDa subunit has been photoaffinity labelled with PK14105, while the 30-35kDa subunit has been labelled with benzodiazepine derivatives; thus implying that both subunits are integral components of the intact receptor. The effect of sulfhydryl reagents and specific inhibitors of mitochondrial transport on ligand binding to the purified receptor has been examined and these results implicate the pBr as a transport protein. The purified pBr has been inserted into phospholipid vesicles towards the goal of reconstituting the transport activity.

Tu-Pos220

MODELS OF G-PROTEIN COUPLED RECEPTORS
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The model illustrated below was developed for the G-ProCoR superfamily. Highly conserved residues in the transmembrane region are located in the interior of each monomer and interact with other highly conserved residues. Helices were positioned to favor formation of salt bridges, hydrogen bonds, aromatic-aromatic interactions, and hydrophobic interactions. Monomers aggregate to form a trimer similar to that of bacteriorhodopsin. Large poorly-conserved hydrophobic faces of helices 1,4,5, and 6 are exposed to lipid on the exterior of the trimer and narrow poorly-conserved hydrophobic faces of helices 3 and 7 are exposed to lipid on the interior of the trimer. Outer helices are tilted relative to inner helices as predicted by helix packing theories. All helices but 7 may extend about two turns beyond the hydrophobic portion of the membrane into the cytoplasm. The post-7 segment forms an amphipathic α helix that is attached to a lipid on the cytoplasmic surface.

Tu-Pos221

DETERMINANTS OF PEPTIDE SECRETION IN RAT ATRIA. Ernest Page and Judy Upshaw-Earley, The University of Chicago, Chicago, IL 60637.

Absolute rates of immunoreactive atrial natriuretic peptide (ANP) secretion were measured *in vitro* at 37° C in noncontracting preparations of combined right and left atria at constant distending pressures of 0 or 5.1 mm Hg in presence of 0.2 mM $(Ca^{2+})_{out}$, 10 μM ryanodine, and 1 μM scxitoxin. Approximately constant mean secretory rates, R ± SE for basal (R_b) and stretched (R_s) atria ((pmoles/min)/mg dry atrium) were .0403 ± .0003 (n=5) and .147 ± .022 (n=6) with apparent activation energies (kcal/mole) of 28 ± 4 for R_b and 19 ± 4 for R_s between 24° and 43° C. By deleting external Na^+, K^+, Mg^{2+}, Cl^-, or HCO_3^-, reducing external Ca^{2+} to 20 μM, and using selective ion transport inhibitors, it was shown that transplasmalemmal influxes of Na^+, Cl^-, Mg^{2+}, and possibly Ca^{2+}, and ryanodine-sensitive release of Ca^{2+} from sarcoplasmic reticulum were not essential for the stretch-dependent activation of ANP secretion. R_s was (a) near zero when both $(Na^+)_{out}$ = 0 and $(Ca^{2+})_{out}$ = 20 μM, or at 18° C; (b) significantly decreased by Cd^{2+}, Ni^{2+}, the isoquinoline H-7, trifluoperazine, and indomethacin, but not by nordihydroguaiaretic acid (NDGA); and (c) increased 3-fold by neomycin. Experiments with NDGA and indomethacin implicated production of arachidonic acid (AA) and its cyclooxygenase metabolites in modulation of R_s, but did not differentiate between AA originating in atrial myocytes and in other cell types. Supported by USPHS grants HL 10503 and HL 20592.

Tu-Pos222

FLUORESCENCE IMAGING OF COMPLEMENT PROTEINS ON HUMAN ENDOTHELIAL CELLS: ENDOCYTOSIS OF THE C5b-8 COMPLEX. Robert D. Fugate, Karen K. Hamilton, and Peter J. Sims. St. Francis Med. Res. Inst., Dept. of Med., OUHSC; Ok. Med. Res. Foundation and Ok. Blood Inst., Oklahoma City, OK. Digital fluorescence imaging was used to probe the distribution of membrane bound complement C5b-8 on human endothelium (EC). C5b67 was deposited on the EC surface from C8-deficient serum, and Texas Red-labeled C8 (TRC8) bound at 4°C. After removal of unbound TRC8, the cells were warmed to 37°C and distribution of fluorescence ($\lambda > 615$ nm) monitored. Although initially diffusely distributed, the C5b-TRC8 complexes coalesced to punctate spots by 5-20 min, migrated to the cell periphery (excluded from nucleus), and thereafter diminished in intensity. At 20 min, fluorescence from TRC8 colocalized with fluorescence from acridine orange, suggesting uptake into acidic endosomes or lysosomes. This redistribution of C5b-TRC8 was arrested at 4°C, and inhibited by cytochalasin D or 2-deoxyglucose, but was unaffected by EGTA. These data suggest that inactivation of nascent C5b-8 occurs by endocytosis, possibly mediated by an EC surface receptor.

Tu-Pos223

HIGH AFFINITY RYANODINE BINDING SITES IN RAT LIVER ENDOPLASMIC RETICULUM Varda Shoshan-Barmatz and Naomi Kraus-Friedmann, Department of Biology, Ben Gurion University, Beer Sheva, Israel, and Department of Physiology and Cell Biology, University of Texas Medical School, Houston, TX 77025 USA

Microsomal membranes prepared from liver or hepatocytes, possess high affinity binding sites for ryanodine. Scatchard analysis of [^{3}H]ryanodine binding revealed a high affinity binding site (K_D 8nM) and maximal binding site of 600 fmol/mg protein, respectively. The smooth ER fraction shows 2-3 fold higher ryanodine binding capacity than rough ER. Ryanodine modified the Ca^{2+} permeability of liver microsomes passively loaded with ^{45}Ca. At submicromolar concentration, ryanodine stimulated Ca^{2+} efflux, while at higher concentrations it blocked Ca^{2+}- permeable pathways. Two high molecular mass proteins (>300k) were cross-reacted with the polyclonal antibodies against the 350k protein of the rabbit sarcoplasmic reticulum. The results suggest the existence of ryanodine binding protein(s)/Ca^{2+} release system in a non-excitable tissue, the liver, and raises the possibility that this system has wider distribution and role that previously considered. Supported by NDDK-36916.

Tu-Pos224

IMMOBILIZATION OF MUTATED IgE RECEPTORS IN TRANSFECTED CELLS S.-Y. Mao, N. Varin-Blank, M. Edidin, and H. Metzger, NIAMS, NIH, Bethesda, MD 20892 and Dept. Biol., Johns Hopkins University, Baltimore, MD 21218

One of the earliest events observed in the degranulation of mast cells induced by aggregation of IgE receptors is immobilization of the receptors. We used site-directed mutagenesis to truncate each of the 5 cytoplasmic domains of the tetrameric rat IgE receptor. Immobilization of the mutant receptors expressed in transfected cells was measured by fluorescence photobleaching and recovery. Native transfected receptors and all but one of the mutants were immobilized by IgE oligomers to the same extent as endogenous receptors. Truncation of the C-terminal domain of the beta chain, created a partial defect in immobilization, suggesting that other domains are also important in this event. Human IgE receptor expressed without any beta chain was also normally immobilized by IgE oligomers. It may be that transmembrane domains of the receptor, rather than cytoplasmic domains are important for its immobilization.

Tu-Pos225

LYMPHOMA GP85 (Pgp-1) IS A PROTEIN KINASE C SUBSTRATE, AND MAY FUNCTION IN GP85-ANKYRIN BINDING. Lilly Y.W. Bourguignon, E. Kalomiris, and D. Kligman, U. of Miami Medical School, (Spon. by W. Loewenstein)

In this study, we have found that the lymphoma plasma membrane contains a 78 kDa polypeptide that exists in a complex with one of the major transmembrane glycoproteins, GP85. This 78 kDa protein appears to be a lymphoma protein kinase C (PKC). Studies we have done to identify the cellular substrate(s) of the lymphoma plasma membrane-associated PKC have shown that GP85 cross reacts with an antibody specifically against a 87 kDa PKC substrate (sequence NH_2-EAAEPEQPEQPEQPAA-COOH) from rat brain. In addition, we have found that GP85 can be phosphorylated by purified brain protein kinase C. Analysis of the resulting phosphoamino acids indicates that phosphorylation of GP85 occurs primarily at serine residues; occurs in minor amounts (~5%) at threonine residues; and does not occur at tyrosine residues. Furthermore, we have established that phosphorylation of GP85 by PKC enhances its binding affinity with the membrane linker molecule, ankyrin. These findings suggest that PKC-mediated phosphorylation of GP85 may be an important part of the lymphoma plasma membrane-cytoskeleton interaction.

Tu-Pos227

MECHANISM OF PROTEIN KINASE C: MEMBRANE INTERACTION

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Fluorescence energy transfer, proteolytic sensitivity, and enzymatic activity have been used to examine the mechanism of interaction of the Ca^{2+}/lipid dependent protein kinase C with model membranes. We show that binding of protein kinase C to model membranes is regulated by the phosphatidylserine content of the lipid bilayer. This is similar to the activation of the kinase by phosphatidylserine, which displays high cooperativity and specificity. Binding is independent of diacylglycerol (an allosteric effector essential for full enzymatic activity), however in the absence of this lipid, the kinase may not insert as far into the hydrophobic core of the membrane. A 32 kd domain of protein kinase C is resistant to proteolysis when bound to membranes containing diacylglycerol. In the absence of diacylglycerol, the membrane-bound enzyme is quantitatively degraded to small peptides.

Tu-Pos226

AGONIST: ANTAGONIST INTERACTIONS AT A_1 ADENOSINE RECEPTORS.

R.D. Green and E. Leung(Intro. by S. Nakajima), Dept of Pharmacol. Univ. Illinois, Chicago IL 60612.

We have probed the mechanism of AdoR agonist:antagonist interactions using membrane-bound and solubilized AdoRs prepared from bovine cortex. Sucrose gradient centrifugation studies suggest that A_1 AdoRs are coupled to a G-protein in the absence of an effector and that AdoR antagonists bind preferentially to free AdoRs to cause the dissociation of "precoupled" receptors. Saturation analyses of antagonist radioligands ± "cold" agonist and agonist radioligand ± "cold" antagonist demonstrate that agonists and antagonists do not interact competitively. Dual isotope experiments using [^{125}I]- and [^{3}H]-radioligands suggest the presence of a species of agonist: AdoR complex that is not detected by filtration assays. This is confirmed by centrifugation assays. Analysis of complex experiments with the computer program EQUIL lead to a "minimal working model". According to this model AdoR antagonists bind to free AdoRs with high affinity while AdoR agonists bind to two coupled forms of AdoRs with high affinity. This model suggests that antagonists may have the potential for exerting effects opposite to those of agonists.

Tu-Pos228

Kinetic analysis of ligand binding to the Fc_ε receptor on mast cells

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Rates of association and dissociation of several specific monovalent ligands to and from the type I Fc_ε receptor ($Fc_\varepsilon RI$) on living mast cells (line RBL-2H3) were measured. The specific ligands employed were a monoclonal IgE and Fab fragments prepared from different, $Fc_\varepsilon RI$ specific mAbs (designated H10, J17 and F4), known to be effective secretagauges. Analysis of the kinetic data and their temperature dependence show that a minimal mechanism to which they can be fitted involves two consecutive steps: $L+Fc_\varepsilon RI \overset{K_{01}}{\longleftrightarrow} (L\text{-}Fc_\varepsilon RI)_1 \overset{K_{12}}{\longleftrightarrow} (L\text{-}Fc_\varepsilon RI)_2$. Thus the initially formed ligand-receptor complex undergoes a conformational transition to a second state. While the binding of IgE and the Fab derived from mAb H10 favour transition to the second state ($K_{12} \gg 1$), Fabs F4 and J17 prefer the former ($K_{12} < 1$ at 25°C). The activation barrier of step 1 is determined by an increase of its enthalpic and entropic part. The activation barrier of step 2, however, is provided by enthalpic contributions compensating a increase of the entropy. The similarity of the temperature dependence observed for all four ligands sugggests that the same mechanism is operative and that the $Fc_\varepsilon RI$ undergoes the same conformational transition upon ligand binding.

Tu-Pos229

TRANSVERSE DISTANCES BETWEEN THE ACETYLCHOLINE BINDING SITES ON THE TORPEDO RECEPTOR AND THE LIPID MEMBRANE SURFACE. Phi Wiegn, Elizabeth Suk, and David A. Johnson, Division of Biomedical Sciences, Univ. of Calif., Riverside, CA 92521-0121.

We estimated the transverse distance between each of the acetylcholine binding sites on the membrane-associated Torpedo receptor and the lipid membrane surface by means of fluorescence energy transfer techniques. Dansyl-C_6-acylcholine was used as an energy donor from the acetylcholine binding sites to 5-(N-dodecanoylamino)-eosin, the acceptor, partitioned into the lipid membrane matrix. Monoclonal antibodies developed by Dowding and Hall [(1987) Biochemistry 26, 6372-6381], that selectively inhibit binding to either the "A" or the "B" acetylcholine binding sites, directed the dansyl-C_6-choline binding to the unblocked sites. The transverse distances were calculated to be the same for each site, 36Å. In the absence of monoclonal antibodies, the magnitude of energy transfer was almost 2-fold (1.8) greater and calculated transverse distance smaller, 31Å. The effect of monoclonal antibodies on the conformational state of the receptor will be discussed.

(Supported by N.S.F. grant #BNS-882137)

Tu-Pos230

INTACT ISOLATED PSEUDOPODIA FRAGMENTS: A MODEL SYSTEM FOR CELL MIGRATION Raouf A. Guirguis*+, Ki Min Eum*, and Jeong Soon Kim*, Cancer Diagnostics Inc.*, Rockville, MD 20852, and Georgetown University+, Washington, DC 20007

Intact Pseudopodial Fragments (IPSF) in the form of liposomal anucleate particles containing desirable surface molecules were isolated from a wide variety of cell systems such as cells of the immune system (e.g. leukocytes, macrophages, and lymphocytes), and a variety of cancer cell lines (e.g. human melanoma, breast cancer, genitourinary cancer, colon cancer, and lung cancer). IPSF were used to replace certain cellular functions otherwise performed by whole living cells. The procedure for isolating pseudopodia from cell lines and white blood cells takes advantage of a number of phenomena such as modulation of cell surface receptor, pseudopodia protrusion and cytokineplasts. Even more, the size and number of IPSF harvested from these cell systems can be fully regulated in a pseudopodia (Guirguis) chamber. Pseudopodia fragments enriched with CD4 receptor, main target of HIV virus, were isolated from T4 and T8 subclones of lymphocytes and macrophages. Pseudopodia fragments isolated from lymphokine activated killer cells (LAK) were used to combat or identify cancer cells in vitro. Preparation and isolation of viable, anucleate pseudopodia fragments was carried out using a multi-chambered vessel (Guirguis Chamber) in which the upper chamber is separated from the lower chamber by a filter plate having pores of sufficient size to permit extension through the pores of cell pseudopodia but small enough to prevent passage of the nuclear cell itself. The pseudopodia fragments which were separated from the parent cells and collected in the lower chamber are viable fragments having many of the characteristics of the parent cells. Immediately after being severed from the parent cell, the fragments assume an otherwise liposomal appearance having a central core of cytoplasm surrounded by an outer plasma membrane which contains a high concentration of receptor cites which migrated to the pseudopodia when initial stimulation occurred. Thus, the viable pseudopodia fragments are adapted for selected targeting and fusion with cells similar to those from which they were derived such as cancer cells or to ingest viruses.

Tu-Pos231

PURIFICATION OF THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR (IP_3REC) FROM SMOOTH MUSCLE MICROSOMES.

Christopher C. Chadwick and Sidney Fleischer. Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

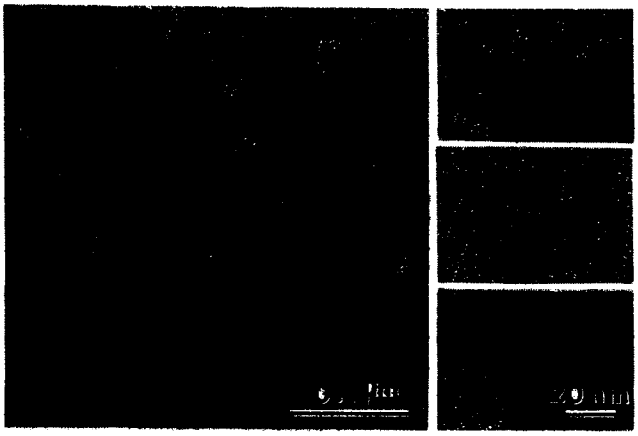
The IP_3Rec has been purified from smooth muscle microsomes (SMM). SMM were solubilized with CHAPS and the solubilized receptor was purified by a combination of column chromatography and sucrose gradient centrifugation. The purified receptor bound 2.7 ± .24 nmol [^{3}H]inositol 1,4,5-trisphosphate ([^{3}H]IP_3) per mg of protein (mean ± S.D., n=4) with a Kd of 2.4 ± .18 nM (mean ± S.D., n=4). The purified IP_3R consists of one polypeptide with M_r ~220,000 as determined by SDS-PAGE. Gel exclusion chromatography suggests a molecular weight in excess of one million, indicating an oligomeric structure. [^{3}H]IP_3 binding is pH dependent with optimum binding at pH 9.5 which is inhibited by heparin ($K_{1/2}$ = 1.5 μg/ml). Inositol 1-phosphate and inositol 1,4-diphosphate do not inhibit [^{3}H]IP_3 binding at concentrations of 10 μM. Inositol 1,3,4,5 tetraphosphate inhibits binding with $K_{1/2}$ ~1 μM. (Supported by NIH HL 32711 to SF and AHA Investigatorship, Tennessee Affiliate to CCC.)

Tu-Pos232

ULTRASTRUCTURE OF THE INOSITOL TRIPHOSPHATE RECEPTOR (IP_3REC) FROM SMOOTH MUSCLE.

Akitsugu Saito, Christopher C. Chadwick and Sidney Fleischer. Dept. Molecular Biology, Vanderbilt University, Nashville, TN 37235.

The IP_3Rec from smooth muscle has recently been purified (C.C. Chadwick and S. Fleischer, Biophys. J., 1989). It consists of an oligomer of a single polypeptide of 220 KD by SDS-PAGE. Sedimentation studies indicate that its size is in excess of one million. The morphology of the receptor has been studied using negative staining with uranyl acetate. The images show structures with four-fold symmetry with pinwheel appearance (see figure). The receptor is in the similar size range as the sarcoplasmic reticulum calcium release channel/ryanodine receptor from heart and skeletal muscle, ~300 Å in diameter. (Supported by NIH HL 32711 and MDA.)



Tu-Pos233

PHOSPHORYLATION STATES OF SKELETAL MUSCLE RYANODINE RECEPTOR AND SMOOTH MUSCLE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR (IP_3Rec).

Anthony P. Timerman, Christopher C. Chadwick and Sidney Fleischer. Dept. Molecular Biology, Vanderbilt University, Nashville, TN 37235.

The ryanodine receptor (RyRec) from fast twitch skeletal muscle has been purified to homogeneity and identified morphologically as the foot structure involved in junctional association of transverse tubule with terminal cisternae of sarcoplasmic reticulum (SR). Functionally, the RyRec of SR is the calcium release channel involved in Ca^{2+} release which triggers muscle contraction in excitation-contraction coupling. Recently, we have isolated the IP_3 receptor (IP_3Rec) from smooth muscle. In this report we describe the phosphorylation of these receptors with protein kinases. The stoichiometry of ^{32}P incorporation from (γ-^{32}P) ATP into both purified receptors by protein kinase A (PKA) and/or protein kinase C (PKC) was analyzed. Results are expressed as pmole phosphate incorporated per pmole of receptor subunit assuming subunit molecular weights of 220,000 and 567,000 for the IP_3Rec and RyRec, respectively. PKA phosphorylated both the IP_3Rec and RyRec with stoichiometries of 0.45 and 0.6, respectively. The IP_3Rec was not phosphorylated by PKC, but the RyRec was phosphorylated by PKC with a stoichiometry of 0.7. Incubation of the RyRec with both PKA and PKC resulted in a stoichiometry of 1.1, indicating the RyRec has separate phosphorylation sites for PKA and PKC. These studies suggest that these receptors may be functionally regulated by the action of protein kinases and phosphatases. (Supported by NIH HL 32711, DK 14632 and MDA.)

Tu-Pos234

ACRIDINE AND ACRIDINE ARAPHANE COMPOUNDS AS MOLECULAR TOOLS IN THE STUDY OF CHANNEL KINETICS AND HOMOLOGY. R.A.M. Reis[1], A.C.S. Costa[1,2], C.M. Himel[2] & E.X. Albuquerque[1,2]. (Intro. by H. Gonzalez-Serratos) [1]Lab. Mol. Pharmacol., IBCCF, UFRJ, Rio de Janeiro, Brazil, [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Med. Sch., Baltimore, MD 21201.

We investigated the effects of 1,2,3,4-tetrahydro-9-aminoacridine (THA), two synthetic analogs 1,2-propane-9-aminoacridine araphane (1,2-pAA) and 1,4-butane-9-aminoacridine araphane (1,4-bAA), and the related agent 9-aminoacridine (9-AA). The cell-attached patch-clamp variant was used to study nicotinic receptors (AChR) on acutely dissociated frog muscle. Whole-cell and outside-out variants were applied to rat cultured hippocampal neurons to evaluate NMDA receptors. THA acted mainly as an open channel blocker both on the AChR and NMDA receptors at the same concentration range. The main effect of 1,2-pAA and 1,4-bAA on the AChR was reduction of channel opening probability, but the major effect of 1,2-pAA on NMDA receptors was reduction of mean open time. 9-AA was also an open channel blocker of NMDA receptors. These studies provide evidence that NMDA receptors and the AChR have a homologous blocking site. Support: CNPq, CAPES, FINEP, US Army Med. Res. Dev. Com. Contr. DAMD17-88-C-8119.

Tu-Pos235

POLYIONIC DRUGS INHIBIT BINDING OF ANTI-CD4 AND HIV GP120 TO LYMPHOCYTES. J. L. Weaver, P. Gergely, P. S. Pine, E. Patzer*, T. Gregory*, and A. Aszalos. FDA, Washington, DC; *Genentech Inc, San Francisco, CA.

We have studied the effect of several polyionic drugs, dextran sulfate, Evans Blue, aurin tricarboxylic acid (ATA), heparin, protamine, and dextran, on the binding of anti-CD4 and of recombinant HIV GP120 to human lymphocytes. We have also studied the effect of these drugs on membrane potential. We have found that dextran and protamine do not affect binding anti-CD4. Dextran sulfate (20 μM), heparin (2 U/ml), Evans Blue (1 μM) and ATA (3 μM) inhibit the binding of anti-CD4, ATA and Evans Blue also inhibit the binding of GP120. All four active drugs alter the uptake of charged membrane potential indicating dyes. However, studies with channel blockers and ionophores suggest that changes in ion fluxes are not involved in the inhibition of ligand binding. Therefore changes in the membrane surface charge due to the negatively charged polyionic drugs block the binding of both anti-CD4 and GP120 to the CD4 receptor.

Tu-Pos236

Evidence for Intra-Membrane Constraints to Cell Surface LDL Receptor Motion

Richik N Ghosh & Watt W Webb, Cornell U, Ithaca, NY

Time-lapse quantitative low-light level digital video microscopy is used for high precision (30nm) automatic tracking of single low density lipoprotein receptor (LDL-R) molecules and small receptor clusters labeled with the fluorescent LDL analogue, diI-LDL. (Ghosh and Webb *Biophys. J.* 55 498a, 1989). Calibration of the fluorescence power emitted by diI-LDL verifies single LDL-R tracking and the number of LDL-Rs in each cluster on a cell. We find that both single receptors and clusters of LDL-Rs can have three modes of motion and frequently can switch amongst them. These are (i)tightly constrained slow diffusion (10^{-12} cm^2/sec) with a fractional power law dependence of mean-squared displacement vs. time reminiscent of percolation; (ii)directionally restrained motion with faster diffusion coefficients (10^{-10} cm^2/sec) and the linear power law expected for diffusion; and (iii)directed motion with linear velocities around 10^{-6} cm/sec and superimposed random motion. This behaviour also occurs for mutant LDL-Rs lacking cytoplasmic tails ruling out direct cytoskeletal coupling. Cytochalasin D stops directed motion leaving only localized random motion. The scale of the constrained motions are 200nm to 1μm. The spatial scale of switching between percolation and fast diffusion or directed motion implies inhomogeneities of the membrane restraints on a scale of a few micrometers.

Tu-Pos237

A STEREOCHEMICAL RELATIONSHIP AMONG COMPOUNDS BIOLOGICALLY ACTIVE IN G-COUPLED PROTEINS. Wilson Radding, Department of Physiology, UAB, Birmingham, AL 35294.

The intense biological activity of arotinoids link retinoid activity with a stilbene-like structure. The stilbene backbone is echoed in tricyclic antidepressants which have a demonstrated stereochemical and biological relationship to biological amines. Both the opsin and biological amine receptors have the putative seven membered transmembrane helix structure of G-linked receptors. The amine competitive activity of tricyclics indicates that the seven helix structure might contain a sub-motif which is organized to bind the stilbene-like region corresponding to a 9-cis double bond of retinal. The identification of this motif might lead to a genetic building block which is responsible for protein structures involved in the first steps of signal transduction (or modulation) but which has evolved to accommodate systems as disparate as light reception or, given the homology of retinoic acid receptors with steroid receptors and the steroid effects of some stilbenes, steroid action.

Tu-Pos238

Lateral mobility of lipid linked and transmembrane proteins in transfected COS-1 cells. F.Zhang, *B.Crise, *D.A.Brown, Y.Hou, *J.Rose, and K.A.Jacobson Dept. of Cell Biol. and Anatomy, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599, *Dept. of Pathology, Yale Univ, New Haven, CT06510. (Intro. by B.Herman)

In biomembranes many transmembrane proteins exhibit lower lateral mobility while most lipid linked proteins are characterized by higher lateral diffusion coeffients(D). To examine factors responsible for restricted lateral mobility, we studied two groups of membrane proteins, expressed in COS-1 cells by transfection. In first group, the diffusion of VSV G, a transmembrane viral glycoprotein, Thy-1, a lipid linked glycoprotein, and GThy-1, a chimeric lipid linked construct consisting of the ectodomain of G and a small part of Thy-1 were compared. VSV G and GThy-1 exhibited low mobility ($D=3.6\pm1.0\times10^{-10}cm^2/s$; $D=5.2\pm1.7\times10^{-10}cm^2/s$ respectively), while Thy-1 exhibited considerably higher mobility ($D=2.7\pm1.0\times10^{-9}cm^2/s$). In the second group, the lateral mobility of the lipid-anchored placental alkaline phosphatase (PLAP) was compared to a transmembrane form (PLAP-G) which is anchored by the transmembrane and cytoplasmic domains of VSV G. Both PLAP and PLAP-G showed high lateral mobility ($D=2.4\pm0.7\times10^{-9}cm^2/s$ and $1.6\pm0.4\times10^{-9}cm^2/s$, respectively). These studies suggest the lateral mobility of lipid linked membrane proteins are determined by their ectodomains.

Tu-Pos239

APPARENT MOLECULAR WEIGHT OF THE ENDOTHELIN RECEPTOR D.L. Bednar, R.B. Stein, V.M. Garsky, and D.L. Williams, Jr. Departments of Pharmacology and Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486. The binding of the vasoactive peptide hormone, endothelin-1, was studied in membranes derived from aorta, atrium, ventricle and kidney. [^{125}I]-endothelin-1 was bound to membrane vesicles and incubated in the presence (aorta) or absence (all tissues) of crosslinking agents. Autoradiography showed that, under non-reducing conditions, a small amount of radiolabelled endothelin-1 was irreversibly bound to a membrane component of Mr about 43,000 even in the absence of crosslinking agents. Treatment of membranes with varying amounts of unlabelled endothelin-1 eliminated this autoradiographic band in parallel with binding competition curves for these tissues. Sarafotoxin, ACM cys 1, 15 endothelin-1 and "big" endothelin-1 (1-39) also eliminated this band in a similar manner. This component may be a subunit of the endothelin receptor.

Tu-Pos240

Imaging of Immune Receptors on Metal Coated Cell Surfaces by Scanning Tunneling Microscopy

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The cell surfaces of fixed, metal coated Rat Basophil Leukemia (RBL) cells have been imaged to approximately 50 Å resolution by Scanning Tunneling Microscopy. Individual IgE Fcε receptor proteins expressed on the RBL cell surface can be identified in the STM images if they are labeled with colloidal gold particles via a protein A-anti-IgE linker. The images show substructure within the label-IgE-receptor complex. In some instances the three-armed geometry expected of IgE molecules is visible. Improvements in coating technology, or improvements in STM instrumentation to make the coating unnecessary, should give resolution high enough to allow the morphologies of the IgE receptor and any associated proteins to be found.

Tu-Pos241

GANGLIOSIDE G_{M3} AND de-N-ACETYL GANGLIOSIDE G_{M3} INTERACTIONS WITH THE EGF RECEPTOR. *Wenxia Song and David A. Rintoul, Biochemistry Dept. and Biology Division, Kansas State University, Manhattan KS 66506*

Previous reports (Hanai, *et al.*, 1988, **JBC** 263:10915) indicate that ganglioside G_{M3} and de-N-acetyl ganglioside G_{M3} respectively stimulate and inhibit the tyrosyl kinase activity of the EGF receptor, when measured *in vitro* in detergent-solubilized membranes. We have confirmed these findings, and further report that these glycosphingolipids can modulate receptor kinase activity in the absence of detergent. Membrane vesicles, prepared from cultures of A431 cells, were incubated with the ionophore alamethicin, which allows access of ATP to the previously latent active site. Incubation of these vesicles with glycosphingolipids results in tyrosyl kinase inhibition (with G_{M3}); stimulation with de-N-acetyl ganglioside G_{M3} was not observed. Fluorescent analogs (N-parinaroyl G_{M3} and de-N-acetyl ganglioside G_{M3}) were synthesized; these analogs also modulate the receptor kinase activity in a similar manner. These non-perturbing fluorescent glycosphingolipids should be valuable probes of the lipid-protein interactions which regulate growth factor receptor kinase activity, and presumably growth control as well.

W.S. is supported by the Wesley Foundation. This work was supported by an American Cancer Society grant (BC-507) to D.A.R.

Tu-Pos242

PARTIAL CHARACTERIZATION OF AN ENDOTHELIN RECEPTOR IN CARDIAC SARCOLEMMAL VESICLES. T.R. Shannon, J.A. Allert, L.M. LeBourveau, H.R. Adams, and C.C. Hale, Dept. of Veterinary Biomedical Sciences and the John M. Dalton Research Center, Univ. of Missouri, Columbia, MO. 65211.

A specific receptor for the endothelial cell-derived vasoactive peptide endothelin-1 (ET1) has been located on the cardiac myocyte sarcolemmal (SL) membrane. ET1 was iodinated (^{125}I-ET1) and utilized in binding studies with bovine cardiac SL vesicles. Competition studies with nonlabeled ET1 in the presence of 1 mM Ca^{2+} at either 22° or 34°C yielded a K_d of 3.2 and 6.2 nM ET1 and a B_{max} of 9.3 and 14 pM ET1/mg SL protein respectively, which were not different. At 34°C, in the presence of 1 mM EDTA, K_d remained unaltered while B_{max} decreased to 8 pM ET1/mg SL protein. Scatchard analysis indicated the presence of a single binding site. Specific binding was not observed in crude cardiac membranes that had been depleted of SL membranes. ^{125}I-ET1 was incubated with SL vesicles in the presence of the cross-linking reagent disuccinimdyl suberate followed by SDS-PAGE and autoradiography. ^{125}I-ET1 covalently cross-linked to a single SL membrane protein with an apparent Mr of 63 kDa. These data suggest the presence of a single, specific receptor for ET1 in the cardiac myocyte which is located on the SL membrane. Supported by NSF DCB-8602234 and the AHA-Missouri Affiliate.

Tu-Pos243

TIME-RESOLVED FLUORESCENCE STUDIES OF P21*ras*. Theodore L. Hazlett, David M. Jameson, Susan E. Neal*, Martin R. Webb* and John F. Eccleston*, Dept. of Biochemistry and Biophysics, University of Hawaii at Manoa, Honolulu HA 96822 and *National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

Time-resolved fluorescence spectroscopy was used to measure the solution dynamics of p21*N-ras* complexed with 2'(3')-O-(N-methylanthraniloyl) (mant) derivatives of GDP and GTP. These nucleotides bind to p21 with an affinity similar to the physiological nucleotides and there is a 3-fold enhancement of fluorescence intensity on binding. The fluorescence lifetime of the mant-nucleotides in solution is 4 ns and this increases to 9 ns on binding to p21. Differential phase and modulation data showed that very little local motion of the fluorophore occurs in the protein-nucleotide complexes and that global motion of the complexes has a rotational correlation time of 24 ns at 4^{o}C. No differences in these values could be detected between the mantGTP and mantGDP complexes or between normal p21 (Gly12) and an oncogenic mutant (Asp12). The rotational correlation times are larger than would be expected from a 21000 molecular weight globular protein. Whether this is due to unusual hydrodynamic properties of the protein or to interaction between monomers is being investigated.

Tu-Pos244

AN ALUMINUM FLUORIDE BINDING SITE WAS NOT DETECTED ON ELONGATION FACTOR TU*GDP. T.L.Hazlett, T.Higashijima*, and D.M.Jameson, Dept. of Biochem. and Biophys., Univ. of Hawaii at Manoa, Honolulu, HI, 96822. *Dept. of Pharm., Univ. of Texas Southwestern Med. Ctr., Dallas, TX, 75235. Fluorescence and NMR methodologies were utilized to measure the extent of association between elongation factor Tu*GDP (EF-Tu*GDP) and aluminum fluoride. Aluminum fluoride is purported to bind the GDP form of several G-proteins at the vacant γ-phosphate position. As in the case of other G-proteins the fluorescence of tryptophan, located within the GDP binding domain, was used to monitor changes in the local environment due to aluminum fluoride binding. The fluorescence spectra and lifetimes remained unchanged upon addition of aluminum fluoride which indicated that aluminum fluoride does not alter the tryptophan environment and implies that the aluminum fluoride does not bind to EF-Tu*GDP. Consistent with this interpretation, and in contrast to data on other G-proteins, the fluoride NMR data did not show an upfield shift of the fluoride peak to the position for protein-bound fluoride (aluminum fluoride). Therefore, the guanine binding sites of EF-Tu and other G-proteins should not be considered structurally equivalent. (Supported by NSF Grant DMB8706440 and NIH Grant GM40676)

Tu-Pos245

AGONIST-INDUCED GUANINE NUCLEOTIDE EXCHANGE IN G_k. Angela de S. Otero, Yongxin Li and Gabor Szabo, Dept. of Physiology, University of Virginia, Charlottesville, VA 22908.

Internal perfusion of atrial myocytes with slowly hydrolyzable GTP analogs (GXP) elicits the muscarinic potassium current I_{KACh} in the absence of agonists. Activation of I_{KACh} by GXP results from direct activation of a G protein, G_K, that promotes opening of K_{ACh} channels. When maximal GXP-supported channel activity is attained, the resulting current resists block by muscarinic antagonists and was thought to be unaffected by agonists. However, we find that in atrial cells perfused with solutions containing both GTP and guanylyl imidodiphosphate (GMP-PNP) at GMP-PNP/GTP ratios of 0.5 to 4, superfusion with acetylcholine leads to a decrease in receptor-independent current upon agonist washout. Thus, agonist receptor complexes can interact with GXP-activated G proteins and induce their return to the inactive, GDP-bound state. This deactivation process does not result from a simple GXP-GDP exchange, but involves release of GXP followed by binding and hydrolysis of GTP.

Tu-Pos246

ACTIVATION OF ATRIAL MUSCARINIC K^+ CHANNELS BY FLUORIDE AND NUCLEOTIDES. K. Okabe, A. Yatani and A.M. Brown. Department Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030. We have shown that atrial muscarinic K^+ channels (K^+[ACh]) in excised I-O membrane patches of guinea pigs are activated by GTP in the absence of agonists and that the activation is dependent on GTP/GDP ratio (Okabe et al., 1989). Theophylline, atropine and prazosin had no effects. To examine the basic properties of the G_k gating K^+[ACh], we studied the effects of fluoride ion (F^-) and nucleotides (NTP). I-O recordings were made with symmetrical 140 mM KCl and agents were applied by the concentration-clamp method which produced concentration jumps within 10 ms. F^- reversibly activated K^+[ACh] in a Mg^{2+}-dependent manner (K_d=5 mM). At Mg^{2+} 20 mM, F^- activation was maximal at 10 mM (K_d=3 mM) and the peak effect was 70 ± 15% (n=8) of that obtained with GTPγS (100 μM). The rate of activation was concentration-dependent and at 10 mM the half-activation time was 2 ± 0.5 sec which was slower than that of GTPγS (800 ± 200 ms). The Al^{3+} chelator, deferoxamine (50-500 μM) inhibited F^- activation. Nucleotides, such as ATPγS activated K^+[ACh] via a nucleoside diphosphate kinase (NDPK) (Otero et al., 1988), thus we compared the potencies of NTPs on K^+[ACh]. The potency was ITP>UTP>ATPγS>CTP>ATP and K_d's were 30,90,600,1000 and 2000 μM, respectively. The sequence was unchanged by agonist. The effects were Mg^{2+}-dependent and the activation reached the maximum value obtained by GTPγS except for ATP which was ~60% of the maximum. AMP-PNP (2 mM) had no effects. Except for ATPγS, the activation was reversed either by washing or by GDP (1-100 μM). Two conclusions are possible: 1) Contamination of nucleotides with GTP or 2) The presence of membrane-bound NDPK (Heidbuchel & Carmeliet, 1989). Supported by NIH grant HL36930.

Tu-Pos247

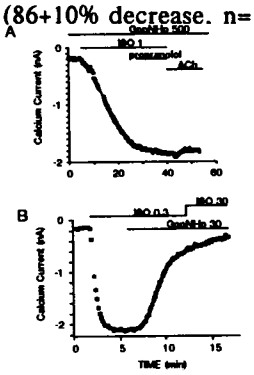
G PROTEINS INHIBIT TRANSMITTER RELEASE AT THE SQUID GIANT SYNAPSE. Stephen D. Hess, Steven S. Vogel and George J. Augustine, University of Southern California, L.A.,CA 90089-0371, NIDDK, NIH, Bethesda, MD and Max Planck Institut, Goettingen, FRG.

To examine the possible role of G proteins in transmitter release, we injected guanine nucleotides directly into giant presynaptic terminals of *Loligo pealei* and *L. opalescens*. Iontophoresis of GTP-gamma-S (20 mM pipette concentration) produced a mean decrease of 70% in postsynaptic currents (PSCs) elicited by presynaptic action potentials (n=5). This effect was largely irreversible. In contrast, iontophoresis of comparable quantities of GTP caused a smaller (25%,n=5) and reversible inhibition of PSCs. Similar effects were also observed with pressure injection of these compounds. Further, UV illumination of preparations injected with DMNPE caged GTP-gamma-S inhibited release (n=2). Preliminary results suggest that the inhibition of transmitter release is at least partially due to changes in presynaptic membrane potential, because GTP-gamma-S, but not GTP, depolarized the presynaptic membrane potential. Supported by NIH NS21624, NS08392 and MPI funds.

Tu-Pos248

AFFINITY OF ADENYLYL CYCLASE FOR SUBUNIT(S) OF IRREVERSIBLY ACTIVATED G-PROTEIN, Gi, DEPENDS ON Gs. T.D. Parsons, A. Lagrutta, R.E. White, H.C. Hartzell. (Intro. by T.R. Nichols) Emory University, Atlanta, GA 30322.

We have internally perfused isolated cardiac myocytes with the non-hydrolyzable GTP analog, GppNHp (GN) to study the regulation of voltage-gated Ca^{2+}-current (I_{Ca}). When cells were stimulated with isoproterenol (ISO, 0.3 μM), in the presence of 500 μM GN, a **persistent** I_{Ca} was observed following the washout of β-agonist, or application of β-antagonist (89±12% of the ISO I_{Ca} was persistent in 14 of 21 cells) (Fig. A). Acetylcholine (ACH, 10 uM) had no effect. In 7 cells stimulated under the same conditions, the initial response to ISO was blocked. However, when cells were first activated with 0.3 uM ISO, and then internally perfused with 30-500 μM GN, a **rapid decrease** in ISO I_{Ca} was observed in all cells (86+10% decrease. n=11) (Fig. B). We interpret these results as: (a) the persistent I_{Ca} results from the irreversible binding of α_sGN to adenylyl cyclase (AC); (b) the inhibitory responses reflect the binding of the irreversibly activated G_i subunit(s) to AC in the absence of ACH receptor stimulation; (c) and that the apparent affinity of AC for G_i is decreased by the binding of α_sGN. (NIH HL21195 to HCH)



Tu-Pos249

DIRECT G-PROTEIN EFFECTS ON ATP-SENSITIVE K^+ CHANNELS IN RAT VENTRICULAR MYOCYTES. G.E. Kirsch, J. Codina, L. Birnbaumer and A.M. Brown. Departments of Anesthesiology, Molecular Physiology and Biophysics, and Cell Biology, Baylor College of Medicine, Houston TX 77030. The regulation of ATP-sensitive K^+ channels was studied in neonatal rat ventricular myocytes. Application of 0.1 mM ATP to the intracellular surface of excised inside-out membrane patches caused a marked decrease in K^+ channel activity which could be partially restored by the addition of the non-hydrolyzable GTP analog, guanosine 5'-O-(3-thiotriphosphate) (GTPγS) at 0.1 mM to the intracellular medium. The effect could be mimicked by application of 0.1 mM intracellular GTP in the presence of 10 μM extracellular adenosine. Hence, the ATP sensitivity of the channel might be modulated by GTP-dependent binding proteins (G-proteins) which are present in the patch membrane and these G-proteins may be coupled to adenosine receptors. We tested the involvement of G-proteins further by applying $G\alpha_i$ subunits, pre-activated with GTPγS to the cytoplasmic face of excised membrane patches, and found that α_{i1}, α_{i2} and α_{i3} mimicked the effect of GTPγS. Application of pre-activated α_o or pre-activated G_s in the presence of Mg^{2+} had no effect, suggesting a specific role for $G\alpha_i$. The ATP-sensitive K^+ channel may be partly responsible for the reduced duration of the cardiac action potential in ischemic or metabolically blocked tissue. Reduction in ATP sensitivity via G-protein activation may provide a cellular mechanism for potentiating channel activity in the presence of subnormal ATP concentrations. Furthermore, the possible involvement of adenosine receptors is consistent with the rise in extracellular adenosine which accompanies ischemia. Supported by American Heart Association (Texas Affiliate) and NIH HL-37044, HL-36930, HL-31154 and DK-19318.

Tu-Pos250

G-PROTEIN INTERACTION MODIFIES THERMAL INACTIVATION OF CARDIAC ADENYLYL CYCLASE. **ROBERT A. COLVIN AND RICHARD A. ALLEN, PHARMACOLOGY DEPT., ORAL ROBERTS UNIVERSITY SCHOOL OF MEDICINE, TULSA, OK 74137.**
Purified canine cardiac sarcolemmal vesicles contain hormone sensitive adenylyl cyclase (AC) activities. When vesicles are incubated at 37°C in 10mM $NaHCO_3$/20mM HEPES (pH 7.4) AC slowly inactivates. Inactivation was fit by a single exponential decay (T½=10 min). Freeze-thawed vesicles had decreased T½ (1.9 min). Addition of protease inhibitors had no effect on T½. Thermal inactivation was the same when either GppNHp or forskolin were used as activators. Addition of ATP slowed the rate of inactivation (EC_{50}=0.3mM). AMP-PNP (EC_{50}=13µM) also increased the T½, indicating phosphorylation was not necessary. Forskolin (EC_{50}=15µM) increased T½. GppNHp (EC_{50}=.22µM) and NaF (EC_{50}=.41 mM) in the presence of 9mM Mg^{2+} increased T½. Addition of Mg^{2+} decreased the EC_{50} for GppNHp and NaF. The results suggest that cardiac AC may undergo spontaneous irreversible thermal denaturation. G-protein ($G\alpha_s$) or forskolin interaction with AC induces a change in the enzyme such that it no longer undergoes denaturation. A binding site for adenine nucleotides exists, which when occupied protects AC from inactivation.

Tu-Pos251

HETEROLOGOUS DESENSITIZATION OF MUSCARINIC AND SOMATOSTATIN ACTIVATION OF AN INWARDLY RECTIFYING K-CONDUCTANCE IN THE CLONAL PITUITARY CELL LINE, AtT-20.

A.G. Dousmanis and P.S. Pennefather, Dept. Physiol. and Fac. Pharmacy. Univ. of Toronto, Toronto, ON. M5S 2S2.

The effect of somatostatin-14 (S-14) and the muscarinic agonist carbachol on membrane currents in AtT-20 cells have been measured using patch electrodes in the whole cell configuration. As reported at last years meeting (Biophys J., 55, 546a), both agents activate an inwardly rectifying K-conductance to a similar extent, via a G-protein. The response was monitored in a medium that contained 20 mM K^+ by voltage steps to -90 mV from a holding potential of -30 mV. By using local superperfusion, which allows bathing solution to exchange within a few seconds, we have found that the response decreases by 60% within 3 min. of exposure to either agonist. Preincubation with S-14 for 5 min. before entering the whole cell configuration also reduces the current and removal of S-14 leads to recovery of the response. The decrement therefore, represents desensitization rather than run-down. Carbachol produces little additional current when applied in the presence of S-14 even after the response to S-14 has desensitized. The desensitization therefore, is heterologous. Supported by an OGS award to AGD and a grant from MRC Canada.

Tu-Pos252

THE SINO-ATRIAL NODAL PACEMAKER CURRENT (I_f) IS DIRECTLY REGULATED BY G PROTEINS.

A. Yatani, K. Okabe, J. Codina*, L. Birnbaumer* and A.M. Brown. Depts. Molecular Physiology and Biophysics and Cell Biology*, Baylor College of Medicine, Houston, Texas 77030. We have proposed that membrane-delimited, direct G protein pathways in the heart make possible the ability of cardiac autonomic nerves to change heart rate within a single beat based on experiments done on muscarinic K^+ (K^+[ACh]) and Ca^{2+} currents in atrial myocytes. To test whether true pacemaker currents are regulated similarly, we measured the time course of the action of carbachol (Carb) on the whole-cell, hyperpolarization-activated pacemaker current, I_f in rabbit SA nodal cells. We used a concentration-clamp to produce step changes within 10 ms. The experiments were done at 22°C. Carb at 1-100 nM reduced I_f within 50 ms. This rapid effect was followed by a much slower inhibition with a half time of 20 sec. Interpretation of the latter was complicated by current rundown. The concentration required to block I_f was about 20-50 times less than the concentration required to activate K^+[ACh] current (DiFrancesco et al., Science, 1989). GDPβS at 800 μM completely blocked the fast inhibition and partially blocked the slow inhibition. Atropine (10 μM) blocked both phases. Direct effects of G proteins were examined by recording multi-channel I_f current in excised inside-out patches. Preactivated human erythrocyte G_s (G_s^*) activated I_f currents while brain G_o blocked them. The effects occurred in the presence of AMP-PNP (2 mM) or after an increase in I_f produced by solutions containing cyclic AMP, ATP and PKA. We conclude that muscarinic and β-adrenegic agonists activate different G proteins which regulate I_f by direct and indirect pathways. Supported by NIH grants HL36930 and HL39262.

Tu-Pos253

BINDING OF A MONOCLONAL ANTIBODY AND ITS FAB FRAGMENT TO SUPPORTED PHOSPHOLIPID MONOLAYERS MEASURED BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

M. L. Pisarchick & N. L. Thompson, Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599

Total internal reflection fluorescence microscopy has been applied to the specific binding of an anti-dinitrophenyl (DNP) monoclonal antibody and its Fab fragment to supported phospholipid monolayers composed of a mixture of dipalmitoylphosphatidylcholine and dinitrophenyl-conjugated phosphatidylethanolamine. Binding curves have been obtained as a function of the antibody and Fab solution concentration and as a function of the concentration of DNP-glycine in solution. Analysis of the binding curves for the Fab fragments indicates that the association constant for surface-bound DNP is ~10-fold lower than the association constant for DNP in solution; analysis of the binding curves for intact antibodies provides an estimate of the association constant that describes the equilibrium between antibodies bound to the surface by one and two antigen binding sites and indicates that the major fraction of antibodies are bound by two sites at equilibrium. Supported by NIH # GM-37145.

Tu-Pos254

SLOW ROTATIONAL MOTIONS OF ANTIBODIES ON SUPPORTED PHOSPHOLIPID MONOLAYERS MEASURED BY POLARIZATION-SENSITIVE FLUORESCENCE PHOTOBLEACHING RECOVERY.

M.M. Timbs & N.L. Thompson, Dept. Chem., University of North Carolina, Chapel Hill, NC 27599

Slow rotational motions of an anti-dinitrophenyl monoclonal antibody (ANO2) specifically bound to distearoylphosphatidylcholine/dinitrophenyldioleoylphosphatidylethanolamine monolayers on alkylated glass substrates have been measured. Antibodies were labelled with a bifunctional carbocyanine fluorophore; steady-state anisotropy measurements showed that the conjugated bifunctional probe had less flexibility than unifunctional probes. Polarized fluorescence photobleaching recovery data of ANO2 on monolayers were consistent with rotationally mobile (~35 sec) and immobile ANO2 populations. The rotational mobility was not sensitive to the ANO2 surface density or to kinetic factors, whereas unlabelled anti-(murine IgG) antibodies significantly decreased the apparent mobile fraction. The ANO2 correlation time was comparable to that of the fluorescent lipid dioctadecyltetramethylindocarbocyanine in the monolayers. Supported by NIH # GM37145 and NSF # DCB-8552986. The carbocyanine fluorophore was a gift of A.S. Waggoner.

Tu-Pos255

INTERACTION OF IgG WITH Fc RECEPTORS IN SUBSTRATE-SUPPORTED PLANAR MEMBRANES MEASURED BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY.

C.L. Poglitsch & N.L. Thompson, Dept. Chem., Univ. of North Carolina, CB# 3290, Chapel Hill, NC 27599

A procedure for constructing substrate-supported planar membranes derived from membrane fragments isolated from the macrophage-related cell line J774A.1 is described. Total internal reflection fluorescence microscopy (TIRFM) is employed to demonstrate that fluorescently-labelled Fab fragments of a monoclonal antibody (2.4G2) specific for a macrophage cell-surface receptor for IgG (moFcγRII) bind to the planar membranes. These measurements show that the planar membranes contain moFcγRII, yield a value for the association constant of 2.4G2 Fab with moFcγRII ~ 10^9 M^{-1} and indicate that the moFcγRII surface density is ~ 60 molec/μm^2. Also, TIRFM is used to investigate the Fc-mediated competition of unlabelled, polyclonal murine IgG with labelled 2.4G2 Fab for moFcγRII in the planar membranes. These measurements indicate that the moFcγRII recognized by 2.4G2 Fab also retain murine IgG Fc-binding activity and yield a value for the association constant of polyclonal murine IgG with moFcγRII ~ 1-5 x 10^5 M^{-1}. Supported by NIH#GM-37145 and NSF#DCB8552986.

Tu-Pos256

RECONSTITUTION OF THE MURINE IgG RECEPTOR moFcγRII IN SUBSTRATE-SUPPORTED PLANAR MODEL MEMBRANES.

C. L. Poglitsch, M. T. Sumner & N. L. Thompson, Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599-3290

The murine IgG receptor moFcγRII was purified from the macrophage-related cell line J774A.1 using affinity chromatography with Fab fragments of the anti-(moFcγRII) monoclonal antibody 2.4G2. The isolated moFcγRII was reconstituted into liposomes by detergent dialysis and the liposomes were allowed to fuse on quartz substrates to form supported planar membranes containing moFcγRII. Total internal reflection fluorescence microscopy (TIRFM) showed that fluorescently-labelled 2.4G2 Fab fragments specifically bound to the supported planar membranes, confirming the presence of moFcγRII, and indicated that the surface density of reconstituted moFcγRII was ≥ 900 molecules/μm^2, which is similar to the natural cell surface density and is at least 15-fold higher than the density in supported planar membranes formed from isolated J774A.1 membrane fragments. TIRFM was also used to characterize the Fc-mediated association of murine IgG with the reconstituted moFcγRII. Supported by NIH # GM-37145 and NSF # DCB-8552986.

Tu-Pos257

KINETICS OF $InsP_3$ INDUCED Ca RELEASE IN SMOOTH MUSCLE A.V. Somlyo, K. Horiuti, T. Kitazawa, S. Kobayashi, D.R. Trentham*, A.P. Somlyo Univ. of VA, *Nat'l Inst. for Med. Res.,London

The rate of Ca release from the sarcoplasmic reticulum (SR), initiated by photolysis of caged inositol trisphosphate ($InsP_3$) in permeabilized portal vein smooth muscle, was measured at 22°c with Fluo-3 (Minta, et al J Biol Chem.264:8171, 1989). Following Ca loading and incubation in caged $InsP_3$, the muscle was exposed to a 2ms or 50ns pulse of near UV light to liberate $InsP_3$. Ca release and force development upon photolysis were concentration-dependent. At saturating [$InsP_3$] force development occurred with a 300ms delay and a rise time (t1/2) of 1.3s.

Ca release was preceeded by a delay of @30ms. The delay in Ca^{2+} channel opening may be due to a slow off-rate of an inactive occupant of the $InsP_3$ receptor, although other mechanisms are also possible. The rate and extent of Ca release were markedly inhibited in the absence of ATP, and were also enhanced by AMPPCP. The $InsP_3$ induced Ca^{2+} transient was not modified by procaine, a blocker of Ca^{2} induced Ca^{2+} release.

Tu-Pos259

CHARACTERIZATION OF THE ANG II INDUCED CALCIUM TRANSIENT IN RAT HEPATOCYTES.
I. Rogulja, C.A. Hansen and J.R. Williamson (Intro. by Björnsson) Dept. of Biochem. & Biophys. U of Penn, Philadelphia, PA 19104.

We have analyzed Ang II induced Ca^{2+} transients in individual hepatocytes loaded with the calcium indicator fura-2. Dose-response experiments showed, that at low agonist conc. (1-100pM), the time to onset of the Ca^{2+} transient was 60-80 sec, and at high hormone conc. (1nM - 50nM) the time to onset was 10-30 sec. At low agonist conc. the duration of the "plateau phase" of the Ca^{2+} transient was dependent on continual exposure of the cell to the hormone, returning to baseline upon removal of the agonist. At high agonist conc. the plateau phase persisted well beyond the time of exposure to the agonist. When a single cell was exposed and subsequently re-exposed to a low conc. of hormone, the onset time of the Ca^{2+} transient was much shorter. The same observation was made at high hormone concentration, however, the difference in the time of onset was not as dramatic. These data suggest that following exposure to Ang II a biochemical modification occurs which enhances the sensitivity of the hepatocyte to a subsequent exposure to Ang II.

Tu-Pos258

DISSOCIATION RATE CONSTANT OF EPIDERMAL GROWTH FACTOR SPECIFICALLY BOUND TO ITS RECEPTOR MEASURED WITH PRISMLESS TIR/FPR
Edward Hellen and Daniel Axelrod
Dept. of Physics and Biophysics Research Div., University of Michigan

Total internal reflection/ fluorescence photobleaching recovery (TIR/FPR) is being used to measure the dissociation rate constant, k_2, of the specific binding of fluorescently labelled epidermal growth factor (EGF), to the EGF receptor molecule on mildly fixed A431 cells. The evanescent wave excites fluorescence at the surface of the cell adjacent to the coverslip. Preliminary results give k_2 in the range .002 to .01 sec^{-1}. This value does not appear to be diffusion limited. Wiley gets .0018 sec^{-1} using I^{125}-labelled EGF dissociation experiments performed on A431 membrane preps.(J. Cell Biol. Vol 107, Aug. 88)

Prismless TIR is achieved by passing the laser beam off axis through the 1.4 numerical aperture microscope objective so that the light is incident at the glass/water interface beyond the critical angle. This avoids the space limitations imposed by the working distance of the microscope objective when a prism is used to achieve TIR.

Tu-Pos260

A MODEL FOR LIGAND BINDING TO MUSCARINIC RECEPTOR SUBTYPES
Margaret E. Kargacin, Cheryl R. Scheid, and Thomas W. Honeyman, Introduced by Roger Craig
Department of Physiology, University of Massachusetts Medical School, Worcester MA.

Pharmacological and genetic studies have defined at least five subtypes of muscarinic receptors and many cells may express more than one subtype. These receptor subtypes may be discreetly coupled to separate processes by G-proteins. It is therefore of interest to define conditions whereby each species of receptor may be independently stimulated by agonist. A series of equations was solved that defined the interaction of agonists and antagonists with each of the 4 species of receptor in gastric smooth muscle (M2 and M3, G-protein coupled and free receptors) and the solutions displayed as a 3D surface representing agonist occupancy of each species as a function of varying concentrations of agonist and antagonist. Comparing the surfaces showed 1) which kinetic parameters have a critical influence on the ability to selectively occupy one receptor subtype 2) if certain parameters are known there are conditions in which individual receptor species may be selectively occupied by agonist thus predicting that the biochemical consequences of stimulation of each receptor species could be examined independently. Supported by NIH HL 41188-02

Tu-Pos261

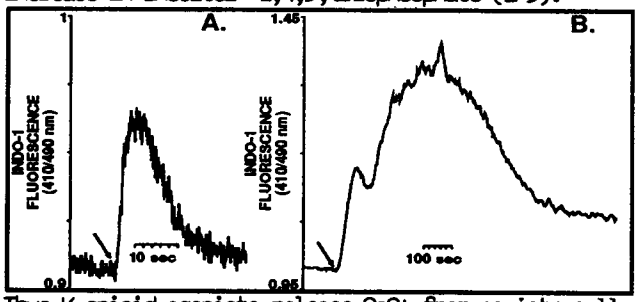
EXTRACELLULAR ATP-INDUCED INCREASE IN $(Ca^{2+})_i$ IN RAT HEPATOCYTES, CARDIAC MYOCYTES, AND A CULTURED NEUROBLASTOMA CELL LINE: STUDIES OF $(Ca^{2+})_i$ RESPONSE IN SINGLE CELLS
I. Rogulja, J.R. Williamson & O.G. Björnsson. Univ. of Penn. Philadelphia, PA, USA.

We examined $(Ca^{2+})_i$ response to extracellular ATP in fura-2 loaded rat hepatocytes and ventricular myocytes, and a neuroblastoma cell line (N1E-115), using a Nikon Diaphot-TMI microscope and fluorometry. ATP ($1x10^{-4}$ M) increased $(Ca^{2+})_i$ ~4-fold in individual hepatocytes relatively independent of $(Ca^{2+})_o$. In myocytes the $(Ca^{2+})_i$ increase was slightly more than ~2-fold (and less in neuroblastoma cells) and the $(Ca^{2+})_i$ response was directly related to extracellular Ca^{2+}. Considerable heterogeneity in $(Ca^{2+})_i$ response was observed in all cell types, rate of rise and decay varied greatly ($t_{0.5\text{-on}}$, range 0.9 - 55.6 sec., $t_{0.5\text{-off}}$, 8.3 - 213.8 sec., n = 30, myocytes). Desensitization to repeated additions of ATP developed rapidly in myocytes but not in hepatocytes or neuroblastoma cells. In myocytes desensitization was associated with inability to respond to electrical stimulation. Bay K 8644 or forskolin reversed the suppressive effect of ATP. Data suggest that the mechanism of ATP regulation differs in cells studied.

Tu-Pos262

K OPIOID RECEPTOR AGONIST CAUSES AN INTRACELLULAR RELEASE OF Ca^{2+} IN CARDIAC MYOCYTES AND NEURONAL CELLS.
C. Ventura, C. Guarnieri, H. A. Spurgeon, E. G. Lakatta, and M. C. Capogrossi, Gerontology Research Center, NIA, Baltimore, MD.

We assessed the effect of the K opioid receptor agonist U-50,488H (U-50) on cytosolic $[Ca^{2+}]$ (indexed as the 410/490 nm ratio of Indo-1 fluorescence) in single rat cardiocytes (Panel A) and neuroblastoma-2A cells (Panel B) bathed in 1.5 mM Ca^{2+}. The rapid addition (arrows) of U-50 from a pipette above the cell (2.5 mM U-50 in pipette) increases cytosolic $[Ca^{2+}]$. This result is not affected by 0 $[Ca^{2+}]$ and EGTA in the bathing medium and is abolished by the specific K antagonist Mr-1452 (not shown). In the continued presence of U-50, a caffeine induced increase in Ca_i is abolished. In rat myocyte suspensions, U-50,(50 μM) also caused a rapid, threefold increase in inositol -1,4,5,trisphosphate (IP3).



Thus K opioid agonists release Ca2+ from an intracellular pool possibly mediated via an increase in IP3.

Tu-Pos263

MOLECULAR DYNAMICS OF LUTEINIZING HORMONE RECEPTORS ON RAT LUTEAL CELLS.
D.A. Roess, N. Kenny, N.A. Rahman, & B.G. Barisas, Colorado State University, Ft. Collins, CO 80523. Intro. by T. Solie.

Fluorescence photobleaching recovery (FPR) studies show that ovine luteinizing hormone (oLH) bound to rat luteal cell LH receptors (LHR) has a diffusion coefficient D of $1.2\pm0.2x10^{-10}$ cm^2 sec^{-1}, a rate comparable to succinyl Con A bound to membrane glycoproteins. In contrast, hCG bound to LHR is immobile ($D < 1x10^{-12}$ cm^2 sec^{-1}). LHR rotational diffusion on individual cells was measured using polarized fluorescence depletion (PFD) to assess possible gonadotropin-induced LHR aggregation. The rotational correlation times for oLH and hCG at 4°C are 42.7 ± 1.6 μsec and 64.1 ± 2.8 μsec, respectively, suggesting that the LHR-hCG complex on rat luteal cells is either physically larger or more asymmetric than that involving oLH. These FPR and PFD results, like those previously obtained from sheep, show that the molecular motions of luteal cell LHR are significantly modulated by subtle structural differences among gonadotropins bound. Supported by NIH grants HD-23236 (DAR) and AI-21873 and AI-26621 (BGB).

Tu-Pos264

CALCIUM SIGNALING IN INDIVUDUAL EGF-STIMULATED A431 CELLS
D. Gross and T. Cheyette, Dept. of Biochemistry, Univ. of Massachusetts, Amherst, MA 01003.

Epidermal growth factor (EGF) elicits a variety of responses in human epidermoid carcinoma A431 cells, including a rise in free cytosolic calcium ion ([Ca]) (Moolenaar et al., J. Biol. Chem. 261: 279,1986). We have previously shown that individual A431 cells respond to EGF challenge with delayed [Ca] increases, the onset of which varies from cell to cell (Gonzalez et al., J. Cell Physiol. 135:269, 1988). Here we report dramatic individual cell variability of Δ[Ca] which is a strong function of [EGF]. At 16.5 nanomolar, a nearly synchronous transient rise in [Ca] is found in all cells after a ~7 sec delay, followed by a persistent elevation of [Ca]. With decreasing [EGF], the initial transient is less rapid and more variable. At 4.1 nM the initial transient and subsequent elevated phase is replaced by pseudoperiodic rises ("osillations") of [Ca]. At 1.7 nM, no changes in [Ca] are observed. We present a kinetic model which indicates that ligand binding and subsequent receptor aggregation of the EGF receptor in these cells cannot alone account for the measured [Ca] kinetics.

Supported by NSF grant DMB-8803826.

Tu-Pos265

DISSOCIATION KINETICS OF BIVALENT HAPTENS BOUND TO IMMUNOGLOBULIN E ANTIBODIES R.Posner, B.Goldstein*, D.Holowka, B.Baird, Dept. of Chemistry, Cornell University Ithaca N.Y. and *Theoretical Division, Los Alamos National Laboratory, Los Alamos N.M.

Crosslinking of IgE-receptor complexes on the surface of mast cells and basophils triggers cellular degranulation. We can characterize binding and crosslinking of bivalent DNP-ligands to fluorescein labeled anti-DNP IgE (FITC-IgE) by monitoring fluorescence quenching. Net dissociation of these bivalent ligands from FITC-IgE is observed as an increase in fluorescence that occurs upon the addition of either excess unlabeled IgE or excess monovalent DNP ligand (which quenches to a lesser extent). We showed previously that unlabeled IgE-induced bivalent ligand dissociation from the cell surface is increasingly reduced as a function of the time that the ligands and cells have pre-incubated. We have recently found that this time dependence does not occur with monovalent ligand as competitor. These results reveal rebinding of bivalent ligands to cell surface receptors that can be prevented by monovalent ligand competitors. The dissociation of bivalent ligands from IgE in solution and on the cell surface in the presence of monovalent ligand has been characterized by a two exponential decay process, yielding values for the two corresponding dissociative rate constants. Analysis of solution dissociation experiments using the unlabeled IgE method has provided some additional information about the forward binding rate constants.

Tu-Pos266

Aggregation of Fc_ε Recptor on mast cells induce an approximation to a novel membrane component

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A monoclonal antibody (G63) against a novel membrane glycoprotein on mast cells was raised recently (Ortega et al. EMBO J. 1988). Crosslinking of this novel membrane protein by mAb-G63 however inhibits the degranulation of the mast cells initiated by $Fc_\varepsilon RI$ aggregation. We have measured energy transfer on single living RBL cells from FITC labelled G63-Fab to different TRITC labelled antibodies binding to $Fc_\varepsilon RI$. We employed the recently developed photobleaching technique (Jo in et al. Ac. Press 1989). In detail we have in vestigated three $Fc_\varepsilon RI$ specific mAbs (H10,J17,F4) known to be effective secretagauges. The mAb most effective in degranulation F4 shows the greatest energy transfer, even though its relative affinity and hence its molar fraction of $Fc_\varepsilon RI$ dimers it formed is the smallest in comparison to the others. This shows that complexes of dimer $Fc_\varepsilon R$ and the membrane protein must exist. Since oligomerisation of the membrane protein in this complex by G63 affects the degranulation, the novel membrane glycoprotein must be involved in the early steps of degranulation.

Tu-Pos267

RECEPTOR-ACTIVATED CALCIUM INFLUX IN HUMAN AIRWAY SMOOTH MUSCLE (ASM) CELLS. R.K. Murray and M.I. Kotlikoff. Depts. of Medicine and Animal Biology, Univ. of Penna., Philadelphia, PA.

We investigated the biophysical mechanisms underlying agonist-induced calcium influx in human ASM cells. Cultured cells were plated on coverslips and loaded with fura-2AM for measurement of cytoplasmic calcium $[Ca]_i$. Application of agonists (bradykinin or histamine) resulted in a biphasic response, consisting of an intitial transient increase in $[Ca]_i$ (from 150 nM to 800 nM) followed by a sustained elevation (300-400 nM). The sustained elevation in steady-state $[Ca]_i$ was: 1) dependent on extracellular calcium; 2) blocked by La^{3+}, Cd^{2+}, or Ni^{2+}; and 3) blocked by agonist removal or receptor antagonists. Incubation of cells with nisoldipine or nifedipine (10 μM for 30 min) had no effect on sustained $[Ca]_i$. To determine the selectivity of the influx pathway, the rates of Mn^{2+}-dependent or Ba^{2+}-dependent changes in fluorescence were determined before and after agonist-induced activation. Activation did not alter the rate of Mn^{2+} or Ba^{2+} permeation, indicating that agonists increase membrane Ca^{2+} permeability via a Ca^{2+}-specific pathway.

Supported by NIH HL-41084 and ALA.

Tu-Pos268

TIME-RESOLVED PHOSPHORESCENCE ANISOTROPY STUDIES OF THE CELL SURFACE ROTATIONAL DYNAMICS OF THE RECEPTOR FOR IMMUNOGLOBULIN E. Jeffrey N. Myers, David A. Holowka, and Barbara A. Baird, Cornell University, Dept. of Chemistry, Ithaca, NY, 14853.

Aggregation of IgE-receptor (IgE-R) complexes on mast cells and basophils triggers cellular degranulation during the allergic response. This activation is readily induced by multivalent crosslinkers, but the response to bivalent ligands is variable. To investigate the structural requirements for IgE-R activation we have constructed a time-resolved phosphorescence spectrometer for measurements of IgE-R rotational dynamics. Addition of multivalent crosslinkers effectively immobilizes the IgE-R, but a bivalent hapten induces only a partial reduction in rotational mobility that is reversible by addition of monovalent hapten. This bivalent ligand binds and crosslinks efficiently, and stimulates a Ca^{++} response,but is a poor stimulus for degranulation. Other ligands are being tested to investigate further structural interactions of IgE-R on the cell surface.

Tu-Pos269

THE LEUCINE BUCKLE: A LEUCINE HEPTAD REPEAT CONTROLLING VOLTAGE DEPENDENCE IN A SLIDING HELIX MODEL OF SODIUM CHANNEL GATING. W.A. Catterall, Dept. of Pharmacol., Univ. of Washington, Seattle, WA 98195.

The highly conserved S4 putative transmembrane α-helices of the voltage-gated ion channels contain repeated 3-residue motifs of 2 hydrophobic amino acids followed by a positively charged residue (Noda et al, *Nature* **320**, 188-192, 1986). These segments are thought to serve as voltage sensors according to a "sliding helix" model of voltage-dependent gating (Catterall, *Trends Neurosci.* **9**, 7-10, 1986). K^+ channels contain heptad leucine repeats which have been suggested to form linear leucine zippers that participate in subunit interaction (McCormack et al, *Nature* **340**, 103, 1989). A similar heptad leucine repeat is present at the junction of the S4/S5 putative α-helical segments in both domains II and IV of the sodium channel α subunits. I now propose that these residues form a new structure, the "leucine buckle," which stabilizes interactions between the S4 and S5 segments. Structure-function studies of sodium channels indicate that these two segments form a transmembrane, anti-parallel helix-turn-helix structure placing the leucine heptads of S4 in position to interact with those of S5. In the resting state, the two halves of the buckle are proposed to be separated. Upon depolarization, the S4 helix slides 5Å outward along a spiral path and rotates 60°. This movement places the leucine heptads in register closing the buckle. The energy of leucine interaction promotes activation of the voltage sensor and stabilizes the open state of the channel.

The role of the "leucine buckle" in promoting channel activation is supported by the effect of conversion of leu_{860} of the R_{II} sodium channel to phe (Auld et al, *PNAS*, in press). This mutation disrupts a proposed "leucine buckle" and shifts the voltage-dependence of channel activation +20mV. The "leucine buckle" motif may provide a versatile mechanism for stabilization of the active state of the voltage-sensitive ion channels.

Tu-Pos270

INHIBITION OF INACTIVATION OF SINGLE SODIUM CHANNELS BY A SITE-DIRECTED ANTIBODY.

Peter Vassilev, Todd Scheuer, William A. Catterall
Dept. of Pharmacology, U. of Washington, Seattle, WA 98195.

The effects of site-directed antibodies on single sodium channel currents in excised membrane patches from primary cultures of rat brain neurons have been examined. Of six antibodies directed against different predicted intracellular domains of the sodium channel α subunit, only Ab_{SP19} directed against a highly conserved intracellular segment between homologous transmembrane domains III and IV induced late single channel openings and prolonged single channel open times during depolarizing test pulses. Also, depolarizing prepulses that caused complete inactivation of antibody-unmodified channels in subsequent test pulses failed to inactivate Ab_{SP19}-modified channels. These effects of Ab_{SP19} was not observed when patches were depolarized to inactivate sodium channels before exposure to the antibody, indicating that the intracellular sequence recognized by the antibody is rendered inaccessible by inactivation. The results suggest that a conformational change involving the intracellular segment between domains III and IV of the α-subunit of the Na channel molecule is required for fast sodium channel inactivation.

Tu-Pos271

POINT MUTATIONS IN THE REGION LINKING DOMAINS III AND IV WHICH SPEED INACTIVATION OF Na^+ CURRENTS. J.R. Moorman*, R.H. Joho, G.E. Kirsch, A.M. Brown. *Univ. of Texas Medical Branch, Galveston, TX; Baylor College of Medicine, Houston, TX. We compared whole cell and single channel inactivation kinetics among wild type (wt) type III Na^+ channels and 3 mutations in the region linking domains III and IV. Mutation M2 converted 2 positively charged Lys to 2 neutral Asp (KK1441/2NN); M4 neutralized another 3 Lys (KKK1453/4/7NNN); M5 converted a positively charged Arg to a negatively charged Glu (R1461E). At test potential 0 mV, M2 and M4 whole cell currents decayed with τ's 8-10 msec (A=0.9) and 30-40 msec (n=6 each). Wild type current is twice as slow (19 msec; 0.85; 75 msec; n=10). M5 was not significantly different from wt. In 4 cell-attached patches at test potential -20 mV, wt and M4 average single channel currents decayed with τ's 1-5 and 50-200 msec. The fast component was 90% of M4 current but only 40-60% of wt. Single M4 channels had shorter open time (0.7 vs 1.5 msec) and many fewer prolonged bursts. We conclude that lysines in the III-IV linker have a role in Na^+ channel inactivation but the lone nearby arginine may not.

Tu-Pos272

MODELING Na CHANNELS IN SQUID GIANT AXON FROM SINGLE-CHANNEL, MACROSCOPIC AND GATING CURRENTS. C.A. Vandenberg, & F. Bezanilla, Dept. of Biological Sciences, UCSB, Santa Barbara, CA 93106, & Dept. of Physiology, UCLA, Los Angeles, CA 90024.

Na channel gating behavior was modeled with Markovian models fitted to currents from cut-open squid giant axon recorded with patch techniques. Optimum models were selected with maximum likelihood criteria using single-channel data, then models were refined by simultaneous fitting of macroscopic ON and OFF ionic and gating currents, and single-channel first latency densities over a wide voltage range. Best models have four or more closed states prior to channel opening, with inactivation from at least one closed state as well as the open state. In the squid, rates of microscopic inactivation are generally slower and show weaker voltage dependence than activation or deactivation rates. Channels reopen several times before inactivating. At positive potentials, there is return from inactivation, producing a low amplitude steady-state current. (Supported by USPHS grants GM 30376 & HL 41656).

Tu-Pos273

AMANTIDINE ACTION ON Na CHANNELS. R. Thomson, R. Eenigenburg, and F. N. Quandt. Multiple Sclerosis Research Center and Dept. Physiology. Rush University. Chicago, Il. 60612.

Amantidine can prolong axonal action potentials and relieve conduction block due to demyelination. This study was initiated to test the mechanism by which amantidine can interact with Na channels in neuroblastoma cells. Amantidine was found to prolong the action potential (300 μM). This action was more apparent after block of K channels with 4-AP. Na currents were isolated in K-free solutions, using the whole cell patch clamp technique. Most of the actions of amantidine were typical of a Na channel blocker. The K_D for block of the peak I_{Na} was 400 μM. The time course of I_{Na} showed little change when scaled to the control amplitude. 700 μM amantidine hyperpolarized the voltage for half maximal apparent steady state inactivation by less than 10 mV. Block increased with conditioning pulses. Conditioned block was enhanced as the pulse duration was increased over a range of 20 to 300 mSec. The time for half recovery from conditioning depolarizations, measured at -120 mV, increased from 60 mSec under control conditions to 300 mSec in the presence of amantidine. The amplitude of currents through single Na channels was unaltered, however the amplitude of tail currents increased in the presence of amantidine, suggesting that amantidine may slow the normal inactivation gating. The rather weak blocking action combined with inhibition of inactivation may explain the prolongation of the action potential.

Tu-Pos274

KINETIC PROPERTIES DISTINGUISH BATRACHOTOXIN-ACTIVATED CARDIAC SODIUM CHANNELS FROM OTHER SUBTYPES IN PLANAR LIPID BILAYERS.
R.J. French[1], D.D. Doyle[2], L. Anscomb[1], M.C. Lee[1], K.J. Mather[1], and Y. Guo[2]. [1]Department of Medical Physiology, University of Calgary, Calgary, AB, Canada T2N 4N1 and [2]Department of Medicine, The University of Chicago, Chicago, IL 60637.

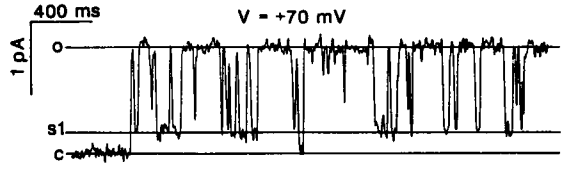
We have incorporated tetrodotoxin(TTX)-insensitive sodium channels from sheep and dog heart sarcolemmal vesicles (Doyle & Winter, 1989, J. Biol. Chem. 264:3811) into planar lipid bilayers in the presence of batrachotoxin. The single channels showed a slope conductance of about 21-22 pS and were blocked 50% of the time by about 1μM TTX (500 mM Na+ outside, 10 mM HEPES-Na, pH 7.0 inside). Two gating properties distinguish the cardiac channels from other subtypes that have been studied under similar conditions. First, for depolarized voltages, at which the channels are open most of the time in the steady state, we observed a conspicuous population of long closed times (mean $\approx$ 100 msec), not seen with brain or skeletal muscle. Second, the activation curve showed a less steep dependence on voltage (9-13 mV/e-fold) than that reported previously for brain or skeletal muscle from other species. In parallel studies of skeletal muscle channels from lamb, we have so far found no overlap in the range of steepness of activation (4-8 mV/e-fold).

Supported by the Medical Research Council (Canada), Alberta Heritage Foundation for Medical Research, and the National Institute of Heart, Lung & Blood (USA).

Tu-Pos275

ZINC-INDUCED SUBCONDUCTANCE EVENTS IN CARDIAC SODIUM CHANNELS PROLONGED BY BATRACHOTOXIN. L. Schild, A. Ravindran and E. Moczydlowski. Department of Pharmacology, Yale Univ. School of Medicine, 333 Cedar St., New Haven, CT 06510.

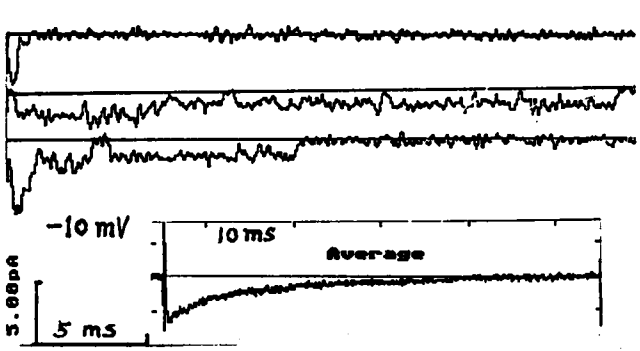
Micromolar concentrations of external Zn^{2+} induce discrete subconductance events (15-25 ms lifetime) in single BTX-modified Na-channels of canine heart in planar bilayers. In symmetrical 0.2 M NaCl, the open state is ohmic with a conductance of 21 pS, while the Zn-induced substate rectifies outwardly with a slope conductance of 3.1 pS at -V and 5.1 pS at +V. Dependence of the open state and substate conductances on [NaCl] indicates that the substate current saturates at a lower g_{max} of 8.6 pS (-V) and 12.6 pS (+V) compared to the open state g_{max} of 35 pS. Using a one-site blocking model, the apparent k_{on} for Zn^{2+} binding is more voltage-dependent (decreasing e-fold per 60 mV) than k_{off} (increasing e-fold per 420 mV) with an apparent K_d of 70 μM at 0 mV and 0.2 M NaCl. However this model cannot explain an apparent increase in k_{off} with increasing [Zn^{2+}]. To explain this result, a four-state kinetic scheme similar to one used by Pietrobon et al. (1989) [J. Gen. Phys. 94: 1-24] for H^+-induced substate behavior in L-type Ca-channels was used to simulate the kinetic and equilibrium behavior of the Zn-induced substate process. Our model suggests that Zn^{2+} induces conversion of the open ohmic Na-channel from a high to a low conductance conformation with an asymmetric energy profile for Na^+ permeation. Since fast block by external Ca^{2+} and other divalent cations displays the same voltage dependence as Zn-induced subconductance block, other divalent cations may also block via brief unresolved substates.



Tu-Pos276

THE μI Na CHANNEL HAS TWO GATING BEHAVIORS. J. Zhou, J. Trimmer, W.S. Agnew and F.J. Sigworth. Dept. Cellular & Molecular Physiology, Yale Medical School, New Haven, CT 06510.

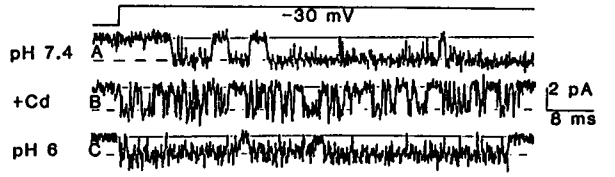
The μI NaCh exhibits a biphasic inactivation time course when expressed in frog oocytes; a large slow component (τ_s~13 ms) and a relatively small fast one (τ_f~2 ms). Single-channel recordings from outside-out patches show that this derives from two types of channel gating; brief openings with short delay and long bursts with variable latencies. Similar gating behaviors are also seen in patches from oocytes injected with μI cRNA plus muscle poly-A mRNA, but the major component of inactivation of the whole cell current is fast. This suggests that the two gating behaviors might be due to differences in channel modulation.



Tu-Pos277

FLICKER BLOCK OF CARDIAC Na CHANNELS BY Cd^{2+} AND PROTONS P. H. Backx, E. Marban, and D.T. Yue, Depts of Biomedical Engineering and Medicine, Johns Hopkins University, Baltimore, MD

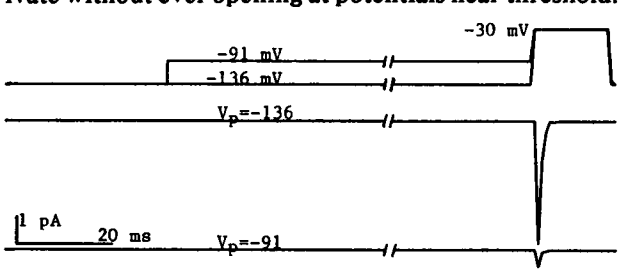
Numerous inorganic cations transiently occlude current flow through Na channels, but the rapidity of individual block events often exceeds the bandwidth of patch clamp records, thereby limiting the value of these blockers as probes of the conduction pathway. We now find that kinetic information of block by Cd^{2+} and H^+ may be resolvable in cell-attached patch clamp records of cardiac Na channels. When inactivation is slowed by DPI 201-106 (10μM, bath), Na channels demonstrate long-lasting openings (A), and the unitary conductance of 31 pS remains unchanged from before drug exposure (pipette contains [mM]: 200 NaCl, .5 $BaCl_2$, 10 HEPES, pH 7.4). Upon addition of $CdCl_2$ (100 μM) to the pipette, openings become chopped by blocking events (B), while unitary conductance remains unchanged. Histogram analysis reveals that block rate (3×10^7 $M^{-1}s^{-1}$, -30 mV) decreases steeply with voltage, while unblock rate ($\sim10^3$ s^{-1}, -30 mV) exhibits only weak voltage-dependence. We estimate that Cd binds about 0.2 of the electrical distance in from the extracellular channel entrance. When $[H^+]$ alone is raised in the pipette, unitary current is markedly reduced (C, no $CdCl_2$, pH 6, MES substituted for HEPES) with a pK_D of 5.5 (at -30 mV), consistent with titration of a carboxylic acid group. Open channel noise is increased under acidic conditions (C), as would be predicted if the timescale of individual blocking transitions just exceeds our current 5 kHz bandwidth. This raises the possibility that kinetic information of proton (un)block in Na channels may also be recovered from patch-clamp records.



Tu-Pos278

INACTIVATION OF SINGLE CARDIAC SODIUM CHANNELS DIRECTLY FROM CLOSED STATES W.C. Rose, J.H. Lawrence, D.T. Yue & E. Marban. Div. Cardiology, Johns Hopkins University, Baltimore MD

While macroscopic current recordings suggest that cardiac Na channels can inactivate without ever opening, brief openings in the conditioning pulse may well have gone undetected. The existence of such openings would make it difficult to exclude gating models in which transition through the open state is obligatory for inactivation. We investigated this question with cell-attached patch clamp, using 200 mM [Na] in pipettes to improve resolution (5kHz, -3db) of single-channel currents from guinea-pig ventricular cells. Eight patches containing 1-4 channels were held at $\leq$ -120 mV, pulsed to V_p ($-100<V_p<-75$ mV) for 185 ms, and then to V_t=-30 mV for 10 ms. Ensemble average currents (Figure) during pulses to V_t confirm that inactivation is at least 80% complete (i.e., $h\leq0.2$) after such prepulses. Therefore, the fraction of V_p sweeps with no openings (corrected for channel number) yields a lower limit for the probability (P) of C→I transitions. At V_p=-91 mV, $P\geq0.80$. Thus, many channels inactivate without ever opening at potentials near threshold.



Tu-Pos279

SPONTANEOUS GATING MODE SHIFTS IN BTX-MODIFIED SODIUM CHANNELS. A. K. Maddock and O. S. Andersen, Cornell Univ. Medical College, New York, N.Y.

The voltage activation of single BTX-modified sodium channels from rat brain shifts spontaneously among different modes. In 1.0 M NaCl, channels in mode I are activated in a steeply voltage-dependent manner with a gating charge z~ 4.5 and midpoint potential (V1/2)~ -90mV. Individual channels spend up to 30-50% of the time in another mode (II) in which the activation curve is shifted about 25 mV in the depolarizing direction (V1/2~ -65mV) and appears to have a shallower voltage dependence. These mode shifts are reversible, occur spontaneously and instantaneously, and last from seconds to minutes, thus allowing separate determinations of mode I and II gating parameters for single channels. The open channel conductance is the same in modes I and II, and in either mode a minimum of three states, 2 closed and 1 open, are necessary to fit the dwell time distributions. The open state time constant and the longer closed state time constant are strongly voltage dependent, and both become shallower functions of voltage concommitant with a mode I to mode II shift. The shorter closed state time constant is only weakly voltage dependent and does not vary with the gating mode shifts. A third gating mode (III) is also observed which channels visit less frequently (<10% of the time). The physiological significance of these gating mode shifts is unclear, but inhomogenous behavior at the single channel level will obscure the interpretation of multichannel gating experiments.

Tu-Pos280

THE VOLTAGE-SENSITIVITY OF SODIUM CHANNEL ACTIVATION IN CRAYFISH AXONS. M.D. Rayner and P. C. Ruben. Dept. of Physiol. and Békésy Lab., University of Hawaii, Honolulu, HI 96822.

From a stongly negative holding potential (V_h -120 mV) both Q(V) and F(V) curves accurately conform to a single Boltzmann distribution with a valence of 2e. From a more "depolarized" holding potential (V_h -85 mV) the gating charge ratio (V_h -85/Vh -120) is less than 1.0 and remains constant across all test potentials. The F(V) curve from V_h -85 also shows a 2e slope at all test potentials more positive than -30 mV and the peak I_{Na} ratio (V_h -85/V_h -120) is constant and equal to the gating charge ratio at these test potentials. By contrast, the F(V) curve from V_h -85 shows a high 4e limiting slope for test potentials between -60 and -30 mV. In this region the peak I_{Na} ratio (V_h -85/V_h -120) increases from near zero at -60 mV to the value found at -30 mV. Examination of I_{Na} traces across this range of near threshold test potentials shows no significant kinetic shifts between records taken from V_h -120 and those from V_h -85 mV. We conclude: i) that "gating particle/channel" stoichiometry changes as a function of test potential at "depolarized" holding potentials or, ii) that "depolarized" holding potentials induce a parallel component of inactivation which is progressively relieved as test potential approaches -30 mV.

Tu-Pos281

SODIUM CURRENTS NEAR THRESHOLD ARE DIFFERENTIALLY AFFECTED BY CHANGES IN HOLDING POTENTIAL.

P.C. Ruben and M. D. Rayner. Békésy Lab. and Dept. of Physiol., Univ. of Hawaii, Honolulu, HI 96822.

Whole-cell I_{Na} was recorded from clonal neuroblastoma cells (N1E-115). I(V) curves were measured from different holding potentials ranging between -120 and -40 mV. When comparing peak I_{Na} amplitudes from different V_{holds}, we note that the fractional suppression is greater near threshold than at more positive test potentials. Although currents from the more positive V_{holds} are smaller than predicted, there is no significant change in I_{Na} rising phase kinetics, indicating uniformity of the channel population and absence of series resistance artifacts. These results, which are similar to those seen in crayfish axons, suggest that steady-state inactivation influences I_{Na} amplitude at near-threshold test potentials measured from "typical" holding potentials. We propose that slow inactivation occurs in parallel to activation (as additional valence is not evident in gating current measurements from crayfish giant axons). This parallel process may lead to high estimates of sodium channel activation valence associated with relief of steady-state inactivation occurring at holding potential.

Tu-Pos282

SODIUM CHANNEL ACTIVATION MECHANISMS: INSIGHTS FROM Δ ^{9}-TETRAHYDROCANNABINOL.

D.A. Alicata and J.G. Starkus . Békésy Lab. of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822

Strichartz et al., (1978, J. Pharm. and Exp. Ther.) have reported for the node of ranvier that Δ^9-Tetrahydrocannabinol (THC) slows sodium channel activation and suppresses ionic conductance (g_{Na}). In our axons we confirm these findings, and additionally observe:

(1) I_{Na} is delayed without corresponding change in gating current kinetics or total charge movement.

(2) THC has no apparent affect on the relative magnitude of the "Cole-Moore" shifts of I_{Na} rising phase.

(3) Sodium channel deactivation (monitored by ionic tail currents), seems insensitive to THC.

(4) THC does not affect the rate of secondary activation after a brief (50 μs) interpulse interval.

Supported by PHS grant #NS21151-04, and partially from Hawaii Heart Association, UHBRSG and NIH RCMI grant RR-03061.

Tu-Pos283

SECONDARY ACTIVATION KINETICS ARE INSENSITIVE TO DEUTERIUM OXIDE IN CRAYFISH AXONS.

J.G. Starkus and D.A. Alicata. Békésy Lab., Univ. of Hawaii, Honolulu, HI 96822

Oxford (JGP 77, 1981) has shown that when two depolarizing pulses are separated by a brief return to holding potential, interpulse interval has a pronounced effect on the activation rate in the second pulse. This "secondary activation" becomes almost mono-exponential for intervals in the order of 0.1 ms in squid axons at 5° C, whereas control "primary activation" kinetics are reestablished at intervals longer than 0.5 ms. Oxford concluded that sodium channels must open and close (deactivate) by the same pathway. By contrast, Schauf and Bullock (Biophys J 37, 1982) showed that deuterium oxide (D_2O) slows channel opening in *Myxicola* without affecting gating current or tail current kinetics. They concluded that channels must close (during tail currents) by a different pathway from that used in channel opening. We have repeated both sets of observations in crayfish axons. We find that D_2O does not affect the rate of secondary activation after a brief interpulse interval (50 μs). We conclude: (i) that primary activation and tail current deactivation involve separate pathways, and (ii) that rapid secondary activation results from reversal of the fast tail current reaction.

Tu-Pos284

BLOCK OF CARDIAC SODIUM CURRENT BY HEPTANOL AND OCTANOL.

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Heptanol blocks sodium current (I_{Na}) in nerve, but its effects on cardiac I_{Na} have not been well characterized. We studied block of I_{Na} by heptanol in 16 internally perfused voltage-clamped cardiac Purkinje cells at reduced Na^+ (45 mM outside, 0 mM inside). Heptanol block of peak I_{Na} was well described by a single site binding curve with half block at 1.3 mM (20°C), and showed no "use-dependence". With 1.5 mM heptanol block increased by 0.7%/°C from 10 to 27°C. With 3.0 mM heptanol steady-state I_{Na} availability (h_∞) shifted by 10 mV in the hyperpolarizing direction and steady-state activation (m_∞) shifted by 7 mV in the depolarizing direction, thus closing off the I_{Na} "window current". Heptanol also slowed the rise (τ_m) and accelerated the decay (τ_h) of I_{Na}. The decrease in peak I_{Na} could be explained by changes in gating kinetics rather than direct channel block. Similar results were found with octanol at lower concentrations. These alcohols have important effects on I_{Na} at concentrations used in studies on cellular uncoupling in heart. *(Supported by HL-01572, HL-20692 & GM-08140.)*

Tu-Pos285

DEVELOPMENTAL CHANGES IN THE ELECTRICAL PROPERTIES OF RABBIT PAPILLARY MUSCLES.

Toshiyuki Osaka, Brian M. Ramza, Rosemarie C. Tan and Ronald W. Joyner, Emory University, Atlanta GA, 30323 (Intro. by John Pooler)

We used Adult (AD) and Newborn (NB) superfused papillary muscles (PM) at 36 C with 2 Hz pacing. In normal $[K^+]_o$ (4 mM) the NB PM (compared to AD at $p<0.05$) had more negative resting membrane potentials (RMP, -83.6 vs. -80.0 mV), higher maximum rate of rise (V_{max}, 134 vs.120 V/Sec) and higher AP amplitude (APA, 115 vs. 108 mV), but a lower conduction velocity (CV, 44 vs 66 cm/S). 8 mM $[K^+]_o$ depolarized AD and NB PM similarly (10.5 vs 10.8 mV) but NB PM (compared to AD, $p<0.05$) had less reduction in V_{max} (9% vs 31%) and less post-repolarization refractoriness (13.8 vs 27.5 mSec). NB PM in 9 mM $[K^+]_o$ had depolarized RMP (-69.6 $\pm$ 0.5 mV) comparable to AD PM in 8 mM $[K^+]_o$ (-69.5 $\pm$ 1.4 mV), but the reduction in V_{max} and APA was still less for NB then for AD PM at this depolarized level. Tetrodotoxin (5 uM) (comparing NB to AD, $p<0.05$) had less effect on CV (31 vs 39% reduction) and on current threshold (72 vs 174 % increase). Conclusions: The lower CV in NB PM is due to a difference in cable properties (greater surface/volume ratio or greater cell-cell resistivity) rather than a decreased excitability. The Na^+ channel density may be higher in NB PM, providing a greater safety factor for conduction when depolarized.

Tu-Pos286

CHARACTERIZATION OF TETRODOTOXIN SENSITIVITY OF CARDIAC AND NEURAL NA CHANNELS EXPRESSED IN XENOPUS OOCYTES.

D. Krafte, W. Volberg, K. Dillon and A. Ezrin, Dept. of Pharmacology, Sterling Research Group, Rensselaer, N.Y. 12144

Sodium (Na) channels from neuronal and cardiac tissue show differing sensitivities to various toxins and pharmacological agents. To further understand potential tissue related differences in Na channel properties we have expressed Na channels from guinea Pig ventricle and rat brain in Xenopus oocytes and compared electrophysiological properties and tetrodotoxin (TTX) sensitivities. Ventricular RNA was extracted using either a LiCl/Urea or LiCl/Guanidine HCl procedure with identical results. Amplitudes for cardiac Na currents were typically 10% of those following equivalent injections of rat brain RNA consistent with lower densities of Na channels in heart vs. neuronal tissue. Brain Na currents showed 50% block at approximately 10 nM TTX (n=15) while 50% block of cardiac Na currents occurred at $\geq$ 650 nM TTX (n=7). The lower TTX sensitivity for cardiac Na channels is consistent with results reported for cardiac tissue and confirms that we are indeed expressing the cardiac Na channel. The expression of Na channels from ventricular mRNA with a higher Kd for TTX presents an interesting tool to explore the kinetic interaction of local anesthetics known to possess cardiac vs. neuronal selectivity.

Tu-Pos287

TMO-MODIFICATION OF Na CHANNELS DOES NOT REMOVE SURFACE CHARGE NEAR THE CONDUCTION PATHWAY.

Diana B. Cherbavaz. Biophysics Program, Brandeis University, Waltham, MA 02254

Batrachotoxin-activated sodium channels were isolated from rat muscle and inserted into planar bilayers. Single channel conductance (γ) was measured over a range of sodium concentrations (1 to 1000 mM, pH 7.0). The resulting curve did not approach zero as the sodium concentration was lowered; instead the conductance approached a finite value. This observation can be explained by the presence of surface charge on the protein that heightens channel conductance at low sodium. One method to determine the functional effect of charged groups is chemical modification by trimethyloxonium (TMO), which methylates carboxyl groups forming uncharged esters. Unexpectedly, modified channel conductance was decreased over the range of concentration measured and it still approached a positive non-zero value. The ratio of (γ modified/ γ control) remained constant as sodium was varied. Therefore, the effect of TMO in reducing channel conductance does not operate via a surface charge mechanism.

Tu-Pos288

RECOVERY KINETICS OF SODIUM CURRENT ARE ACCELERATED BY DECREASE IN EXTERNAL pH.

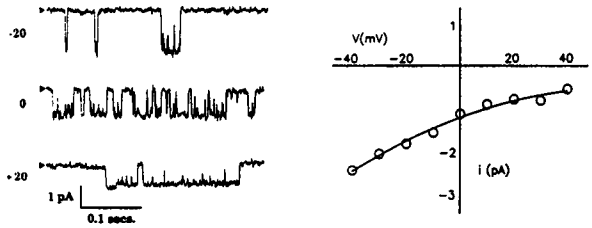
D Wendt, F Starmer, A Grant (Intro. by J Kootsey) Duke Univ Med Ctr, Durham, NC 27710.

It has been proposed that the slowing of recovery of sodium current (I_{Na}) at low pH during exposure to local anesthetics in atrial cells is a direct result of slowing of recovery from normal inactivation (Am. J. Physiol. 225:H1554,1988). This contrasts with observations in neuronal tissue where low pH accelerates normal recovery from inactivation. We explored the recovery kinetics of I_{Na} at extracellular pHs of 6.8 and 7.7, by measuring I_{Na} in atrial myocytes using a whole-cell voltage clamp technique at 17°C. From a holding potential of -120 mV, a 100 msec conditioning pulse was applied to -20 mV followed by test pulses at varying recovery intervals to the same potential. Recovery time constants (τ_r) were obtained from the normalized peak current during the test pulses. The recovery process could be fitted with a single exponential at both pH values. τ_r was 12.1 $\pm$ .6 ms at pH 6.8 and 19.7 $\pm$1.3 ms at pH 7.7 (mean $\pm$ SE n=5; $p<.001$). Peak I_{Na} and the potential for 1/2 inactivation was 6.7 $\pm$ 1.1 nA and 67 $\pm$ 14 mV at pH 6.8 and 7.7 $\pm$ 1.6 nA, and 72 $\pm$ 11 mV at pH 7.7. Under the same recording conditions, recovery of I_{Na} was slowed in the presence of local anesthetics as pH was lowered from 7.7 to 6.8. We conclude that the slower recovery of I_{Na} during drug exposure at low pH does not result from a slowing of the recovery from normal inactivation.

Tu-Pos289

CALCIUM CHANNELS AND TONE IN NORMOTENSIVE AND HYPERTENSIVE RAT RESISTANCE SIZED ARTERIES. J.M. Quayle, J. McCarron*, W. Halpern*, M.T. Nelson. Depts. of Pharmacology and Physiology*, University of Vermont, Burlington, VT 05405.

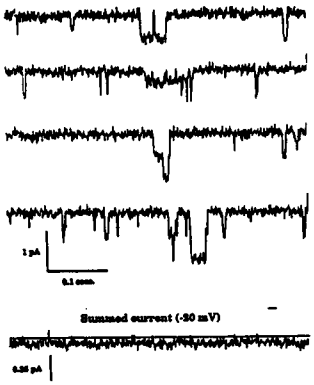
In established hypertension, cardiac output is normal whilst total peripheral resistance is increased. We measured the diameters of pressurized, resistance-sized branches of rat posterior cerebral arteries (diameters~150 μm), and have isolated single cells for single channel recordings from the same. Pressurized arteries from both normotensive (WKY) and hypertensive (SHRSP) rats developed spontaneous tone which was dependent on extracellular calcium and abolished by nimodipine (5 nM), a dihydropyridine calcium channel antagonist. Single cells were isolated by perfusion of the artery with a low calcium physiological saline solution containing collagenase (Sigma, Type 4, 4 mg/ml) and elastase (Sigma, Type 2A, 0.3 mg/ml), followed by mechanical dispersion. Single calcium channels opened on depolarization. Channel openings were prolonged by the agonist Bay R 5417 (0.5 μM). The most frequently observed channel had a conductance of 30 pS at 0 mV (80 mM barium as charge carrier).



Tu-Pos290

CALCIUM CURRENTS THROUGH SINGLE CALCIUM CHANNELS IN SMOOTH MUSCLE CELLS FROM CEREBRAL ARTERIES. John M. Quayle and Mark T. Nelson. Department of Pharmacology, University of Vermont, Burlington, VT 05405.

Voltage-dependent calcium channels provide an important route for calcium entry into arterial smooth muscle cells, being involved in regulation of tone and vasoconstrictor action. Barium ions have been used as the charge carrier in most studies of single calcium channels. In this study, we have recorded calcium currents through single calcium channels in on-cell patches of smooth muscle cells enzymatically isolated from rabbit cerebral (basilar) arteries. The pipette solution contained 80 mM $CaCl_2$ and the bath solution contained 0.1-0.5 μM Bay R 5417 to increase channel open times. At -20 mV, two unitary current levels were observed with means of -0.60 pA and -0.32 pA. The conductances of these two levels measured between -20 and +30 mV were ~9 pS and ~5 pS, about one-half of that with 80 mM barium as the charge carrier. Open state probability (P_O) of these channels increased with membrane depolarization, with P_O being maximal around +20 mV. Summed currents on depolarization from -70 to -20 mV showed little decay, with openings throughout the 0.5 second test pulse. Inactivation during the pulse was seen for test pulses positive to 0 mV.



Tu-Pos291

DEPOLARIZATION TRIGGERED CAPACITANCE INCREASES IN SINGLE ISOLATED NERVE TERMINALS *Nancy H. Fidler[v], Martha C. Nowycky*, and Richard J. Bookman[‡]* Depts of Biophysics[v] and Physiology[‡], and HHMI[‡] Univ. of Penn. and Dept. of Anatomy*, Med. Coll. Penn., Phila, PA.

A sensitive assay for membrane area increases was used to study excitation-secretion coupling in single nerve terminals isolated from rat posterior pituitary. To monitor changes in membrane capacitance, terminals were stimulated with sinusoidal excitation under whole-cell patch clamp and the resulting currents were analyzed with a phase sensitive detector. Voltage-gated Ca^{++} currents were activated with step depolarizations applied while the sine wave was interrupted. These techniques permit the relationship between Ca^{++} entry and exocytosis to be determined quantitatively. In response to a single 30 to 150msec pulse, we observed a rapid capacitance (C_m) increase ranging from 10 to 100fF. This represents the exocytosis of up to 100 peptide-containing secretory vesicles (avg. diam. ≈ 170nm). Using low frequency stimulation (< 0.1Hz), the secretory response in a given terminal was reproducible for 5-20 minutes. Total C_m increases corresponding to the fusion of hundreds of vesicles were observed over the duration of the recordings. The C_m increases were correlated with Ca^{++} entry through voltage-gated channels. The peak of the C_m response occurs at the peak of the I_{Ca}-V relation, and capacitance jumps were not observed with depolarizations to the reversal potential for Ca^{++}.

Tu-Pos292

CALCIUM CURRENTS AND CAPACITANCE RESPONSES TO REPETITIVE DEPOLARIZATIONS IN SINGLE MAMMALIAN NERVE TERMINALS *Martha C. Nowycky*, Nancy H. Fidler[v] and Richard J. Bookman[‡]* Dept. of Anatomy*, Med. Coll. Penn. and Depts of Biophysics[v] and Physiology[‡], and HHMI[‡], Univ. of Penn., Phila, PA.

Nerve terminals isolated from rat neurohypophysis are 1 - 10 μm in diameter and suitable for patch clamp studies. Using standard techniques, we have recorded two types of voltage-gated calcium currents which coexist in single, isolated nerve terminals (Lemos & Nowycky, 1989, Neuron, 2:5). Recently, we have succeeded in studying exocytosis in these peptide-releasing terminals by utilizing capacitance measurements of membrane area increases (Fidler et al., this meeting). Although exocytosis has a strong dependence on Ca^{++} entry and buffering, multiple pulse protocols reveal non-linear relationships between Ca^{++} entry and secretion. Terminals stimulated with five 50 msec depolarizing pulses @1.33Hz show progressively larger capacitance jumps (~10-100fF), peaking at the 3rd or 4th pulse. This 'facilitation' occurs despite partial inactivation of the Ca^{++} currents. The jumps in response to subsequent pulses are smaller, possibly indicating vesicular depletion or inactivation of the secretory machinery. The recovery of the Ca^{++} currents is faster than that of the secretory response. Taken together, these findings suggest that the observed 'facilitation', 'depression' and recovery occur after the Ca^{++} entry step in excitation-secretion coupling.

Tu-Pos293

IMAGING Ca^{++} TRANSIENTS IN SINGLE, VOLTAGE-CLAMPED BOVINE ADRENAL CHROMAFFIN CELLS

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Howard Hughes Medical Institute,
University. of Pennsylvania, Phila., PA., 19104.

Our capacitance measurements of secretory responses to depolarizing pulses have shown the Ca^{++} dependence of fast and slow phases of exocytosis in cells. Since the time course of the $[Ca^{++}]_i$ transient may help to explain these phases, we have measured fluorescence signals from single, ~spherical, cultured chromaffin cells internally perfused with Fura-2 free acid. Cell to cell variability in the extent and time course of dye-loading was large. Dye binding within some cells was indicated by the relative brightness of the nucleus with either 340nm or 380nm excitation. Most, but not all, cells showed spatially uniform Ca•Fura-2 responses to depolarizations. With 0.1-0.5mM Fura + 0 EGTA in the pipette, the initial, rapid decrease in the ΔF_{380} signal in response to a single 20-200ms pulse was correlated with the amount of Ca entry and was absent for pulses to E_{Ca}. The ΔF_{380} required 15-30s to recover. With five repetitive pulses @ 1Hz, saturation of the signal was likely since the response to the 5th pulse was always smaller despite similar Ca entry. This can be prevented by either increasing $[EGTA]_i$ to 5mM, in which case the Ca•Fura-2 responses are rapid transients (recovering within 100-330ms), or by increasing $[Fura\text{-}2]_i$. Generous support from Dr. Robert G. Johnson, Jr. and HHMI is gratefully acknowledged.

Tu-Pos294

CALCIUM CHANNEL SELECTIVITY IN SQUID NEURONS. L. Tabares, R.H. Chow, and C.M. Armstrong. Marine Biological Laboratory, Woods Hole, MA 02543

Calcium channels in giant fiber lobe neurons of the squid are characterized by a high voltage threshold for activation, a fast deactivating time course, and minimal inactivation for 100 ms depolarizations. These channels are highly selective for Ca at physiological divalent concentrations, and they conduct Ba better than Ca. At negative potentials, however, monovalent cations become permeant when the external divalent concentration is in the low millimolar range. Instantaneous IV curves were obtained in different mixtures of Na and Ca or Ba. In general, for small hyperpolarizations, Na partially blocked Ba or Ca current; while for more negative steps, Na itself passed through the channels (more readily in the presence of Ba than of Ca). The degree of block or permeation was highly dependent on the type of and the concentration of divalent ion present in the external solution. Supported by NIH Grant No. NS 12547.

Tu-Pos295

REEVALUATION OF DISSOCIATION CONSTANTS FOR 1,4-DIHYDROPYRIDINES (DHP) BASED ON MEMBRANE DRUG CONCENTRATION. R.P. Mason, D.G. Rhodes and L.G. Herbette - Biomolecular Structure Analysis Center, University of Connecticut Health Center, Farmington, CT. 06032

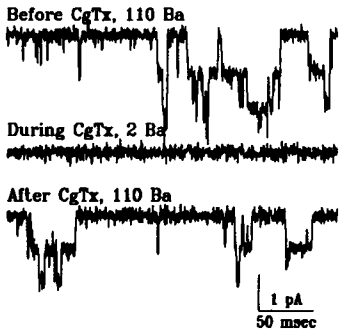
The dissociation constant (K_d) for a bimolecular reaction is the ratio of the concentration of complex to the product of the concentrations of drug and free receptor. Based on several lines of supporting evidence, DHP's approach their binding site on the calcium channel from within the membrane bilayer by partitioning and then diffusing laterally. Thus, in calculating K_d using Scatchard analysis, the appropriate concentration of free drug is that present in the membrane. We have calculated K_d's for a number of DHPs by using the membrane-based partition coefficient ($P_{k[mem]}$) to determine the concentration of free drug in the environment to which the receptor site is exposed. Because the $P_{k[mem]}$ for DHPs are high, it is not surprising that the apparent DHP affinities are significantly lower than previously thought. Drugs which have an apparently high affinity may acquire this overall property from high partition coefficients, high specific affinity or a combination of these effects. (Supported by AHA. CT Affiliate; American Health Assistance Foundation; HL-33026; NSF# CTS-8904938)

Tu-Pos296

SELECTIVE MODULATION AND BLOCK OF N-TYPE CALCIUM CHANNELS. M.R. Plummer, M. Kanevsky, & P. Hess. Dept. Cellular & Molecular Physiology, Harvard Medical School, 25 Shattuck St., Boston, MA, 02115.

A variety of transmitters and toxins have been shown to act on the calcium current in vertebrate neurons. Determining which of the neuronal calcium channel types is affected, however, has been difficult. To examine the selectivity of different substances, we have used the dihydropyridine agonist (+)-(*S*)-202-791 to augment specifically the L-channel contribution to whole-cell tail currents. During the reduction in peak current induced by intracellular application of 100 μM GTPγS and extracellular application of 100 μM acetylcholine or 50 μM noradrenaline, the amplitude of the prolonged portion of the tail current remained constant. This indicated that these substances, like ω-CgTx (Plummer *et al.*, *Neuron* 2: 1453 - 1463, 1989), had no effect on the L-channel and must have been acting only on DHP-insensitive channels.

To examine directly the behavior of single channels in response to extracellularly applied ligands, we have developed a method for recording from perforated outside-out membrane vesicles in a way similar to that recently described by Levitan *et. al.* (*Soc. Neurosci. Abstr.* 15(1): 178, 1989). With this technique, channel activity persists for up to thirty minutes, and we have been able to confirm the absence of ω-CgTx block on the L-channel.

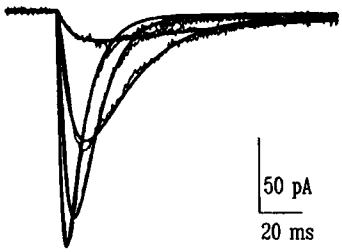


Tu-Pos297

A KINETIC MODEL FOR T-TYPE CALCIUM CHANNEL GATING IN 3T3 FIBROBLASTS.

Chinfei Chen and Peter Hess, Dept. of Physiology and Program in Neuroscience, Harvard Medical School, Boston, Ma 02115.

Our analysis of whole-cell T-type currents in 3T3 fibroblasts reveals that channel closing, inactivation and recovery from inactivation each include a voltage independent transition which becomes rate limiting at extreme potentials. These results can be modeled by a cyclic scheme with two closed, one open and two inactivated states (bold lines in figure below). The opening and closing steps and the inactivation from the open state are voltage independent. This model is further supported by our cell attached recordings, which show that each T-type channel opens once in a bursting fashion before inactivating. Comparison of the kinetics of T-type Ca current in neuronal cells to those in fibroblasts reveals significant differences which suggest that T-type Ca currents in various cells represent a heterogeneous population of channel types.



Tu-Pos298

CALMODULIN MODULATES CALCIUM CHANNELS IN HELIX ASPERSA NEURONS.

Juan Bernal and J.David Johnson (Intro. by L.B. Cohen), Univ. of CT, Farmington CT. and Ohio State University, Columbus, OH.

It has been reported that trifluoperazine, an anticalmodulin drug, blocks the calcium currents in Helix neurons (Bernal J. et al. Biophys. J. 51:33a, 1987). These results suggested that calmodulin (CaM) may modulate calcium channels. Here we report that the complex calcium-calmodulin (Ca^{2+}-CaM*) modulates voltage-dependent calcium channels in Helix neurons. The cells were superfused with a solution containing: (in mM) TEA-Cl 70; 4-AP 5; 3,4 DAP 5; CsCl 5; $CaCl_2$ 20; Cs-Hepes 10; pH 7.5. Intracellular recording experiments under current and voltage clamp conditions were done. When Ca^{2+}-CaM* was injected into the cell by pressure, the duration of the calcium action potential increased by 145% $\pm$ 40, n=5. The injection of Ca^{2+}-CaM* into clamped cells, increased the magnitude of the calcium currents, ICa^{2+}Max (at +30 mV) by 57% $\pm$ 8 n=5. The effect of Ca^{2+}-CaM* was slow in onset ($t^{1/2} \cong 5$ min.) and was mantained over 12 minutes. These results suggest that Ca^{2+}-CaM* or a Ca^{2+}-CaM-dependent process modulates calcium channels.

Tu-Pos299

PRESYNAPTIC CALCIUM CONCENTRATION EXCEEDS ONE MILLIMOLAR IN FROG SACCULAR HAIR CELLS.

W.M. Roberts[1], R.A. Jacobs[2], and A.J. Hudspeth[2], [1]Institute of Neuroscience, University of Oregon, Eugene, OR 97403 and [2]Dept. of Cell Biology and Neuroscience, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

Voltage-clamp recordings, serial transmission electron micrographs, and freeze-fracture electron micrographs of saccular hair cells from *Rana pipiens* indicate that each of a cell's ~20 afferent synaptic active zones is associated with a 200-nm-diameter plaque of intramembrane particles that includes ~100 Ca^{2+} channels and ~35 K_{Ca} channels. The close association between K_{Ca} channels and active zones allowed us to use the channels' Ca^{2+} sensitivity to assay the local $[Ca^{2+}]_i$ at synaptic sites. After blocking the Ca^{2+} current, we calibrated the K_{Ca} channels by measuring their voltage dependence in the presence of buffered $[Ca^{2+}]_i$ solutions introduced through whole-cell pipettes. Using these calibrations, we deduced that when the Ca^{2+} current is active, the local $[Ca^{2+}]_i$ rises steeply upon depolarizations above -60 mV, and exceeds 1 mM at -40 mV. This high local $[Ca^{2+}]_i$ is predicted by a simple diffusion model in which each active zone is assumed to contain 5% of the cell's Ca^{2+} channels, scattered randomly within a 200-nm-diameter disk.

Supported by NIH grants NS07904 and NS22389.

Tu-Pos300

ATP INCREASES THE Ca CURRENT OF SINGLE RAT CARDIAC CELLS.

F. Scamps & G. Vassort (intr. by D. MORNET), INSERM U-241, Orsay, FRANCE.

Exogenous applications of ATP (μM) and adenosine compounds induce positive inotropic effects in the rat ventricle and after pertussis toxin treatment in the rat auricle. When applied to single rat cells under whole cell voltage clamp, ATP increased the L-type Ca current (I_{Ca}) recorded in the presence of TTX and Cs to block Na and K currents respectively. The maximal increase was up to 60% at 10 μM ATP as compared to 120% with 1 μM isoprenaline. Adenosine was less effective. ATP further increased the maximally β-adrenergic or cAMP stimulated I_{Ca}. However, this was preceded by a transient decrease which could not be attributed to P_1-purinostimulation. This decrease was not elicited by adenosine nor by Mg-free ATP ; it was still elicited by ATP after pertussis toxin treatment. In conclusion, purinostimulations of I_{Ca} by ATP occur independently of the cAMP-dependent pathways and provide another control mechanism of cardiac inotropy.

Tu-Pos301

THE EFFECT OF COMPARTMENTALIZED Ca^{2+} IONS ON BURSTING PANCREATIC β-CELLS

TERESA REE CHAY, BIOLOGICAL SCIENCES, UNIVERSITY OF PITTSBURGH, PITTSBURGH, PA 15260

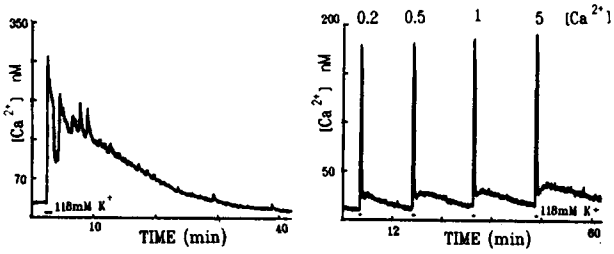
Based on the observations that (i) the whole cell Ca^{2+} current has two functionally distinct components (fast and slow), (ii) a fast component is inhibited by intracellular Ca^{2+} ions, (iii) a slow component is inactivated by depolarization, and (iv) a significant fraction of the outward current is carried by the calcium-sensitive, voltage-gated K^+ channels, we present a model for bursting pancreatic β-cells. The model contains a feature that the Ca^{2+} concentration in the submembrane compartment, $[Ca^{2+}]_s$, is different from that in the cellular phase. At the plateau phase, $[Ca^{2+}]_s$ is high enough to activate the K(Ca,V) channels. The model also contains a voltage-activated Ca^{2+} channel that is quickly blocked by Ca^{2+} ions and slowly inhibited by voltage. Since the Ca^{2+} channel has a Ca_i-dependent inactivation gate, an increase in $[Ca^{2+}]_s$ can inactivate the Ca^{2+} channels. According to this model, the spikes during the plateau phase are caused by a rapid movement of Ca^{2+} ions into and out of the compartment. Because of a rapid change in $[Ca^{2+}]_s$, the two competing currents, I_{Ca} and $I_{K(Ca,V)}$, fluctuate rapidly; the fluctuation leads to an emergence of spikes. The slow underlying wave is due to a voltage-dependent inactivation gate of the Ca^{2+} channels, which slowly closes as a result of depolarization. While the existing models give plateau fractions (the ratio between the plateau duration and cyclic time) to be far less than unity, the present model is the first of its kind that allows plateau fractions to be in the near-unity range.

Tu-Pos303

$[Ca^{2+}]_i$-TRANSIENTS ARE NOT ALWAYS RELATED TO Ca^{2+} CURRENTS.

A.P. Fox, M.K. Dahmer, R.L. Perlman, & C.R. Artalejo, The University of Chicago, Dept. Of Pharm/ Phys

$[Ca^{2+}]_i$ homeostasis of bovine chromaffin cells was explored using 118 mM K^+ to depolarize cells. The left panel shows that after a 30 second depolarization was terminated there was a broad shoulder of elevated $[Ca^{2+}]_i$ lasting over 40 minutes. The right panel shows $[Ca^{2+}]_i$-transients induced by high-K^+ depolarizations in extracellular Ca^{2+} solutions that were varied from 200 μM to 5 mM. All the $[Ca^{2+}]_i$-transients were the same amplitude, in contrast to patch-clamp experiments which predicted different sized currents using the assorted extracellular Ca^{2+} concentrations. Long shoulders were suppressed by keeping cells in 0 mM Ca^{2+} between depolarizations. These results indicate that $[Ca^{2+}]_i$-transients (and perhaps secretion) are relatively uncoupled from Ca^{2+} influx into the cells, suggesting that under these conditions neither $[Ca^{2+}]_i$-transients nor perhaps secretion are good indicators of Ca channel activity.



Tu-Pos302

DRUG BINDING SITES ON THE α_1 SUBUNIT OF L-TYPE CALCIUM CHANNELS.

Pal L. Vaghy, Amy Atzel and Kazuaki Naito, Dept. of Pharmacology and Cell Biophysics, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0575

We have shown that specific photoaffinity probes (+)[^{3}H]PN200-110, [^{3}H]LU-49888 and [^{3}H]azidobutyryl diltiazem representing the three major groups of calcium antagonists bind to the 165 kDa α_1 subunit of the purified skeletal muscle L-type calcium channels. To determine the location of binding sites for structurally different calcium antagonists within the α_1 subunit, the purified, photoaffinity labeled receptor was subjected to partial tryptic digestion and the fragments were separated with SDS-PAGE. The labeled fragments were identified by measuring radioactivity in gel slices. In partially digested receptor preparations 80 and 30 kDa fragments were labeled with all three drugs. In fragmented receptors reversible drug binding was retained and allosteric interactions between the three drug binding sites were unaltered. These data suggest that 80 and 30 kDa fragments of the 165 kDa α_1 subunit may contain intact binding sites for all three major classes of calcium antagonists. (Supported by NIH grant R01 HL41088 to P.L.V).

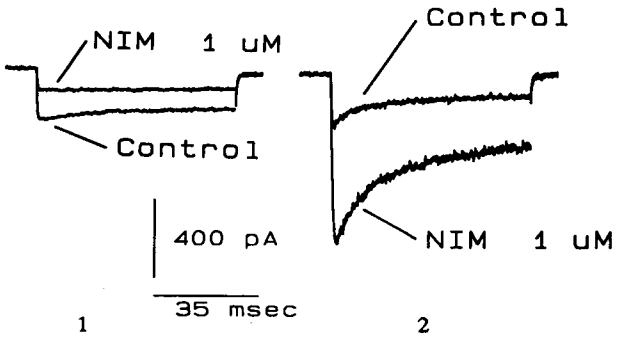
Tu-Pos304

NIMODIPINE EXHIBITS VOLTAGE-DEPENDENT AGONIST AND ANTAGONIST EFFECTS ON Ca CURRENT IN HIPPOCAMPAL CA3 NEURONS

DJ Mogul & AP Fox (Intr. by M Villereal), Dept. of Pharm/Phys, Univ. of Chicago, Chicago, IL

The effects of 1 μM Nimodipine (NIM) on Ca current (i_{Ca}) in acutely dissociated guinea pig hippocampal CA3 pyramidal neurons were studied. Cells were voltage-clamped using the whole-cell configuration. Solutions contained, in part (mM): Internal=Cs 100, GTP .35, ATP 2, Mg 5, EGTA 10; External=Ba 5,TEA 135, TTX 0.3 μM. Ba^{2+} was the external charge carrier. With a holding potential (HP) = -40 mV and test potential (TP) = -10 mV (Fig 1), exposure to NIM caused a large decrease in i_{Ca}. However, with HP=-80 mV and TP=-30 mV in a different cell (Fig 2), i_{Ca} significantly increased. Thus some dihydropyridine antagonists may show significant voltage-dependent agonist effects in neurons.

HP=-40 mV, TP=-10 mV HP=-80 mV, TP=-30 mV



Tu-Pos305

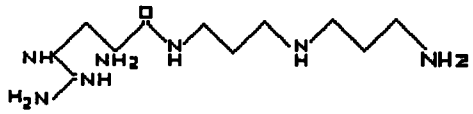
EXPRESSION OF α_1 AND α_2 SUBUNIT cDNAs OF THE SKELETAL MUSCLE L-VOLTAGE DEPENDENT CALCIUM CHANNEL IN MOUSE Ltk$^-$ CELLS: POSSIBLE FUNCTION OF α_2. G.Varadi[1], S.Tang, H.Kim[2], E.Perez-Reyes[2], A.E. Lacerda, A.M. Brown, L.Birnbaumer[2] & A. Schwartz[1]. [1]Univ Cinti Coll Med-Dept Pharm, & [2]Baylor Coll Med-Depts MolPhys & CellBiol, Houston,TX 77030.

The L-type voltage dependent calcium channel (L-VDCC) is important in E-C coupling in skeletal muscle. The L-VDCC consists of five subunits, α_1, α_2, β, γ and δ. The primary structures of rabbit α_1, α_2 and β were determined by sequencing complementary cDNAs. The α_1 may have domain characteristics for channel activity. Our attempts to express cRNAs for skeletal muscle α_1 and α_2 in oocytes have not as yet been successful. The α_1 cDNA linked to a zinc-inducible promoter has been expressed in mouse cells (Lca11). Binding characteristics of Lca11 for drugs/calcium channel are similar to native T-tubules; however, L-VDCC activity is different from intact muscle. The α_2 and perhaps other subunits may be required for native expression. Accordingly, we linked α_2 cDNA to glucocorticoid-inducible promoter and transfected into Lca11 cells. The effect of α_2 on α_1 and L-VDCC function, will be presented. Supported by NHLBI grants.

Tu-Pos306

SYNTHETIC FTX-LIKE TOXIN BLOCKS P-TYPE CALCIUM CURRENTS.Cherksey, B., Lin, J.W., Sugimori, M. and Llinas, R. NYU Medical Center

FTX, a toxin fraction from Agalenopsis aperta venom (Cherksey, et al, Biophys.J. 55:438,1989),has been shown to block the calcium dependant spikes in Purkinje cells and the presynaptic calcium current (ICa) in the squid stellate ganglion and was used to construct an affinity gel for the isolation and characterization of P-type calcium channels from squid optic lobe and mammalian CNS (Llinás, et al PNAS 86:1698,1989) Purification and structural analysis studies of FTX have been performed. Cation exchange HPLC permitted a high level of purification of FTX. Purified FTX exhibited a sharp uv absorption at 220 nm. No ring (aromatic) structure was detected. Fourier transform Infrared spectroscopy (FT-IR) indicated the presence of C-C, C-N, N-H, C-H and the absence of C=O groups. These results suggest that FTX is a polyamine. On the basis of these results, model compounds were constructed with the general structure of arginine-polyamine;



The compounds exhibited the selectivity of FTX but not its potency. Compounds of the structure arginine-polyamine-arginine were ineffective as blockers. Thus, the free terminal amine is critical for efficacy. The differences between FTX and the model compounds may be due to the negative charge on the carbonyl of the model compound, which on the basis of FT- I R spectra is absent from FTX. Supported by AFOSR85-0368 and the FIDIA Foundation.

Tu-Pos307

FTX, A LOW MOLECULAR WEIGHT FRACTION OF FUNNEL WEB SPIDER VENOM, BLOCKS CALCIUM CHANNELS IN NERVE TERMINALS OF VERTEBRATES.

B.M. Salzberg, A.L. Obaid, K. Staley, J-W. Lin, M. Sugimori, B.D. Cherksey and R. Llinás, U. of Penn, N.Y.U. Medical Center, and the M.B.L.

Specific channel blocking agents, FTX (Llinás, et. al., PNAS, 86:1689, 1989) and w-conotoxin, (Cruz and Olivera, J.Biol.Chem. 261: 6230,1986) were utilized to characterize the calcium channel populations in the intact nerve terminal of the Xenopus neurohypophysis. Optical measurement techniques (Salzberg et. al. Nature 246: 508,1973), demonstrated that 100 µM of synthetic FTX, a 385 Da polyamine (Cherksey, et.al., Biol.Bull. 177: 2, 1989) eliminated the undershoot of the normal action potential and reduced the magnitude of the calcium spike in TTX-TEA pretreated preparations. Both of these effects are indicative of Ca-channel block. In preparations maximally blocked by 10 µM synthetic w-conotoxin GVIA, FTX exhibited further effect but did not entirely eliminate the active calcium response. Further addition of 200 µM Cd^{++} left only the passive electrotonus. These results suggest that at least two, and probably three populations of calcium channels are present in the intact terminals of the frog neurohypophysis.

Supported by NS16824, NS13742 , EY08002 and AFOSR85-0368

Tu-Pos308

Isoproterenol Shifts Cardiac Calcium Channel Gating Currents. I.R. Josephson and N. Sperelakis. Department of Physiology and Biophysics, University of Cincinnati, College of Medicine, Cincinnati, OH 45267-0576.

Non-linear charge movement (gating current) was studied by the whole-cell patch clamp method using cultured 17-day-old embryonic chick heart cells. Na^+ and Ca^{++} currents were blocked by the addition of 10 µM TTX and 3 mM $CoCl_2$; Cs^{++} replaced K^+ both intra- and extracellularly. Linear capacitive and leakage currents were subtracted by a P/5 procedure. The small size (15 u in diameter) and the lack of an organized internal membrane system in these myocytes permits a rapid voltage clamp of the surface membrane. Ca^{++} channel gating currents were activated positive to -60 mV and displayed a rising phase which was not due to the system response time. The addition of isoproterenol (Iso) (10^{-6} M) caused a shortening of the time to peak of the Ca gating current, and a small negative shift in the Q_{on} vs. Vm curve. Q_{max} was unchanged by Iso. The effects of Iso were reversed by ACh (10^{-5} M) and could be mimicked by 8-bromo-cyclic AMP (2 mM). The results suggest that a cyclic AMP-dependent phosphorylation of one or more of the Ca^{++} channel subunits alters the kinetics and shifts the voltage-dependence of gating. These changes in the gating currents can explain the parallel changes in the macroscopic Ca^{++} currents.

Tu-Pos309

THE LA-N-5 HUMAN NEUROBLASTOMA CELL LINE HAS L-TYPE CALCIUM CHANNEL mRNA SIMILAR TO THAT FOUND IN SKELETAL MUSCLE.

R.E. Weiss, M.A. Hediger+, J. Hu, N. Sidell, & J.A. Talvenheimo#*. Depts. of Ped. Cardiology & *Pathology, UCLA, L.A., CA 90024, +Brigham & Womens Hosp., Renal Div., Harvard Med. Sch., Boston, MA 02115, & #Dept. of Pharmacology, Univ. of Miami, Miami, FL 33101.

Northern analyses using three short oligonucleotide probes (23-24 bases) complementary to the rabbit skeletal muscle dihydropyridine receptor (DHPR) mRNA, and a long probe (821 bases) complementary to rat skeletal muscle DHPR mRNA, were performed on mRNA isolated from rabbit and rat skeletal muscle, and from a human neuroblastoma cell line, LA-N-5, derived from a sympathetic nerve tumor of the adrenal medulla. The long probe (rat) and one of the short probes (rabbit) hybridized to a 6.5 kbase mRNA from all three samples. Two short probes (rabbit) hybridized only to the rabbit mRNA. We conclude that the Ca channel mRNA in the LA-N-5 cell line is similar, though not identical, to the skeletal muscle DHPR mRNA. Whole-cell Ba^{2+} currents measured with the patch clamp technique from LA-N-5 cells bathed in 50 mM Ba^{2+} confirmed that functioning L-type Ca channels exist in the membrane. The DHPR density in LA-N-5 cells, measured by specific [^{3}H]PN200-110 binding, is 78 fmol/mg membrane protein. This receptor density is similar to the estimate obtained from the whole-cell currents, suggesting that most of the dihydropyridine receptors are functional Ca channels.

Supported by grants from MDA and Calif. Affiliate of AHA to REW, NIH to JT, and NIH to NS.

Tu-Pos310

GLUCOSE-INDUCED OSCILLATIONS OF INTRACELLULAR Ca^{2+} AND MEMBRANE POTENTIAL IN SINGLE MOUSE ISLETS OF LANGERHANS

L.M. Rosario, R.M. Santos, D. Contreras, B. Soria and M. Valdeolmillos Department of Physiology, University of Alicante, Alicante, Spain

Intracellular Ca^{2+} levels were monitored in single, acutely isolated mouse islets of Langerhans by dual emission Indo-1 fluorometry. High-frequency (3.1 min^{-1}) $[Ca^{2+}]_i$ oscillations with a brief rising time (1-2 s) and 10 s half-width were detected in 11 mM glucose. These $[Ca^{2+}]_i$ oscillations were coincidental with the bursts of electrical activity as recorded intracellularly with microelectrodes. Raising glucose concentration to 16.7 mM increased the duration of the $[Ca^{2+}]_i$ oscillations, which were otherwise absent in 5.5 mM glucose. The data indicate that the $[Ca^{2+}]_i$ oscillations are the consequence of β-cell bursting electrical activity, and suggest the existence of extensive networks of electrically coupled cells in the islet.

Tu-Pos311

A POSSIBLE CROSSTALK IP3-cAMP DEPENDENT PHOSPHORYLATION OF Ca CHANNELS IN EPIDERMOID CELL LINE.

T JULLIEN, C COCHET*, J VERDETTI & Y CHAPRON, BMC-CENG - *BRCE-CENG. FRANCE.

A new, non-independent Ca channel running in BMC (Bursting Multi Channel) mode has been found in A431 cells after EGF receptor activation using the patch-clamp technique*. In the inside-out excised patch we have shown that a Ca channel was activated by IP3 exhibiting the same conductance (8pS) without voltage dependence and similar kinetic parameters (τ_f = 0,6 ms ± 0.3, τ_s = 7 ± 3 ms)*. Recently, either in cell-attached configuration after forskolin addition or in inside-out excised patch with cAMP and ATP we got similar inward Ca-current. Inhibition of kinase or activation by catalytic subunit confirms that Ca-channel activation could be processed by phosphorylation from the PKA.

Power spectral density functions of EGF, IP3 and cAMP + ATP show 2 frequency corners (f_r = 250 ± 100 Hz, fs = 23 ± 9 Hz) in the same frequency range indicating similar kinetics for all 3 pathways.

As shown in other conditions a crosstalk between IP3 and cAMP activated kinase might exist to modulate Ca-channel activation.

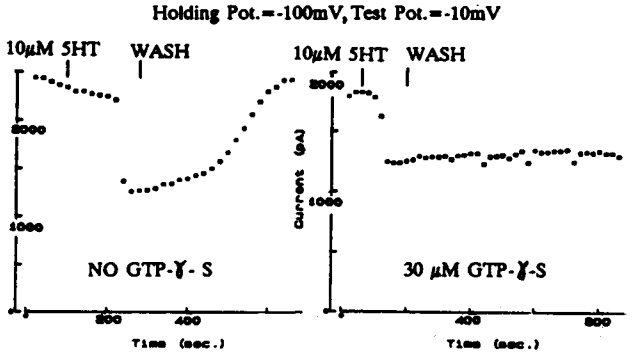
*Y CHAPRON et al (1989) BBRC, 158: 527.

Tu-Pos312

G-PROTEIN INVOLVEMENT IN THE INHIBITORY ACTION OF 5-HT ON Ca^{2+} CURRENTS IN ACUTELY ISOLATED ADULT RAT DORSAL RAPHE NEURONS.

N.J.Penington*+, A.P.Fox+, J.S.Kelly*, and R.J.Miller+. (Intro. by D. Hanck) Univ.of Edinburgh U.K*. and Univ. of Chicago+, Dept of Pharm/Phys, Chicago, Il. U.S.A.

Serotonin (5-HT) reversibly inhibits the calcium current of acutely isolated raphe neurons by an action on a 5-HT_1-like or a 5-HT_{1A} receptor (N.J.P. Proc. Phys. Soc. August 1989). I_{Ca} was isolated and whole cell currents carried by 5mM Ba^{2+} were voltage clamped. Inclusion of 30μM GTP-γ-S in the patch pipette with the usual 300μM GTP prevented or occluded the recovery (lower Fig. right) of that component of I_{Ca} which is inhibited by 5-HT (without GTP-γ-S lower Fig left). Both 5-HT (10μM) and GTP-γ-S alone frequently slowed the rate of activation of I_{Ca} measured at its peak. These data indicate that 5-HT activates a G-Protein to modulate the opening of Ca^{2+} channels in these neurons.



Tu-Pos313

pH REGULATION OF THE Ca^{2+}-ACTIVATED K^+ CHANNEL IN RABBIT PORTAL VEIN CELLS. H.S. Kim and S. Hu. Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901

Regulation of the Ca^{2+}-activated K^+ channel (B_K channel) by changes in extracellular (pH_o) and intracellular pH (pH_i) in enzymatically dissociated rabbit portal vein cells was studied. Effects of pH_o change from pH 7.3 were examined with the whole cell voltage-clamp technique. Outward K^+ current (mainly B_K channel current) was decreased at pH_o 6.8 by 40% and increased at pH_o 7.8 by 15%. The inside-out patch-clamp technique was used to measure channel activity at any precise pH_i levels (±0.01). Little change in channel behavior, but a 12% increase (n=4) in B_K channel conductance was observed at pH_i 8.3. Acidic pH_i greatly enhanced the B_K channel activity. At pH_i 7.0 and 6.7, the channel mean open-time at 20 mV was, respectively, 1.1 and 1.3, and mean closed-time 0.3 and 0.1 of that at pH_i 7.3 (n=6). Acidic pH_i (but not alkaline pH_i) also potentiated inward opening of the B_K channel at potentials more negative than E_K. All pH effects were reversible. Acidic pH_i-induced activation of the B_K channel, accompanied by cell repolarization and relaxation, could be a beneficial compensatory mechanism in acidosis under hypoxia.

Tu-Pos314

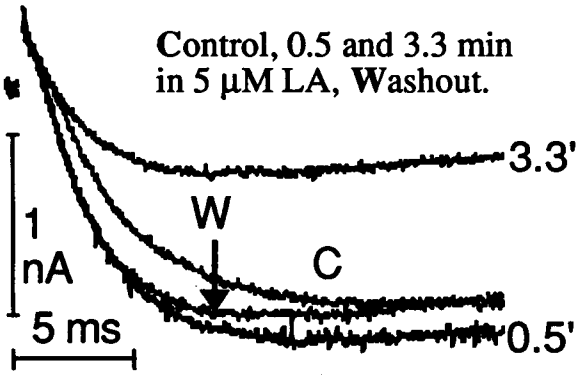
EFFECTS OF BRL 34915 AND P 1060 ON THE Ca^{2+}-ACTIVATED K^+ CHANNEL IN RABBIT PORTAL VEIN CELLS. S. Hu, H.S. Kim and G.B. Weiss. Research Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

The effects of BRL 34915 and P 1060 (Leo), a pinacidil derivative, on the Ca^{2+}-activated K^+ channel (B_K channel) in enzymatically dissociated cells were studied with single channel and whole cell recording techniques. The channel conductance is 264 pS (n=14, 140 mM K_o/140 mM K_i) and is dependent on voltage (14 mV/e-fold) and Ca^{2+}_i (45 mV/10-fold). In cell-attached patches, P 1060 (3-100 nM) did not alter channel conductance, but increased the channel open-state probability (p_o) mainly by reducing the mean closed-time. P 1060 (10 nM) increased channel voltage sensitivity by about 20%. BRL 34915 (0.1-5 uM) had similar actions, but was about 30-fold less potent. In inside-out patches, 100% of those exposed to 1 uM BRL 34915 (n=7) and 50% of those with 10 nM P 1060 (n=10) showed a 40-120% increase in p_o, indicating that BRL 34915 and P 1060 act directly on the K^+ channel. The whole cell recording revealed that 10 nM P 1060 reversibly increased outward rectification of K^+ current, mainly B_K channel current. These results indicate that B_K channel opening may be an important vasodilation mechanism.

Tu-Pos315

Calcium Agonist and Antagonist Effects of Fatty Acids in Guinea Pig Atrial Myocytes. C.J. Cohen, T. Bale and M.D. Leibowitz. Merck Inst., Rahway, NJ 07065

Arachidonic and linoleic acids (AA, LA; 0.5-10 μM) reversibly block L- and T-type Ca channels and Na channels in atrial cells [see G. Katz abstr. for GH3 cells]. Block of L-type Ca channels by LA and AA is similar to that caused by the therapeutically useful Ca antagonists: 1) the rate of current decay during a test pulse is speeded; 2) the effect on decay rate increases with the degree of channel activation; 3) block is more potent when binding equilibrates at less negative potentials; 4) currents sometimes increase when drug is first applied (see Fig). Block of Na and Ca channels is not prevented by inhibitors of 5-lipoxygenase or cyclooxygenase.



Tu-Pos316

Tissue Specific Regulation of T-type Ca Channels by Fatty Acids. M.D. Leibowitz, T. Bale, and C.J. Cohen. Merck Inst. Ther. Res., Rahway, NJ 07065

T-type Ca channel currents were recorded from the A10 cell line (derived from rat aortic smooth muscle) and guinea pig atrial myocytes using the whole-cell patch-clamp technique. At 10 μM, arachidonic acid (AA) reversibly blocked the T-type channels in A10 cells by >60%, while linoleic acid (LA) had no effect on T-type channels. In myocytes, LA and AA (0.5-5 μM) were approximately equipotent, reversible blockers of T-type Ca channels (see abstract by Cohen, Bale & Leibowitz). Inhibitors of 5-lipoxygenase and cyclooxygenase failed to prevent the depression of T-type current by AA in both myocytes and A10 cells. In contrast to the effects on L-type channels in myocytes, T-type currents in A10 cells: 1) never increased in response to fatty acid and 2) the block of T-type channels showed little or no voltage-dependence. In addition, LA and AA are ~equipotent modulators of Na, K, and L-type Ca channels in a variety of cell types. These data suggest that the activity of T-type calcium channels can be modulated by fatty acids via multiple pathways.

Tu-Pos317

CALCITONIN GENE-RELATED PEPTIDE MODULATES Ca^{2+} CURRENT IN BULLFROG ATRIAL MYOCYTES.
K. Ono, T. Nakajima, H. Irisawa and W.R. Giles. University of Calgary, Calgary, Canada.

The effects of calcitonin gene-related peptide (CGRP) on the action potential (AP), and the calcium current (I_{Ca}), have been identified in single cells from bullfrog atrium, using a tight-seal whole cell voltage clamp technique. CGRP increased the AP plateau height and shortened the AP at relatively low doses. CGRP dose-dependently increased I_{Ca} (approx. 10x at 3×10^{-7} M) without a significant change in its voltage-dependence ($ED_{50} = 1.64 \pm 0.55 \times 10^{-8}$ M, n=4). This effect was completely blocked by Cd^{++} (3×10^{-4} M) but not by propranolol (3×10^{-7} M). When I_{Ca} had been maximally activated by either isoproterenol (ISO, 10^{-6} M) or intracellular application of cAMP (10^{-4} M), CGRP did not increase I_{Ca} any further. Forskolin (5×10^{-7} M) significantly potentiated the effect of CGRP (10^{-8} M). Intracellular application of GTPγS (10^{-4} M) and GDPβS (2×10^{-3} M) greatly potentiated and partially inhibited the effect of CGRP, respectively. These results indicate that CGRP enhances I_{Ca} by activation of adenylate cyclase in a specific receptor, G-protein dependent manner.

Tu-Pos318

SECONDARY MODULATION OF OUTWARD Na/Ca EXCHANGE CURRENT IN GIANT EXCISED CARDIAC SARCOLEMMAL MEMBRANE PATCHES.
D.W. Hilgemann & D.P.Cash / Dept. of Physiology, UTSW, Dallas, TX, 75235
Secondary stimulation of outward I_{NaCa} by internal Ca^{++} and MgATP involves separate mechanisms [Hilgemann & Collins, this meeting]. Since Ca^{++} stimulates in nominal absence of ATP, involvement of a protein kinase would presuppose residual ATP in patches. However, the ATP-hydrolysing enzyme, apyrase, is without effect. Also, secondary I_{NaCa} activation by Ca^{++} is found in presence of a nonhydrolyzable ATP analogue, AMP-PNP; in absence of ATP, both ATP-γ-S and AMP-PNP (2 mM, ±2.5 mM Mg) are not effective, except as Mg^{++} chelators. [Note: Outward I_{NaCa} is inhibited by 75% with increment of Mg^{++} from 1 to 4 mM]. As a first test for calmodulin involvement in Ca^{++} modulation, a peptide, corresponding to the calmodulin-binding domain of myosin LCK [Blumenthal et al., Pro Nat Ac Sci 82,3187,1985] was examined. Inhibition by >80% is achieved in <40s with 10 μM, within 4 min with 1μM. Calmodulin involvement has not been proven, however, because calmodulin, itself (1-4 μM), is without effect.

Tu-Pos319

OUTWARD Na/Ca EXCHANGE CURRENT (I_{NaCa}) IN GIANT EXCISED CARDIAC SARCOLEMMAL MEMBRANE PATCHES.
D.W. Hilgemann & A.Collins / Dept. of Physiology, UTSW, Dallas, TX, 75235.
A new giant cardiac sarcolemmal patch preparation (12-18 μm ϕ with 5-25 GΩ seals) allows studies of outward I_{NaCa} with free access to the inner membrane surface and without contamination by other current mechanisms [Hilgemann, Pflügers Arch, in press]. With 5 mM Ca^{++} in the pipette and 0.2-1 μM Ca^{++} on the cytosolic side (20 mM EGTA), the I_{NaCa} activated by 100 mM internal Na^+ (>20 $\mu A/cm^2$) apparently inactivates by 40-90% over 0.5 to 5 s. I_{NaCa} is stimulated by internal Ca^{++} and inhibited by its removal with a similar time course (K_d for $Ca^{++} \approx 1\mu M$). I_{NaCa} in patches from ATP-depleted cells is stimulated many fold by MgATP with little or no reversal upon ATP removal (patch life-times, 20-60 min); effects of internal Ca^{++} remain rapid and reversible. Thus, ATP and Ca^{++} act by separate mechanisms. I_{NaCa} is stimulated irreversibly by chymotrypsin, whereby inactivation, inhibition by low internal Ca^{++}, and stimulation by ATP are abolished. Thus, digestion of peripheral protein deregulates I_{NaCa}.

Tu-Pos320

Is Intracellular Free Calcium Influencing the Function of Voltage-Dependent Na-Channels ?
A.K.Bulatko and N.G.Greeff, Physiologisches Institut, Universität Zürich, CH-8057 Zürich

We previously reported about a significant enhancement (factor 1.8) of macroscopic sodium current I_{Na} in mouse neuroblastoma cells when increasing intracellular free calcium concentration from subnanomolar to 100 nanomolar (Experientia 44:A70, 1988). Here we show that this phenomenon is not due to shifts in the current-voltage relation, the steady-state inactivation curves or unequal recovery of the channels from slow inactivation. After clamping the membrane to a holding potential of -70 mV the relative increase in I_{Na} was equal for all calcium concentrations. Influence of cell size was excluded by calculating current densities (peak I_{Na} in pA for pulses to 0 mV divided by the cell membrane capacity at the beginning of the experiment). Analysis of membrane capacity time lapse curves excluded endo- or exocytosis to explain the higher I_{Na} at pCa7.

As non-stationary fluctuation analysis of I_{Na} yielded equal single channel currents for pCa 9, pCa 8 and pCa 7, it seems reasonable to explain the significantly higher current densities at pCa 7 with a higher number of activatable sodium channels. (Supported by Swiss National Fund 3.143-0.85)

Tu-Pos321

HYPERPOLARIZATION-ACTIVATED CATION CHANNELS IN SINGLE SMOOTH MUSCLE CELLS ARE SENSITIVE TO STRETCH. Tetsuya Hisada, Richard W. Ordway, Michael T. Kirber, John V. Walsh Jr., Joshua J. Singer, Department of Physiology, Univ. of Massachusetts Medical School, Worcester, MA. The properties of hyperpolarization-activated channels (HACs) were studied in single smooth muscle cells from the toad stomach using the patch clamp technique. In cell-attached patches, inward channel currents were activated with variable latency by hyperpolarizing pulses from a holding potential of -20mV to voltages more negative than -40 to -80mV (130mM K^+ in the bath). The open probability (Po) of the HACs increased and the latency of activation decreased with further hyperpolarization. Permeation characteristics and the single channel conductance of HACs were similar to those of stretch-activated channels (SACs) reported previously in these cells. Further, stretch of the membrane by suction applied to the back of the pipette increased the Po and decreased the latency of activation of HACs. Thus, at least some SACs would appear to be HACs. However, in excised, inside-out patches, channel currents elicited by hyperpolarization disappeared over a period of minutes, whereas those elicited by stretch remained. (Supported by NIH DK-31620.)

Tu-Pos322

ACTIVATION OF Ca-DEPENDENT K-CHANNELS IN TRACHEAL MYOCYTES BY PHOSPHORYLATION. A. Takai, H. Kume & H. Tokuno (introduced by M. Sokabe), Dept of Physiology, Sch of Med, Nagoya Univ, Nagoya 466, JAPAN.

The clinically important relaxant action of β-adrenergic agonists on airway smooth muscle is known to be accompanied by elevation of the level of intracellular cyclic AMP and hyperpolarization of the membrane. In isolated smooth muscle cells of rabbit trachea we have examined the effect of isoprenaline and cyclic AMP-dependent protein kinase (A-kinase) on Ca^{2+}-dependent K^+ channels (K_{Ca}), using the patch-clamp method. The open probability of K_{Ca} was reversibly increased by either extracellular application of isoprenaline or intracellular application of A-kinase. This effect was reversibly enhanced and prolonged in the presence of okadaic acid ($C_{44}H_{66}O_{13}$; 10μM), a potent inhibitor of protein phosphatase-2A and -1. These results support the idea that the β-action is mediated by cyclic AMP dependent phosphorylation of K_{Ca} which are densely distributed in the membrane of tracheal myocytes.

Tu-Pos323

WHOLE CELL RECORDING OF VASOPRESSIN ACTIVATED CHLORIDE CONDUCTANCE IN CULTURED RAT MESANGIAL CELLS.
J.W. Hanrahan[1], S.G. Kremer[2] and K.L. Skorecki[2]. [1]Dept. Physiol., McGill Univ., Montreal, Que. and [2] Dept. Medicine, Univ. of Toronto, Toronto, Ont.

Mesangial cells of the renal glomerulus contract in response to a variety of vasoactive agents and are implicated in the regulation of filtration surface area. Previous studies suggested vasopressin and angiotensin II depolarize mesangial cells by activating chloride channels but the chloride conductance was not directly measured. We used whole cell recording to examine the effects of ion substitutions, K channel blockers and vasopressin on membrane voltage and whole-cell currents in a clonal mesangial cell line. The resting membrane potential in cells that had been deprived of serum for > 1 hr was -62.5 mV; similar potentials were obtained in whole cell patches when pipette solutions contained K, Na or TEA as the principal cation. Control whole cell currents showed little time-dependence and rectified slightly in the outward direction. Replacing extracellular Na and K with TEA and Ba reduced whole cell conductance from 27.8 to 4.5 nS and depolarized the cells to -12 mV. Adding vasopressin (<200 nM) in the continued presence of K channel blockers increased whole cell conductance to 36.3 nS. The results suggest that K channels account for > 80 % of the resting conductance of mesangial cells, that ADH activates a chloride conductance of similar magnitude to the resting K conductance, and that dialysis of adherent mesangial cells by patch pipette solution is slow, presumably due to their flattened profile.

Tu-Pos324

FLOW AND EXTRACELLULAR Ca^{++} ACTIVATE A K^+-CHANNEL IN SMOOTH MUSCLE CELLS.
Michael T. Kirber, Richard W. Ordway, Joshua J. Singer, and John V. Walsh Jr., Department of Physiology, Univ. of Massachusetts Medical School, Worcester, MA.

Smooth muscle cells freshly dissociated from the stomach of the toad *Bufo marinus* contain a K^+-selective ion channel which is sensitive to the presence of Ca^{++} at the extracellular surface of the plasma membrane (J. Gen. Physiol. 94:36a, 1989). In the absence of Ca^{++} (2 mM EGTA) at the extracellular surface of the patch, channel openings were rarely seen. If micromolar or greater concentrations of calcium (0 EGTA) were present at the extracellular surface of the patch, channel activity was seen in virtually every patch. When the solution bathing the extracellular surface of outside-out patches contained Ca^{++}, flow from a pressure ejection pipette (≈1 μm tip diameter) containing the same bathing solution caused the channel to open more frequently. This effect of fluid flow was more pronounced when the concentration of Ca^{++} in the bathing solution (and pressure ejection pipette) was increased. Flow of Ca^{++}-free (2 mM EGTA) but otherwise identical solutions from a pressure ejection pipette failed to activate the channel and inhibited activity induced by the flow of Ca^{++}-containing solutions. [Supported by NSF DCB-8511674 and NIH DK-31620.]

Tu-Pos325

BOTH FATTY ACIDS AND STRETCH ACTIVATE LARGE CONDUCTANCE, Ca^{++}-ACTIVATED K^+ CHANNELS IN RABBIT PULMONARY ARTERY SMOOTH MUSCLE CELLS. Richard W. Ordway, Michael T. Kirber, Lucie H. Clapp, John V. Walsh, Jr., and Joshua J. Singer, Dept. of Physiology, University of Massachusetts Medical School, Worcester, MA. Patch clamp techniques were utilized to study large conductance, Ca^{++}-activated K^+ (CAK) channels in smooth muscle cells isolated from rabbit pulmonary artery (J. Gen. Physiol. 94:37a, 1989). Fatty acids (FAs) were applied to the cytosolic face of excised, inside-out membrane patches by pressure ejection from a micropipette. Arachidonic (AA), linoelaidic and myristic acids (each at 20 uM) activated CAK channels with no apparent change in the slope of the ln NP_O versus voltage relationship at low P_O. The latter two of these FAs are not substrates for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes which convert AA to active metabolites. These results, coupled with FA activation in the absence of nucleotides and Ca^{++} (5 mM EGTA in both the pipette and bath), indicate that FAs activate these channels directly rather than through COX, LOX, or NADPH-dependent cytochrome P450 pathways. Additionally, stretching the patch of membrane by applying suction to the patch pipette increased CAK channel activity in inside-out patches even in the absence of Ca^{++} (5 mM EGTA). [NSF DCB-8819750 and NIH DK-31620]

Tu-Pos326

CHLORIDE CHANNEL REGULATION IN SKELETAL MUSCLE OF NORMAL AND MYOTONIC GOATS.

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External intercostal muscle biopsies from normal and congenitally myotonic goats were studied in vitro at 30 °C with standard two microelectrode square pulse cable analysis. Resting chloride conductance (G_{Cl}) was calculated from the difference between mean membrane conductance in chloride-containing and chloride-free media. The protein kinase C (PKC) activator, 4-β-phorbol-12,13-dibutyrate, (0.1 to 2.0 μM) blocks up to 80-90% of G_{Cl} in normal goat fibers and induces myotonic hyperexcitability. The G_{Cl} block was antagonized by pretreatment with the PKC inhibitor, staurosporine (10 μM). The "inactive" 4-α phorbol-12,13-didecanoate had no effect at 50 μM, whereas the "active" 4-β isomer blocked G_{Cl} at 1 μM. The genetically low G_{Cl} of myotonic goat fibers was not altered by treatment with high concentrations of the PKC inhibitors: staurosporine, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7), or tetrahydropapaveralone. Also, forskolin and cholera toxin, which may increase cAMP levels, or clofibric acid analogs, which increase G_{Cl} in normal fibers, were ineffective in restoring G_{Cl} in myotonic goat fibers. The data suggest that PKC may be a regulator of G_{Cl} in normal goat skeletal muscle fibers. The molecular defect of myotonic goat fibers does not appear to be due to excessive activity of PKC.

Supported by NIH GRANTS NS-03178 & HL-22619-IIb.

Tu-Pos327

MEMBRANE CURRENTS OF CANINE GASTRIC SMOOTH MUSCLE CELLS AND EFFECTS OF ACETYLCHOLINE. Stephen Sims. Dept. Physiology, Univ. Western Ontario, London, Ontario, Canada

Membrane currents of smooth muscle cells freshly dissociated from circular muscle of dog stomach were studied using conventional whole-cell recording as well as the perforated-patch technique (Horn & Marty, 1988. J. Gen. Physiol. 92:145). Cells were initially relaxed and contracted reversibly in response to acetylcholine (ACh). With K^+ in the patch electrode, depolarization evoked inward Ca^{2+} current followed by outward K^+ current. Tail currents reversed direction at -70 mV, close to E_K, and the outward current was blocked by TEA. I_{Ca} was studied with Cs^+ in the patch pipette. In solutions containing 2.5 mM Ca^{2+} the threshold for activation of I_{Ca} was ≈-40 mV and peak inward current was at +10 mV. I_{Ca} showed inactivating and sustained components which were enhanced by BAY K 8644 and blocked by nifedipene. Cholinergic responses were studied with perforated-patch recording, where ACh was found to elicit two changes (i) ACh suppressed outward K^+ current (ii) ACh induced an inward current. The inward current was accompanied by an increase in current noise, consistent with activation of channels. The ACh-induced inward current was activated between ≈-50 and 0 mV. Thus, ACh depolarises gastric smooth muscle by at least two changes: suppression of a g_K, and activation of another conductance, whose selectivity has not yet been defined. (Supported by MRC Canada).

Tu-Pos328

ATP INDUCES DEPOLARIZATION BY INCREASING INTRACELLULAR FREE Ca^{++} AND H^+. M. Pucéat, F. Scamps, O. Clément, P. Cornec, G. Vassort, INSERM U-241, Orsay, FRANCE.

Exogenous application of ATP causes perturbation of membrane permeability which leads to depolarization in cardiac tissues. Isolated rat ventricular cardiac myocytes were loaded with the fluorescent Ca and H indicators, Indo-1 and BCECF respectively. Measurements were carried out on cell suspensions (≥ 90% yield) under constant stirring and temperature (37°C). Upon exposure to 10 μM ATP we confirm the rapid increase in Ca_i which declines within 2-3 min. to a new steady value (Sharma & Sheu, 1986; De Young & Scarpa 1987). Alkalinization was also observed (Wallert & Frölich, 1989) it was preceded by a transient acidification. In EIPA, a sustained acidification (0.05 pH unit) was observed. Alterations in H_i and Ca_i upon ATP application required the presence of Mg. Other nucleotides were ineffective. There was also no transient effects of ATP in Cl poor (2 mM) or in the presence of DIDS. Caffeine and ryanodine did not prevent increase in Ca_i. It is proposed that MgATP is required for an ecto-ATPase to activate the $Cl\text{-}HCO_3$ exchanger; this leads to internal acidification and Ca overload.

Tu-Pos329

REGULATION OF IP_3-INDUCED Ca RELEASE BY Mg^{2+} AND cAMP-DEPENDENT PROTEIN KINASE.
B. Alderson-Lang, M. Tzinas, and P. Volpe. Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

The effects of Mg^{2+} and cAMP-dependent protein kinase (PKA) on IP_3-mediated Ca release and IP_3 binding to canine cerebellar membrane fractions were investigated. Increasing $[Mg^{2+}]_{free}$ from ~30 - ~300 μM reduced the extent of a Ca release 2-3 fold (K_i of ~ 25 μM free Mg^{2+} and increased the K_m from ~0.5 to ~1.1 μM). Mg^{2+} also inhibited Ca release by $GPIP_2$, a poorly metabolized analog of IP_3, further suggesting that the inhibitory effects of Mg^{2+} are not primarily due to IP_3 hydrolysis through IP_3ase activation. In medium designed to minimize IP_3ase activity, Mg^{2+} increased the K_d of IP_3 binding and decreased the B_{max} approximately 3-4 fold. The catalytic subunit of PKA increased the extent of IP_3-induced Ca release but had no effect on the Ca loading rate and Ca capacity of the preparation and did not affect the K_d and B_{max} of IP_3 binding.
[Supported by NIH grant GM-40068-02].

Tu-Pos330

CYCLIC AMP (cAMP) ACTIVATES DIRECTLY A K^+-SELECTIVE CHANNEL IN *Drosophila* LARVAL MUSCLE.
Ricardo Delgado, Ramon Latorre, Pedro Labarca. Centro de Estudios Científicos de Santiago, Casilla 16443, Santiago 9, Chile and Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Single-channel recording from dorsal longitudinal muscle in *Drosophila* larvae reveals the presence of a K^+-selective channel activated directly and reversibly by cAMP. In the absence of cAMP this channel displays a low voltage-independent probability of opening (Po≈1%). The current-voltage relation is nonlinear yielding a P_{K^+}/P_{Na^+}≈10 and slope-conductance, measured at voltages ≥0 mV, of 47 pS. In excised-patches, exposure of the cytoplasmic side to micromolar concentration of cAMP activates the channel in reversible, dose-dependent fashion. Maximum activation (Po≈10%) is obtained at 80 μM cAMP with an apparent dissociation constant of 50.8 μM and Hill number of 2.9. Channel activation is not observed in the presence of AMP, cGMP, IP_3 or Ca^{2+} at micromolar concentration or ATP at 1 mM. Further studies carried out in cell-attached patches show that muscle exposure to 200 μM 8-Br cAMP results in channel activation to levels similar to those observed in excised-patches, revealing that elevation of intracellular cAMP increases the probability of channel opening.
Supported by NIH, FONDECYT 1167/88, 451/88 and Tinker Foundation.

Tu-Pos331

A CYCLIC AMP-ACTIVATED K^+ CHANNEL IN *Drosophila* LARVAL MUSCLE IS PERSISTENTLY ACTIVATED IN *dunce*^{M14}.
Ricardo Delgado, Ramon Latorre, Pedro Labarca. Centro de Estudios Científicos de Santiago, Casilla 16443, Santiago 9, Chile and Departamento de Biología , Facultad de Ciencias, Universidad de Chile.

Wild type dorsal longitudinal *Drosophila* larval muscle possess a K^+-selective channel that displays a low probability of opening (Po≈1%) and is activated directly, reversibly and specifically by cAMP. *dunce*^{M14} mutants lack a form of phosphodiesterase, have intracellular level of cAMP that are 3-5 fold higher than the wild type and displays poor retention in a classical conditioning test. Studies performed in cell attached patches in *dunce*^{M14} muscle reveal the presence of a channel displaying identical P_{K^+}/P_{Na^+} and slope conductance than the K^+ channel recorded from wild type muscle, but with a much increased probability of opening (Po≈13%). Excising the patch from the muscle cell leads to a decrease in the probability of opening to levels similar to those found in wild type muscle. Furthermore, as in a wild type, exposure of *dunce*^{M14} excised patches to micromolar concentration of cAMP results in an increase in the probability of channel opening. In short, the cAMP-activated channel present in wild type larval muscle is persistently activated in *dunce*^{M14}. It remains to be established whether this channel is also present in adult muscle or in other excitable tissues in *Drosophila*. (Supported by NIH, FONDECYT 88/1167, 88/451 and Tinker Foundation).

Tu-Pos332

NOREPINEPHRINE AND GTPγS ACTIVATE A TIME-INDEPENDENT 'BACKGROUND' CURRENT IN RAT PARASYMPATHETIC CARDIAC NEURONS.
Z. Xu and D.J. Adams, Dept. Pharmacology, Univ. of Miami Sch. Med., Miami, FL

The ionic basis of a background current activated by norepinephrine (NE) and GTPγS in cultured parasympathetic neurons from rat intracardiac ganglia was investigated using the whole-cell patch clamp technique. Exposure to NE (<100 μM) activated a time-independent current which was observed in the external presence of 0.3 μM TTX, 50 mM TEA^+ and 0.1 mM Cd^{2+} and antagonized by the α-blocker, phentolamine. The current-voltage relationship exhibited outward rectification and a reversal potential near 0 mV. This background current was carried by small monovalent cations (MW <140) but not L-$arginine^+$ or $Bis\text{-}Tris^+$. Replacement of external Cl^- with SO_4^{2-} or presence of 100 μM ouabain did not affect this current. The activation of the background current by NE was mimicked by the addition of 100 μM GTPγS (but not GDPβS) to the pipette solution and inhibited by incubation with 200 ng/ml pertussis toxin (PTX). These data suggest that the activation of a time-independent 'background' current by NE is mediated via a PTX-sensitive G-protein. This non-selective cation current may be superimposed to the effect of α-adrenergic inhibition of the high threshold calcium current observed in rat parasympathetic cardiac neurons. Supported by NIH grant HL 35422.

Tu-Pos333

CHEMOTRANSDUCTION MECHANISMS DETERMINED BY TWO METHODS OF WHOLE-CELL RECORDING. A. Stea and C.A. Nurse (Intro. by R.E. Garfield). Dept. of Biology, McMaster University, Hamilton, Ontario, L8S 4K1.

The carotid body is a chemosensory organ that is excited by hypoxic, hypercapnic and acidic stimuli in the blood. We are investigating chemotransduction mechanisms by application of conventional whole-cell and perforated patch (J. Gen. Physiol. 92: 145-159, 1988) recording to cultured glomus cells, the main cell type in the rat carotid body. These cells contain voltage-gated Na^+ and K^+ channels characteristic of excitable cells. Under conventional whole-cell and perforated patch recording hypoxia causes a reversible decrease in outward K^+ current suggesting that an intact cytoplasm is not necessary for the response. This effect was specific since the inward current was unaffected and the O_2-sensitive K^+ conductance was not present in a closely related cell type, i.e. cultured SIF cells. We are currently testing whether acidic and hypercapnic stimuli also affect membrane currents in both dialysed and "intact" glomus cells.

This work is funded by NIH grant #1 R01 HL 43412-01. A. Stea is supported by a NSERC postgraduate scholarship.

Tu-Pos334

ACTIVATION OF KINETICALLY DIFFERENT POPULATIONS OF K CHANNELS BY ADENOSINE IN RAT ATRIA. Donghee Kim and Richard Duff. Department of Physiology and Biophysics, Chicago Medical School, North Chicago, IL 60064.

Adenosine binds to A1-receptors in atrial cells and activates the muscarinic-gated K channel via G proteins. The K channel has been reported to have a mean open time of ~1 msec and a conductance of ~40 pS in symmetrical 140 mM K. We found that in many rat atrial patches (~40%), adenosine-induced K channel activity was several fold higher in inside-out (with GTP in the bath) than cell-attached patches. We therefore examined the possibility that changes in K channel kinetics account for the difference in the channel activity. Single channel analysis indicated that in cell-attached patches, adenosine activated a single population of K channels with a mean open time of ~1 msec and a conductance of ~40 pS. However, in inside-out patches, adenosine activated a single population of K channels with a mean open time of 3-4 msec. There was no change in the channel conductance. These channels were not resting K channels, since GTP washout completely shut them and reapplying GTP opened them again. These findings suggest that certain cytosolic component(s) may modulate the kinetics of muscarinic-gated K channel or that there may be more than one population of G protein-coupled K channels in atrial cells.

Tu-Pos335

High $[Ca^{2+}]_o$ causes membrane depolarization and $[Ca^{2+}]_i$ increase in a calcitonin-secreting rat cell line. Naohide Yamashita and the late Susumu Hagiwara, BRI;JLNRC. Univ. of Calif., LA, CA 90024. (Intro. by E. E. Serrano)

Secretion of calcitonin is regulated by $[Ca^{2+}]_o$. We examined changes of membrane potential and current with the perforated whole cell clamp technique using nystatin in a rat calcitonin-secreting cell line (rMTC 44-2). $[Ca^{2+}]_i$ was simultaneously measured using fura 2/AM. Under current clamp Na^+- and Ca^{2+}-dependent action potentials were observed. Increasing $[Ca^{2+}]_o$ from 0.5 mM to 2~5 mM depolarized the membrane potential and simultaneously elevated $[Ca^{2+}]_i$. Hyperpolarization inhibited action potentials and drastically reduced the rise in $[Ca^{2+}]_i$. When the cells were voltage clamped, high $[Ca^{2+}]_o$ increased the holding current and the membrane conductance. This was not observed in Na^+-free media, indicating that membrane depolarization was caused by a permeability increase to Na^+ ions. Even when the membrane potential was clamped at the potential more negative than the threshold for Ca^{2+} channels, a small increase of $[Ca^{2+}]_i$ was observed in high $[Ca^{2+}]_o$, indicating the involvement of mechanisms other than Ca^{2+} influx through voltage-gated channels.

Tu-Pos336

DIACYLGLYCEROL ALTERS HIPPOCAMPAL CALCIUM CHANNEL CURRENT. D. Doerner and B. E. Alger. Dept. Physiol., Univ. Maryland Sch. Med, Baltimore, MD.

Phorbol esters depress whole-cell barium current through hippocampal calcium channels (I_{Ba}) apparently via protein kinase C (PKC) (Doerner, et. al., J. Neurosci., 8:4069-4078, 1988). Pressure application of the synthetic diacylglycerol, 1-oleoyl-2-acetyl-glycerol (OAG), also depressed I_{Ba}, although a detailed study of OAG was not made. Reported differences between phorbol esters and diacylglycerols prompted reexamination of OAG effects on I_{Ba}.

OAG (20-300 μM) was bath-applied to guinea pig hippocampal neurons isolated by an enzymatic and mechanical dispersion method and I_{Ba} was recorded. Lower doses of OAG (20-60 μM) had only slight effects, often a small enhancement prior to a small depression of I_{Ba}. At higher doses (180-300 μM) OAG prominently depressed I_{Ba}, although initial transient increases were still frequently seen. OAG effects were reduced by the kinase inhibitor H-7. The vehicle, DMSO ($\leq$ 0.3%) had no effect. Interestingly, depressant effects of OAG were usually transient, i.e., I_{Ba} partially recovered despite continued presence of OAG. Phorbol-ester-induced I_{Ba} depression was never transient. Thus, OAG effects on hippocampal Ca channels differ from their effects on DRG cells or cardiac myocytes.

Tu-Pos337

CHARACTERIZATION OF A CATION SELECTIVE CHANNEL AND AN ACTIVATING PROTEIN FROM SEA URCHIN EGG.

Ture Lii Lu and David G. Levitt, Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Vesicles isolated from sea urchin eggs contain a monovalent cation selective channel that can be reconstituted into planar bilayers. After channel incorporation into the bilayer, the channel number tends to decay within minutes at room temperature. This phenomenum can be prevented by the addition of sulfhydryl reducing reagents (DTT or glutathione) to the cis side of the bathing solution. The channel can be inactivated by sulfhydryl oxidizing reagents (DTNB, diamide) and can be re-activated by excess amount of DTT. The channels can also be activated by addition of the egg homogenate supernatant. It is activated by very low concentrations of DTT (10 μM) or supernatant (1/10,000 dilution). The activation factor from the supernatant has been partially purified. It is a heat stable protein, with a m.w. of 10-15K and pI of 5-5.5. These properties are similar to that of thioredoxine, a known sulfhydryl reducing reagent.

Tu-Pos338

CALCIUM AND SODIUM ACTIVATE DIFFERENT POTASSIUM CHANNELS IN CULTURED RAT OLFACTORY BULB NEURONS.

T. M. Egan, D. Dagan and I. B. Levitan, Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

Single potassium-selective ion channel activity was recorded from cultured rat olfactory bulb neurons in vitro. Calcium-activated potassium channels resembled maxi K^+ channels of other preparations in conductance (220 pS) and sensitivity to $[Ca^{++}]_i$ and voltage. The catalytic subunit of protein kinase A increased the open probability of the channel.

Sodium-activated potassium channels had conductances of 140-170 pS when $[K^+]_o$ = 150 and $[K^+]_i$ = 90 mM. The channel was active when $[Na^+]_i$ exceeded 20 mM. In on-cell patches the channel was activated by bath perfusion of veratridine (50-110 μM)when the external solution contained sodium (but not when lithium replaced sodium). This effect of veratridine was blocked by TTX. Channel gating was characterized by the presence of distinct and frequently occuring substates. Channel activity often ran down with a variable time course.

Tu-Pos339

ARACHIDONIC ACID AND LTC_4 MODULATE MUSCARINIC POTASSIUM CHANNEL ACTIVATION IN HEART.

R. W. Scherer and G. E. Breitwieser. Johns Hopkins U. School of Med. Baltimore, MD. Activation of muscarinic acetylcholine receptors (mAChR) or internal perfusion of hydrolysis-resistant guanine nucleotide analogs (GXP) results in stimulation of an inwardly rectifying potassium current ($I_{K(M)}$) in bullfrog atrial cells. The maximal rate of GXP-mediated activation of $I_{K(M)}$ is a measure of GDP release from G_k. Superfused arachidonic acid (AA; 20 μM) increases this rate (from 0.29 to 0.48 min^{-1}), an effect which is inhibited by 10 μM NDGA, but not by 100 μM indomethacin. 10 μM LTC_4 is more potent than AA in increasing this rate (0.65 min^{-1}); a response not inhibited by NDGA. Nanomolar ACh increases the rate of GXP-mediated $I_{K(M)}$ activation. AA causes a rightward, and LTC_4 a leftward shift in the ACh concentration-dependence of this increase in rate. Neither AA nor LTC_4 elicit $I_{K(M)}$ in the absence of GXP, or alter $I_{K(M)}$ previously activated by GXP. These data suggest that AA metabolites alter both the basal turnover and mAChR-mediated activation of G_k in vivo. Supported by NIH and AHA.

Tu-Pos340

THE MODULATION OF SINGLE DELAYED RECTIFIER CHANNELS BY ISOPROTERENOL AND cAMP IN FROG ATRIAL CELLS.

Isabelle Duchatelle-Gourdon and H. Criss Hartzell. Emory University. School of Medicine. Atlanta, GA 30322.

The patch-clamp technique with two pipets was used to record single delayed K^+ channels (cell-attached electrode) and to control the potential and the composition of the intracellular compartment (whole-cell electrode). With 30 μM cAMP in the cell and normal potassium concentrations inside and outside the patch, a channel carrying an outward current was characterized. Its open probability was very low and the channel was present in only 50% of the patches. This channel had the characteristics expected of a delayed rectifier channel. The time-course of its ensemble average resembled the whole-cell current in the same cell. The current-voltage relationship exhibited inward rectification, and had a slope conductance of 20 pS in the linear portion, and a reversal potential close to E_K. In the absence of intracellular cAMP, the channel was present in only 5% of the patches. However, the channel could be made to "appear" in 50% of the silent patches upon stimulation with either cAMP in the cell or isoproterenol outside the cell. β-adrenergic stimulation did not change the conductance of the channel, but increased its probability of opening. Supported by NIH HL21195 to H.C.H.

Tu-Pos341

THE EFFECTS OF STRESS ON ARACHIDONIC ACID METABOLISM IN NEURONS

K.S. Madden, P.G. Lysko[1] and H. Knapp[2] (Intro. by J. Kasianowicz) LNP, NINDS, NIH Bethesda, MD 20892, [1]SKF Laboratories, King of Prussia, PA 19406, and [2]Div. of Clin. Pharm., Vanderbilt University, Nashville, TN 37232

Arachidonic acid (AA) and its metabolites have been implicated in the events of signal transduction such as the modulation of membrane conductance mechanisms. The multi-enzymic pathways mediating AA metabolism are reportedly stimulated by stress including the structural deformation of cells*. Since stress is imposed on cells by many electrophysiological protocols used to study conductance mechanisms, we reassessed its effects on the release and metabolism of AA using standard biochemical methods and primary cultures of rat (P8) cerebellar granule cells pre-loaded with 1-^{14}C-AA. Cells were bathed in modified Locke's medium containing glucose or galactose (5.6 mM, each). They were left to rest or were stressed by: 1) K^+-depolarization (50 mM), 2) hypertonicity (415 mosm), 3) glutamate (10^{-4}M), 4) scraping with a pipette tip, or 5) exposure to distilled H_2O (3 plates/condition; n=6 experiments). Glucose-deprivation doubled the release of label evoked by stresses 1-3 but it did not affect the quantity of label under 4 or 5 which increased release >10-fold. However, the types of metabolites produced by glucose-deprived cells under 4 and 5 were altered. These stress-induced changes in AA metabolism may account for some diversity in electrophysiological recordings and also contribute to transient events such as the "run-down" of single channel activity.

*Gilmore, N., Vane, J.R. and Wyllie, J.H. (1969) In: *Prostaglandins, Peptides and Amines*, eds. P. Mantegazza and E.W. Horton, Academic Press, London/New York, pp. 21-29.

[This work was done while KSM held a National Research Council-NIH Research Associateship.]

Tu-Pos342

ROLE OF ARACHIDONIC ACID METABOLISM IN MODULATING THE INWARD RECTIFIER OF BRAIN NEURONS.

K. Koyano, J.J. Grigg, S. Nakajima and Y. Nakajima, Dept. of Anat. and Cell Biol. and Dept.of Pharmacol., Univ. of Illinois at Chicago, Chicago IL, 60612.

Our previous studies have shown that somatostatin inhibits excitability of brain neurons by inducing an inwardly rectifying K-current, whereas substance P excites them by suppressing this current. We have studied the role of arachidonic acid metabolism in these responses using the whole-cell clamp on cultured locus coeruleus and nucleus basalis neurons of the rat. The somatostatin-induced increase in K-conductance was reduced by a phospholipase A_2 inhibitor (quinacrine, 50μM), a lipoxygenase inhibitor (NDGA, 3-5μM) and 5-lipoxygenase inhibitors (AA-861, Takeda, 50μM; A-63612, Abbott, 50μM). In contrast, a cyclooxygenase inhibitor (indomethacin, 50μM) and a 12-lipoxygenase inhibitor (baicalein, 50μM) were ineffective. The substance P-induced decrease in the K-conductance was also suppressed by AA-861. Our results suggest that 5-lipoxygenase metabolites act to maintain or to enhance the functional state of these K-channels. Supported by NIH grant, AG06093.

Tu-Pos343

DIFFERENT EFFECTS OF INTRACELLULAR Ca AND A PHORBOL ESTER ON CARDIAC T AND L Ca CHANNELS

G-N Tseng, PA Boyden. Dept Pharmacology, Columbia U, New York NY 10032 In canine Purkinje cells (P), α_1-adrenoceptor stimulation (α_1-stim) has variable effects on T (T) and L (L) Ca currents. Since α_1-stim generates intracellular IP_3 and DAG, which increases intracellular free Ca (Ca_i) and protein kinase C activity (PKC), respectively, we studied the effects of changing Ca_i and a phorbol ester (TPA, an activator of PKC) on the T and L currents in canine ventricular (V) and P cells. Whole-cell Ca currents were recorded in isolation of other interfering currents; Ca_i was changed by intracellular dialysis with pipette solutions of different EGTA/Ca. Increasing Ca_i by changing EGTA/Ca from 10/0 to 1/0.9 decreased L in 4/4 V (by 55±25%) and in 6/7 P (by 26±14%), and increased T in 3/3 V (max. 70±37%). In 2 of the 3 V, the increase in T was biphasic: it rose to a peak in 9 or 12 min and then declined with the continuous elevation of Ca_i. Increasing Ca_i increased T in 3/5 P (38±25%) but decreased T in 2/5 P. Decreasing Ca_i by changing EGTA/Ca from 0.2/0 to 40/0 increased L in 2/2 V (by 36 and 5%) and in 5/5 P (by 52±39%), and decreased T in 2/2 V (by 56 and 14%) and in 5/5 P (by 22±20%). These results indicate that Ca_i decreases L but increases T. The differences in response to changing Ca_i between V and P suggest that the "basal" Ca_i is higher in P than in V. TPA (10^{-8}-10^{-7}M) decreased T in 6/6 P (by 28±8%) and abolished T in 1 V in which T was present. TPA transiently increased L or prevented L run-down in 6/7 V and 2/6 P. In the remaining cells, TPA decreased L. Our results show that elevating Ca_i and activating PKC have different effects on cardiac T and L Ca channels and may underlie the variable effects of α_1-stim on these Ca channels.

Tu-Pos344

MODULATION OF DELAYED RECTIFIER K^+ CHANNELS BY ADRENERGIC AGONISTS IN FROG SKELETAL MUSCLE.

J.A. Sánchez, J. Arreola and D. Elias. Dept. of Pharmacology, CINVESTAV. A. Postal 14-740, México 07000, D.F. México.

We have previously shown that Ca^{2+} channels are modulated by adrenaline and cAMP (J. Physiol. 1987, 393, 307). The present experiments show that delayed rectifier K^+ channels of frog skeletal muscle are modulated by α and β agonists.

Techniques: The triple vaseline-gap voltage clamp technique for cut fibers of Rana montezumae was used (Hille-Campbell, J. Gen. Physiol. 1976, 67, 265) at 20°C. Solutions (mM) external: Mg^{2+}=10, TMA=106, $CH_3SO_3^-$=131.6, K^+=5.6, MOPS=4, pH=7.2. Internal: EGTA-$(TMA)_2$=19, K^+-aspartate=87, MOPS=4, pH=7.1.

Results: Isoproterenol ($1-5x10^{-6}$M) increased g_{Kmax} of K^+ channels in ca. 30% without major changes in their voltage dependence. Intracellular cAMP (1 mM) increased g_K by ca. 20%. In contrast, forskolin (10 μM) decreased g_{Kmax} in ca. 60%, the effect was partially reversible. Phenylephrine (10^{-6}M) plus propanolol ($2x10^{-6}$M) decreased g_K by ca. 50% and shifted its voltage dependence by ca. 10 mV towards more negative potentials. These effects were attenuated by phentolamine (10^{-6}M). Phorbol esters (PMA= 150 nM) had similar effects.

We conclude that K^+ channels of frog muscle are modulated by α and β agonists probably by activation of protein kinase C and A respectively.

Supported by CONACYT grant No. P228CCOX880183.

Tu-Pos345

EFFECTS OF PERTUSSIS TOXIN AND CHOLERA TOXIN ON A CA^{2+}-DEPENDENT CHLORIDE CHANNEL IN DISTAL NEPHRON CELLS.

Yoshinori Marunaka & Douglas C. Eaton
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We have previously described a Ca^{2+}-dependent, outwardly-rectifying Cl^- channel in the apical membrane of a distal nephron cell line, A6 (Marunaka & Eaton, Am. J. Physiol., in press). The outward rectification is reduced when $[Ca^{2+}]_i \geq 10\mu M$. Insulin reduces rectification by increasing inward current through an alteration in the $Ca^{2+}{}_i$-sensitivity of the channel (Marunaka & Eaton. J. Gen. Physiol., in press). Since some actions of insulin are mediated by G-proteins, we examined the effects of pertussis or cholera toxin on the channel. Pertussis toxin increases the open probability and induces some inward current in a manner similar to insulin-treated cells. Cholera toxin also increases the open probability of the channel.

This work was supported by NKF grant to Y. Marunaka and NIH grant (DK-37963) to D. C. Eaton.

Tu-Pos346

THE PROPERTIES OF VOLTAGE GATED DELAYED RECTIFIER OUTWARD CURRENT IN GUINEA PIG HEPATOCYTES.

S. Koumi, R. Sato, Y. Kushikata, H. Muramatsu, T. Horikawa, T. Aramaki, H. Okumura, Nippon Medical School, Tokyo, Japan

The membrane ionic currents of guinea pig hepatocytes were examined using whole cell patch clamp techniques. The two types of membrane currents were observed: 1) the time-independent currents showing a linear current-voltage relationship, or 2) the voltage gated outward currents having the outward going delayed rectifier component. We investigated the hepatocytes having the latter current systems. The delayed rectification of this current was more pronounced at the more positive depolarizing potentials. This outward current was suppressed in Ca^{2+}free external solution. In contrast, $10\mu M$ noradrenaline markedly enhanced this delayed rectifier component with increasing the conductances. After external application of $20\mu M$ quinidine, the delayed rectifier component was blocked reversibly. Moreover, $0.1\mu M$ apamin irreversibly suppressed this outward current. These observations indicate that this outward current might belong to the Ca^{2+} activated K^+ current.

Tu-Pos347

ANALYSIS OF THE PUTATIVE SUBUNITS OF SKELETAL MUSCLE CALCIUM CHANNELS AND THEIR DIFFERENTIAL PHOSPHORYLATION BY PROTEIN KINASES.

C. F. Chang, C. M. Weilenmann, J. Ptasienski and M. M. Hosey (Intro. by N. Owen). Dept. Pharmacol., Northwestern University, Chicago, IL 60611.

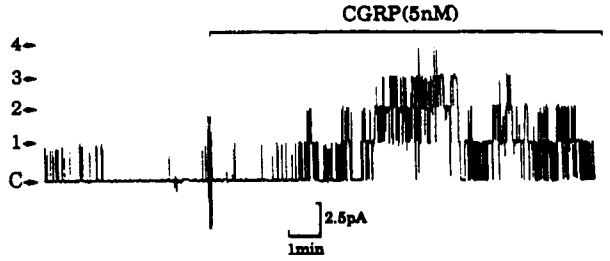
L type Ca channels in skeletal muscle have been suggested to be multi-subunit proteins. We have demonstrated by purification, photoaffinity labelling, phosphorylation and immunological analysis that the native complex of skeletal muscle Ca channels migrates as a protein of ~900 kDa in a digitonin-based non-denaturing PAGE. This complex dissociated into the putative subunits of 165 (α_1), 140 (α_2), 52 (β), 32 (γ) and 27-25 (δ) kDa in SDS-PAGE with a stoichiometric ratio of approximately 1:1:1:1 for α_1: α_2: β: γ peptides. In order to assess the extent of phosphorylation of the putative subunits, phosphorylation was performed in skeletal muscle membranes. The results demonstrated that the α_1 and β peptides were differentially phosphorylated by cAMP-dependent protein kinase, protein kinase C and an endogenous protein kinase present in skeletal muscle membranes. The overall results suggest that L type Ca channels are multi-subunit proteins and the regulation of these channels by different signal transduction pathways may involve differential phosphorylation of the putative subunits.

Tu-Pos348

CALCITONIN GENE-RELATED PEPTIDE (CGRP) DILATES ARTERIES BY ACTIVATING ATP-SENSITIVE K^+ CHANNELS (K_{ATP}) IN ARTERIAL SMOOTH MUSCLE.

M.T. Nelson, Y. Huang, J.E. Brayden, J.K. Hescheler, N.B. Standen*. Dept. of Pharmacology, University of Vermont, Burlington VT 05405; *Dept. of Physiology, U. of Leicester, Leicester, LE1 9HN U.K.

CGRP, one of the most potent vasodilators, is present both in perivascular nerves and in the blood stream suggesting that it plays a role in the control of blood flow. CGRP dilated norepinephrine (10 μM)-contracted rabbit mesenteric arteries (half relaxation with 1.2 nM CGRP) and these dilations were reversed by blockers of K_{ATP} channel, glibenclamide (1 μM) and barium (50 μM). Further, CGRP (5 nM) hyperpolarized arterial smooth muscle by 19 mV (n=5) and blockers of K_{ATP} channel reversed this hyperpolarization. Charybdotoxin (30 nM), a blocker of the Ca^{2+}-activated K^+ channel, had no effect on CGRP-induced dilations or hyperpolarizations. Finally, CGRP opened single, K_{ATP} channels in patches on single smooth muscle cells from these arteries (see Figure), with 5 nM CGRP increasing the open state probability 28.8 fold (n=7) at 0 mV in physiological external K. We propose that activation of K_{ATP} channels underlies a major part of the relaxation produced by CGRP, and forms an important general mechanism for vasodilation.



Tu-Pos349

PROTEIN TRANSLOCATION IN THE VOLTAGE GATING MECHANISM OF THE COLICIN E1 CHANNEL.

A.R. Merrill and W.A. Cramer, Dept. of Biological Sciences, Purdue University, W. Lafayette, IN, 47907. [Intro. by W.L. Pak]

Translocation of 1/3-1/2 of the colicin E1 thermolytic channel peptide (178 residues) into the membrane bilayer of liposomes by a *trans*-membrane voltage was demonstrated through a potential (-100 mV)-dependent increase in peptide labeling by two different lipophilic photoaffinity probes (TID, INA) incorporated into the bilayer, and the decrease in labeling of peptide tyrosines by a membrane-impermeant iodination reagent. The labeling change was reversible with a half-time of ~1 min upon elimination of the potential, a time similar to that for reversal of channel gating. Protein translocation associated with gating of the colicin E1 channel had also been inferred from the effect of protease added *cis* and *trans* to planar bilayers in the open (*trans*-negative potential) and closed states (Slatin *et al.*, 1986). It is concluded that the mechanism of voltage gating of the colicin E1 channel involves translocation of a significant fraction of the channel protein into the membrane bilayer. Sequencing of labeled peptide segments will allow identification of the translocated amino acids. (supported by NIH GM-18457)

Tu-Pos350

THE TRANSLOCATION-COMPETENT STATE OF THE COLICIN E1 CHANNEL.

A.R. Merrill, F.S. Cohen[1], and W.A. Cramer, Dept. of Biological Sciences, Purdue University, W. Lafayette, IN, and [1]Dept. of Physiology, Rush Medical College, Chicago, IL [Intro. by J.L. Smith]

The acidic pH conditions required for optimum *in vitro* activity of colicin E1 result in increased susceptibility to proteases, and increased accessibility to acrylamide of a fluorescence probe attached to Cys-505. Treatment with dilute (10^{-3}%) SDS also results in a large increase of activity and protease susceptibility. Such data for protein import and secretion have been interpreted as indicative of an unfolded structure associated with a translocation-competent state. However, the translocation-competent state of the thermolytic colicin E1 channel peptide at pH 4 is apparently not unfolded because its hydrodynamic radius (22-23Å) is not affected by pH. The large (factor of 10^2-10^4) increase of activity with 10^{-3}% SDS at pH 6 is also not linked to any increase in Stokes radius, although some further increase in activity with 10^{-2}% SDS may be linked to a change of radius. It is proposed that increased side chain mobility may be important in translocation competence (supported by NIH GM-18457 and GM-27367).

Tu-Pos351

MUSCARINIC MODULATION OF SODIUM CURRENTS IN RODENT PANCREATIC B-CELLS

B. Soria, D. Contreras, J.V. Sanchez-Andrés. Department of Physiology, University of Alicante, Alicante (Spain).

Rodent pancreatic B-cells have an inward current which is due to Na^+ permeation of tetrodotoxin (TTX)-sensitive, voltage-activated channels. These channels were almost fully inactivated at -70 mV (midpoint of the h_∞ curve, - 80 mV). The muscarinic agonist carbamylcholine (CbCh, 1 μM) blocked the Na^+ current evoked from a holding potential of -100 mV. The mechanism of the CbCh block was unlike that of TTX since CbCh blocked the current by displacing the h_∞ curve to more negative membrane potentials. Although TTX had no effect on the "steady-state" glucose-induced electrical activity, both TTX and muscarinic agonists (CbCh and methacholine) were found to delay the onset of the depolarizing response to glucose. It is suggested that neurotransmitter-modulated Na^+ channels of hitherto unknown function are involved in the initial depolarizing action of glucose.

Tu-Pos352

ION CHANNEL KINETICS - A RANDOM OR A DETERMINISTIC PROCESS?

Larry S. Liebovitch and Tibor I. Tóth
Dept. Ophthalmology, Columbia Univ.,NY

Models of channel gating have assumed that the switching between states is a random process. In recent years it has been shown that deterministic processes with a small number of degrees of freedom can have properties that appear to mimic random behavior. This is now called chaos.

We present a deterministic channel gating model based on iterated maps. Depending on the functional form of the map, the open and closed time distributions of this model can have Markov, stretched exponential, or power law behavior. Thus, such dwell time distributions could be due to either random or deterministic processes.

We show how phase space portraits can tell if the gating is random or deterministic.

Supported by Amer. Heart Assoc., Whitaker Found., and NIH EY6234.

Tu-Pos353

VOLTAGE-DEPENDENCE AND STABILITY OF THE GATING KINETICS OF THE FAST Cl CHANNEL FROM CULTURED RAT SKELETAL MUSCLE. David S. Weiss and Karl L. Magleby. Dept. of Physiol. and Biophys., University of Miami School of Medicine, Miami, FL 33101.

The voltage dependence and stability of the gating kinetics of the fast Cl channel were examined in excised patches of membrane from cultured rat skeletal muscle. Five patches, each containing a single channel were examined. Up to 10^6 open and shut intervals were analyzed per channel. Gating stability was examined from plots of the running means of open and shut interval durations and from plots of consecutive dwell-time distributions. Both methods demonstrated that sufficiently stable data could be collected for a detailed examination of the mechanism of the voltage-dependence of the channel. Depolarization increased P_{open} e-fold per 17 mV (gating charge=1.6 electronic charges). This voltage sensitivity arose from a large decrease in the mean shut time (e-fold per 19 mV) and a smaller increase in the mean open time (e-fold per 109 mV). The number of components in the dwell-time distributions were independent of voltage (-100 to -30 mV), but the areas and time constants of the components changed in a systematic manner. Thus, the voltage dependence results from changes in the transition rates among states and not from changes in the number of states. (NIH AR32805 and the MDA).

Tu-Pos354

A CATION CHANNEL IN HUMAN T LYMPHOCYTES. C. Grygorczyk, L.C. Schlichter, Department of Pharmacology, Merck-Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe-Claire, Quebec H9R 4P8.

We have found a cation channel in single-channel recordings of cell-attached patches from normal human T lymphocytes. This channel is apparently common in the membrane since it was seen in almost all membrane patches from cells of ~ 10 donors. Channels that were active in cell-attached recordings became inactive after excision, hence the current is probably absent from traditional whole-cell recordings. This observation restricted as to recording from cell-attached patches, making interpretation of the channel more difficult. For example, the amplitude was often irregular and sloped toward the baseline, as though current flowing through the open channel was altering the cell membrane potential. Whenever possible, I-V relations were constructed and indicate a single-channel conductance of 20-25pS, with some inward rectification. From the reversal potential, measured with NaCl or KCl Ringer's in the bath, the channel is cation selective, but apparently not very discriminating between Na^+ and K^+. Activity was often seen as bursts of openings separated by quiescent periods. Activity increased when the membrane was depolarized (negative pipette potentials); however, activity was often present at the resting potential. With TEA^+ substituted for Na^+ in the pipette no channel activity was present. Moreover, active channels could be blocked by adding quinine to the bath, suggesting membrane permeation and block from the inside. Supported by MRC and NCIC (Canada).

Tu-Pos355

AMILORIDE DIRECTLY BLOCKS THE MECHANOSENSITIVE CATION CHANNEL IN *XENOPUS* OOCYTES. J. Lane, C. Levine, K. Levine and O. Hamill. Department of Neurobiology and Behavior, Cornell University, Ithaca, NY

Amiloride, a potent diuretic, is known to block mechanosensitivity in the lateral line organ of *Necturus* and has recently been shown to block mechanosensitivity in hair cells of the inner ear. In this study we demonstrate directly with patch clamp recording that amiloride blocks, in a voltage and concentration dependent manner, the mechano-activated cation channel in *Xenopus* oocytes. At negative membrane potentials 0.1 mM amiloride applied to the extracellular membrane increases the number of brief interruptions and burst duration of single stretch activated channel currents. As concentration is increased the frequency of interruptions increases and there is an apparent reduction in channel current amplitude, most likely due to the inability to resolve completely very brief openings. At positive membrane potentials amiloride block is greatly relieved so that even with 1 mM amiloride channels appear similar to control. The dose and voltage dependence of the amiloride channel block is very similar to that reported for the amiloride block of mechano-electrical transduction in chick hair cells indicating the underlying mechano-sensitive channels may share basic similarities.

Tu-Pos356

CATIONIC CHANNELS PRESENT IN THE 210 KDa PROTEIN FRACTION ISOLATED FROM PLASMA MEMBRANES OF SEA URCHIN SPERM. E. Morales, A. Liévano, V. Vacquier*, L. de De la Torre, L. Possani# and A. Darszon. Dept. of Biochem., CINVESTAV-IPN, Apdo. Postal 14-740, 07000 México City; *Scripps Institute of Oceanography, La Jolla, CA 92093; #Dept. of Biochem., CIINGEBI, UNAM, Apdo. Postal 510-3, Cuernavaca, Morelos 62270, México.

There is pharmacological evidence suggesting that ionic channels are very important during the sea urchin sperm acrosome reaction (AR). In this cell, there is a 210 KDa protein which probably is the receptor to the "egg jelly", the natural inductor of the AR. This protein has been purified and when reconstituted into BLMs it induced single channel activity. In 90 % of the assays (n= 70) an anionic channel was incorporated (Biophys. J. 155:491a). In 10 % of the experiments cationic channels of 25, 70 and 130 pS were observed. The 70 pS channel was blocked by Co^{2+}, whereas the 130 one was sensitive to the venom of the scorpion *Oxyuranus scutellatus scutellatus* in a reversible manner. Since these two blockers inhibited the egg jelly induced AR, these channels may be involved in the AR. Supported by CONACYT and WHO.

Tu-Pos357

VOLTAGE CLAMP OF ISOLATED GROWTH CONES. C.E.Morris & R.Horn, U. of Ottawa, Ottawa, Ont., K1N 6N5 & Roche Inst., Nutley, NJ 07110. Growth cones of cultured snail (Lymnaea) neurons were severed to enable measurement of ensemble I_K from the cells' ubiquitous mechanosensitive (MS) K^+ channels. Isolated growth cones (igc) were suitable for the purpose: a) isolation did not induce cytoplasmic Brownian motion, b) perforated patch recording yielded 4 v-dependent currents, including an I_{Ba} which did not "wash out", c) cell-attached recordings of igc's showed stretch-activated (and stretch-inactivated (SI)) K^+ channels with the expected conductance parameters. The membrane was stepped below -50 mV or ramped (10 s) on either side of E_K. The igc's were subjected to pressure-ejected bath solution and difference records were made (I with and without stimulus). Surprisingly, no mechanically-activated I_K was seen, even when pressure almost blew the igc off the pipette. The only MS effect observed was a **decrease** of at most 4pA of I_K (driving force, 25 mV, 17 mM K^+ to carry I_K) per 300 μm^2. For SI K^+ channels carrying 0.2 pA, this could correspond to say, 100 channels reducing their P_{open} by 1/5. (CEM supported by NSERC).

Tu-Pos358

SINGLE CHANNEL EVENTS EVOKED BY IMMUNOLOGICAL STIMULATION OF RAT MUCOSAL MAST CELLS (RBL-2H3 LINE).
Chr. Romanin, M. Reinsprecht, I. Pecht, H. Schindler

Aggregation of the receptors for Fc domains of IgE (Fc RI) on the surface of mast or RBL cells leads to secretion of mediators of immediate type hypersensitivity. As early events, potentially involved in stimulation secretion coupling, a large increase in chloride permeability (measured by whole cell experiments) and a calcium influx have been observed. The purpose of this study was to investigate changes in membrane permeability at the single channel level following immunological stimulation (Fc RI aggregation) of mast cells of the RBL-2H3 line by i) IgE + specific antigen and ii) F4 antibody to the Fc RI. In the cell attached configuration a stimulation-induced chloride channel (23-42 pS) was observed after application of either i) or ii) in 25 % of cells (n=40) studied. In control experiments 15 of 16 cells exhibited no channel events. A stimulation-induced change in barium (or calcium) permeability could not be observed in the cell attached configuration. In the inside out patch configuration, however, calcium or barium selective channels (13 pS) could be detected in either unstimulated or stimulated cells. (supported by Austrian Res. Fonds: S-45)

Tu-Pos359

A MAXI-Cl CHANNEL IN NORMAL HUMAN T LYMPHOCYTES. L.C. Schlichter,*† R. Grygorczyk†, P.A. Pahapill*, C. Grygorczyk†, * Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8 and †Department of Pharmacology, Merck-Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe-Claire, Quebec H9R 4P8.

Cl channels have been proposed to play roles in lymphocyte volume regulation, cytotoxicity and mitogenesis. We have found a large, multiple-conductance Cl channel in ~ 50% of excised patches from normal human T lymphocytes. There are three prevalent subconductance states, the most common being the largest, ~365pS. Other levels are observed at intervals of about 45 pS. The probability of opening in inside-out patches is about 1 between -15 and +15 mV and closes sharply with further voltage excursions. Boltzmann fits suggest two gates in series with equivalent charges of 5.7 and 9.6. The selectivity for Cl^- over Na^+ or K^+ is about 30-fold and the selectivity sequence among anions $I^->NO_3^->Br^-$, $Cl^->F^-$, isethionate, $HCO_3^->SO_4^{2-}$>gluconate, propionate > aspartate, determined from reversal potentials and, where possible, from conductance. The channel was rapidly and reversibly blocked by 1 mM Zn^{2+} or Ni^{2+} added to the cytoplasmic face. Supported by MRC and NCIC (Canada) grants to L.C.S.

Tu-Pos360

DIVALENT CATION EFFECTS ON CGMP-ACTIVATED SODIUM CURRENTS IN PHOTORECEPTOR PATCHES.

J.C. Tanaka and R.E. Furman. Depts of Neurology and Biochemistry & Biophysics, Univ. of Pennsylvania, Philadelphia, Pa.

Current-voltage relations were measured in voltage-clamped inside out patches from Rana pipiens rod outer segments by exchanging solutions on the cytoplasmic side. The pipette contained 120 mM NaCl, 2 mM EGTA and 5 mM Hepes, 7.4. Test solutions contained either 200 μM or 20 μM cGMP and either Ca^{+2} or Mg^{+2} ranging from 10^{-8}M to 10 mM.

In 200 uM cGMP, the membrane inward sodium current showed little change as Ca was varied from 10^{-9}M to 10^{-5}M. At ~10^{-4}M Ca, the current increased by 20-30% falling to <20% of the initial value at 10 mM. With Mg^{+2}, the initial current changed very little until ~10^{-4}M, where it slowly decreased to ~50% of the initial value at 10 mM. Outward currents showed a similar profile of divalent block.

Currents activated with 20 uM cGMP were strikingly different. from currents at saturating nucleotide concentrations. With either divalent, the current decreased 80-90% as the concentration was varied from ~10^{-9}M to 10^{-8}M. The current recovered to about 30% at 10^{-7}M and remained at this value until > 1mM. The strong block at 10^{-8}M divalent cation was seen for both inward and outward currents.

We find these phenomenon in qualitative agreement with a 2 site multi-ion model in which the inner site has a high binding affinity and the outer site a much lower affinity.

Tu-Pos362

RAPID PRODUCTION OF AXOSOMES: A NOVEL ION CHANNEL PREPARATION FROM SQUID GIANT AXON.

Kirti P. Tewari & Harvey M. Fishman, Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550-2779.

Giant vesicles, "axosomes," form spontaneously in the subaxolemmal region of squid axons after transection in ASW (B.J., 55:588a). Formation requires Ca^{2+} or Mg^{2+}, apparently involves organelles (e.g. ER) and axosomes carry ion channels. To study these channels, rapid production (3 min) was achieved by cutting 3-5 mm segments out of an axon bathed in 494 TMACl, 5 TrisCl and sequentially adding 50 μL of 1 M $MgCl_2$ and 10 μL of 1 M $CaCl_2$ to 1 ml of TMA solution bathing the axon segment. Axosomes form-ed instantly. Axon segments were transferred by glass capillary to another dish containing test solution and immobilized with one glass rod while another was used to force axoplasm out of each cut end. Axosomes exited cut ends with stable attachment to axoplasm. Pipets applied to axosomes yielded seals ca. 100 GΩ, often lasting several hrs. Single-channel recordings from excised patches showed K^+, Ca^{2+} (Fishman & Tewari, these absts.) and other channel types.

[Aided by ONR contract N000-14-87-K-0055]

Tu-Pos361

CHARACTERIZATION OF MACROSCOPIC CHLORIDE CURRENT IN HUMAN NEUTROPHILS.

Schumann M.A., Heller D., Tanigaki T., and Raffin TA. Division of Respiratory Medicine, Stanford University Medical Center, Stanford, CA 94305-5236.

Whole-cell voltage-clamp recordings were obtained using human neutrophils following isolation utilizing Ficoll-Hypaque density gradient centrifugation. The current was carried by Cl^- ions based on the shift in reversal potential derived from voltage-current relationships as a function of transmembrane Cl^- gradient. In addition, the current was inhibitable by the anion transport inhibitor, diisothiocyanostilbene-2,2'-disulfonic acid (DIDS, $5x10^{-5}$M) when included in the pipette or bath solution. Two components of the outward current were observed: a time-dependent current and a time-independent current. The time-dependent component was discernable at depolarized potentials >20 mV. It demonstrated apparent voltage-dependent inactivation: 1) Current decline at depolarized potentials (<80 mV) was approximated by a single exponential function; and 2) With higher depolarizations current inactivation followed a double exponential function. Bath application of 1μM fMLP, a chemoattractant peptide for neutrophils, produced a 50% increase in peak current amplitude within 2-4 minutes with a faster activation rate and slower decline. This is the first report of a chloride current in human neutrophils. The increase in anion current may serve as a marker of neutrophil activation.

Tu-Pos363

ANION CHANNELS FROM MAMMALIAN NERVE GROWTH CONES.

J.A. DeBin and G.R. Strichartz, Dept. of Biol. Chem. & Mol. Pharm., Harvard University, and the Anesthesia Research Labs of Brigham & Womens Hospital, Boston, MA 02115.

Anion channels (AC) from embryonic rat brain growth cone particles (GCPs), prepared using the method described by Pfenninger et al. (Cell 35:573, '83), have been reconstituted into neutral lipid bilayers. AC activity was found to be 100-fold greater in the GCPs compared to adult brain synaptosomes, as determined from membrane incorporation rates. The biophysical properties of the channel resemble those described by Franciolini and Nonner (J.Gen.Phys. 90:453, '87) for ACs patch clamped in embryonic rat hippocampal neurons. The channel is remarkably unselective for anions over cations with a P_{Na}/P_{Cl} of only 0.26, and the halide selectivity sequence ; I(1.92)> Br(1.73)> Cl(≡1)> F(0.34), is indicative of ion binding to a site of low field strength. The ACs rectify in symmetric 200mM NaCl, with a chord conductance of 40pS at +30mV. Gating is relatively voltage-independent up to roughly +50mV, where the channel inactivates, or below -30 to -50mV where brief closings increase in frequency causing P_o to fall off. The most effective pharmacological agents are furosemide which produces a ≥80% reduction in current amplitude at 1mM, an indanyloxyacetic acid derivative (94-IAA, from Landry et al., J.Gen.Phys. 90:779, '87) with $K_I \approx 7\mu$M, and dinitrostilbene disulfonic acid with $K_I \approx 5\mu$M. The AC is also blocked by Mg^{++} and 9-Anthroic acid, but is unaffected by Ca^{++} ions up to 25mM©

Tu-Pos364

ADAPTATIONAL BEHAVIOR OF A MECHANOSENSITIVE ION CHANNEL IN *ESCHERICHIA COLI*. M. Buechner, B. Martinac, C. Kung, and J. Adler. Depts. of Biochemistry and Genetics and Laboratory of Molecular Biology, University of Wisconsin, Madison, WI, 53706.

We isolated a mutant strain of *Escherichia coli* which forms round cells without treatment by drugs, and which is large enough to be studied directly by the patch-clamp technique (Buechner *et al.*, submitted to *Biophys. Biochim. Acta*). The activities of the large-conductance mechanosensitive channel reported in wild-type *E. coli* spheroplasts (Martinac *et al.*, *Proc. Natl. Acad. Sci. USA* 84, 2297-2301, '87) were studied in excised inside-out patches from these mutant cells. The channel here rectifies and has the same reversal potential as in wild-type spheroplasts, but the most frequent conductance level is about 30% larger. The kinetics are also different; the channel shows brief openings (tens to hundreds of milliseconds) upon application of pressure, but activity then decays within 2 to 10 seconds even in the continued presence of pressure. Activity is regained upon reapplication of pressure after release. By summation of currents from repeated applications of pressure, time constants for the decay can be obtained. The amount of channel activity depends upon the rate of pressure application, which suggests that the decay in activity is caused by adaptation of the channel to continued pressure. Supported by NIH DK39121.

Tu-Pos365

FUNCTIONAL RECONSTITUTION AND PARTIAL PURIFICATION OF A CHLORIDE CHANNEL FROM TORPEDO CALIFORNICA. A.G. Goldberg, D.J. Woodbury and C. Miller, HHMI, Grad. Dept. of Biochemistry, Brandeis University, Waltham, MA 02254

We have developed a functional assay for purification of the chloride channel from Torpedo californica electroplax. The assay measures passive leakage of radioactive chloride out of phospholipid vesicles into which we have reconstituted functional ion channels. CHAPS extraction of electroplax membranes yields a soluble fraction containing DIDS-inhibitable chloride channels. Addition of exogenous phospholipids, removal of detergent, and a freeze-thaw cycle in the presence of $^{36}Cl^-$, forms a vesicle population whose internal space is equilibrated with the radioactive tracer. Dilution and exchange of the external medium removes external radioactivity, and trapped internal tracer is measured. Since $^{36}Cl^-$ flux out of channel-containing vesicles is fast with respect to our measurement, a "burst" of chloride release is observed. By quantifying the size of the burst (relative to an impermeant marker), the fraction of vesicles which contain channels can be determined. Using this method of assay, we have obtained an initial purification of approximately 10-fold by FPLC anion-exchange chromatography.

Tu-Pos366

PARTIAL PURIFICATION OF Na/Ca EXCHANGER FROM HEART. A. Ambesi, E.E. Bagwell & G.E. Lindenmayer, Depts. of Pharmacology & Medicine, Med. Univ. of South Carolina

The following sequence was employed for partial purification of the Na/Ca exchanger from canine myocardium: (1) Isolation of sarcolemma (SL); (2) alkaline extraction, pH 12.1; (3) solubilization with 9 mM CHAPS in salt/buffer (160 mM NaCl, 10 mM MOPS, pH 7.4); (4) DEAE chromatography with exchanger bound at 160 mM and eluted at 225 mM NaCl; (5) concentration of active fraction; (6) gel filtration with apparent MW for exchanger in CHAPS/lipid of approximately 375 kDa; (7) reconstitution by addition of 1% cholate with asolectin and 480 mM NaCl (final concentrations) followed by 3.2-fold dilution with salt/buffer. Steps (4) and (6) required 0.12% phosphatidylcholine for stability of exchanger. Specific activity was increased from 5.2 (SL) to 2791 nmol/mg/sec with recovery of 28.4% activity and 0.064% protein. SDS-PAGE revealed prominent proteins of 135, 120 and 70 kDa. Most of the 135 kDa protein binds to WGA-Sepharose in the presence of cholate/asolectin whereas most exchange activity and the 120 and 70 kDa proteins do not.

Tu-Pos367

CHARACTERIZATION OF A PURIFIED CHLORIDE CHANNEL. M. Akabas, A. Edelman, C. Redhead, D. Landry, Q. Al-Awqati. Dept. of Medicine, Columbia Univ., New York, N.Y.

Using an indanyloxyacetic acid affinity column we have purified chloride channels from bovine kidney. Four major proteins, 97, 64, 40, and 27 kD, are eluted from the affinity column. We have prepared an antibody against the 64 kD protein. Using the anti-64 kD antibody we can immunoprecipitate chloride channel forming activity from solubilized kidney membranes suggesting that the 64 kD protein is a component of the channel. By N-terminal sequence analysis the 97 & 27 kD proteins are unlikely to be components of the Cl channel. The Cl channels observed following reconstitution of the purified proteins into planar lipid bilayers are heterogeneous and display multiple subconductance states. Whether these differences are due to copurification of different Cl channels or are different manifestations of a single protein remains to be resolved.

Tu-Pos368

EFFECTS OF BILAYER LIPID COMPOSITION ON ION CHANNEL RESPONSES TO A GENERAL ANESTHETIC. M. M. Chang & Raphael Gruener. Dept. Of physiology, University of Arizona, College of Medicine. Tucson, AZ 85724.

We tested how the lipid bilayer charge density and fluidity affected the ability of a general anesthetic (halothane) to alter the Ca-K channel kinetics. Channel proteins were isolated from rat brain (Krueger, 1983) and incorporated into lipid bilayers of different phosphatidylethanolamine (PE) to phosphatidylserine (PS) ratios. The charge density on the bilayer, is related to the PS concentration, and the fluidity is inversely proportional to the PS concentration (Ohyashiki et al. 1986). HAL (0.7-2.8 mM) superfused the trans-side of the bilayer. Single channel activity was observed with a patch-clamp amplifier and analyzed with P-Clamp^t software. The bilayer was bathed with 100 mM KCl (trans-side) and 300 mM KCl (cis-side). The solutions were buffered with 10 mM HEPES and contained 0.1 mM $CaCl_2$, at pH 7.4. HAL reduced the channel opentime, open probability and the opentime constant in a concentration-dependent manner in all bilayer compositions. Channel conductance was unaffected. However, when comparing the effects of HAL across bilayers with PE/PS ranging from 3/7 to 9/1, no significant differences were observed. The data show that under the present conditions, neither charge density nor changes in fluidity alter the efficacy of anesthetic-channel interactions. We are now testing whether more drastic changes in bilayer fluidity (e.g. by doping the bilayer with cholesterol) may attenuate the effects of HAL on channel kinetics.

Supported by a grant from the Flinn Foundation (to RG) and a Graduate Student Fellowship from the U of AZ (to MMC).

Tu-Pos369

STRETCH SENSITIVITY OF ALAMETHECIN CHANNELS, L. R. Opsahl, D. D. Mak and W. W. Webb, Cornell U.

Alamethecin in lipid bilayers assembles into multisubunit ion channels which switch amongst several conductance states. We have used single channel recording to measure the effect of the applied transmembrane pressure difference, p, on the relative occupation probabilities P_i of the various states of alamethecin. Preliminary measurements show that an increase in pressure results in an increase in the P_i of higher conductance states relative to lower conductance states. Using Boltzmann statistics we show that the relative equilibrium occupation probabilities of states i and j are related to the difference in area occupied, $A_i - A_j = \Delta A_{ij}$ and membrane tension t by $ln(P_i/P_j) = -(G_0 - t\Delta A_{ij})/kT$, where G_0 is independent of t. The membrane tension is related to pressure by $t = pr/2\cos\theta$, where r is the membrane patch radius and θ is the contact angle. Uncertainty of the geometry of the membrane in the pipette has limited determination of the quantitative proportionality between t and p. The results show a clear increase with pressure of the relative occupation probabilities of the higher conductance states implying larger effective occupied areas for these states and an increase of the mean channel conductance with transmembrane pressure.

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Tu-Pos370

CHANNELS AND TUNNELING MECHANISMS IN FUNCTION OF MACROMOLECULES AND MEMEBRANES. V. Vasilescu, Mioara Tripsa, Cornelia Zaciu, Eva Katona Biophysics Dept., Medicine Fac., Bucharest

This decade has been dominated by the breakthroughs in the knowledge of mechanisms in cells and functional macromolecules. This has been achieved by improving the methods of tracking the role of channels and the tunneling processess in transport phenomena. Theory and experiment prove that certain macromolecules, such as DNA, have a tunnel structure and act as genuine channels. The experimental data shown here refer to the part played by water molecules in the architecture and operation of various types of channels. Data obtained following heavy water for water substitution are also given. Some of these data can be extrapolated to explain the interaction of photons with photoreceptor cells.

Tu-Pos371

PATCH-CLAMP STUDIES OF POINT AND REGULATORY MUTANTS OF YEAST PLASMA MEMBRANE ATP-ASE (PMA1)

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Earlier studies showed that a single point mutation (*pma1-105*, Phe→Ser368) of the gene for the proton-pumping ATP-ase caused the normally ATP-insensitive voltage-gated K^+ channels to be activated by ATP (Ramirez et al., PNAS 86, in press). We report here patch-clamp experiments on other ATPase-mutant yeast and on Xenopus oocytes injected with yeast mRNA which reveal further aspects of the linkage between the ATPase and the K^+ channels. A revertant of *pma1-141* (Phe→Ser368), *pma1-141-rev29* (Val→ Phe368), restores the channel gating to its wild-type phenotype. Another PMA1 mutant, *pma1-114* (Asp→Gly158) shows ATP-dependendent gating kinetics. *Mop2*, a regulatory mutant which reduces the expression of PMA1also reduces the number of K^+ channels in inside-out patches. To further test the linkage to the PMA1 gene, we injected Xenopus oocytes with mRNA constructed by linearizing a PSH9 plasmid with a Hind III fragment containing PMA1. The injected oocytes showed new ion channels having a 17 pS conductance, identical to that of the native yeast K^+ channels.

Tu-Pos372

COMPARISON OF NUCLEAR PORES DENSITY AND K-NUCLEAR CHANNELS DISTRIBUTION IN MURINE CELLS.

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The nuclear envelope functions as a selective barrier between nucleus and cytoplasm for a variety of molecules. During cycles of cell division, the nuclear envelope must repeatedly disassemble and reassemble; presumably, previous functional and structural properties are restored after each division. Whether the nature of selectivity changes after repeated rounds of cell division, such as during differentiation for example, is unknown.

Using the patch-clamp technique and electron microscopy, we compare ionic permeability and nuclear pore density in murine germinal vesicles, pronuclei, nuclei from two cell embryos, and liver nuclei. A large conductance K-channel is present in all nuclear envelopes we tested. Further, liver nuclei exhibit a greater density of both the K-channel and the nuclear pores, in contrast with the other nuclei examined. We show here that the nature of the conductance changes in different nuclei, and hypothesize a close relationship between the channel and the pore complex.

Tu-Pos373

ADP-EVOKED CATION CHANNELS AND VOLTAGE-GATED K CHANNELS IN HUMAN PLATELETS. M.P. Mahaut-Smith, S.O. Sage and *T.J. Rink. Physiological Laboratory, Cambridge, U.K. and *Smith, Kline and French Research Ltd, Welwyn, U.K.

Using patch clamp and potentiometric dye techniques we are studying the role of ionic channels in activation of human platelets by ADP. In cell-attached patch recordings, ADP transiently evoked single channel inward currents when back- filled into the pipette, but not when added to the bath. Slope conductance was 10-11 pS at the cell resting potential (pipette filled with 110 mM $BaCl_2$ saline, standard NaCl saline or Na gluconate saline; the extrapolated reversal potential was -50, -65 and -60mV (applied potential) respectively). ADP- evoked activity was blocked by 5mM Ni^{2+} added to NaCl saline in the pipette; these channels may therefore underly the ADP-stimulated (Ni-sensitive) Ca influx measured in fura-2 loaded platelets. Cell-attached and whole-cell patch recordings also reveal a high density of voltage-gated K channels (approx. 300-400/platelet). The threshold for activation was near 0 mV (applied) in cell-attached patches and -60 to -70 mV when measured whole cell. Measurements using the fluorescent probe $DiSC_3(5)$ show a resting Em of about -60 mV; stimulation by ADP results in 5 mV or less depolarization. K channels with the above properties could be responsible for setting the membrane potential at rest and during stimulation, thus maintaining the driving force for Ca flux.

Tu-Pos374

NEUROSECRETORY GRANULE CHANNEL RESEMBLES GAP JUNCTIONS. Cheol Joo Lee, José R. Lemos, Worcester Found. Exp. Biol., Shrewsbury, MA 01545.

The neurosecretory granules (NSG) from bovine posterior pituitary, isolated on Percoll gradients, were reconstituted into lipid bilayers (PE:PS=3:1). We have studied the effects of gap junction uncouplers on the larger of the two channels observed (Lemos et al, 1989).

The conductance of the NSG channel is variable, but most (17/24) were between 100 and 200 pS. Channel openings were decreased at lower (6.5) pH (11/13) and increased at higher (7.4) pH (3/7) when compared to controls (pH=7.0). Channel openings were completely abolished in 0 Ca^{++} (n=5) and in higher than 10 µM (n=5) internal free Ca^{++}. 1-5 µM decamethonium (C10) effectively blocked channel openings (5/7). Although 0.1 mM hexanol had no effect on channel openings, heptanol and octanol (0.1 mM - 0.1 vol%) strongly blocked the channel (n=8). Under most conditions (22/24), the channel openings showed voltage dependence.

These results indicate that the larger NSG channel of the posterior pituitary resembles gap junctions and lend support for the possibility that they are involved in the secretory process.

Tu-Pos375

EFFECT OF MUTATION ON THE CHANNEL PROPERTIES OF BACTERIAL PORIN STUDIED BY PATCH-CLAMP. A. H. Delcour, B. Martinac, J. Adler, and C. Kung. Depts. of Biochemistry and Genetics, Lab. of Molecular Biology, University of Wisconsin, Madison, WI, 53706.

We have prepared and reconstituted outer membrane fractions from *Escherichia coli* strains which express OmpC proteins with altered pore properties (Misra & Benson, *J. Bacteriol.* 170, 3611 (1988)). The channel characteristics were studied by the patch-clamp technique on blisters formed on the reconstituted liposomes. The channels are open most of the time but display frequent brief transitions to closed levels. Multiple conductance levels are observed and appear to correspond to the cooperative gating of identical channels. Single-channel conductance, selectivity, and gating pattern were compared between the parent strain (RAM105) and a mutant strain (RAM276) whose OmpC porin has a single amino acid substitution and is postulated to have a wider pore. We found in the mutant channel a small but significant (9%) increase in the single-channel conductance, while the reversal potential in asymmetric solutions was not altered. The channel mean open time and gating cooperativity also appear different between the two strains. These observed phenotypical differences have some implication as to the structure-function relationships of these channels.

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Tu-Pos376

EFFECT OF AMPHIPATHS ON PRESSURE SENSITIVITY OF THE MECHANOSENSITIVE ION CHANNEL IN ESCHERICHIA COLI. B. Martinac*, Mark W. Tengowski#, J. Adler&, and C. Kung*, Lab of Molecular Biology and Dept. of Genetics*, Dept. of Biochem. and Genetics$, Integrated Microscopy Resource of Biomedical Research#, University of Wisconsin, Madison, WI 53706

We have studied the effect of amphipathic compounds on the pressure sensitivity of the mechanosensitive ion channel in E. coli. Independent of their chemical structure all the amphipaths tested activated the channel in a reversible manner. There was a time lag between exposure to the drugs and their effect on the channel. Cationic amphipaths appeared to be more effective than anionic ones. Amphipaths with opposite charge were able to cancel each others effect. The results could be interpreted most consistently according to the bilayer couple hypothesis (Sheetz and Singer, Proc. Natl. Acad. Sci. USA 71:4457, 1974.)

Among amphipaths tested the most effective one was chlorpromazine. We have isolated several chlorpromazine resistant mutants in E. coli. Studies on properties of mechanosensitive channels in these mutants are in progress. Supported by NIH DK 39121.

Tu-Pos377

NONEQUILIBRIUM EFFECTS IN CELL MEMBRANES

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Mole fractions effects have been observed in both ion channels and in model peptides such as gramicidin. A typical example is the dependence of transmembrane potential of starfish eggs. Replacement of KCl with $TlNO_3$ generates a deviation from resting potential with a minima at mole fraction of .4 and a maxima at mole fraction of 1. This behavior can be simulated with the nonequilibrium Henderson equation. This equation describes the junction potential formed by differing rates of transport of ions across the cell membrane, and indicates a kinetic basis for the experimental observations.

Tu-Pos378

FACTORS CONTROLLING THE MITOCHONDRIAL INNER MEMBRANE ANION-CONDUCTING CHANNEL. M.J. Selwyn* and S.C. Halle-Smith**, *Department of Biochemistry, National University of Singapore, Singapore 2158 and **School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K. (Introduced by H. Tedeschi).

The pH-dependence of the conductivity of the mitochondrial inner-membrane anion-conducting channel is one of its diagnostic features and it has been suggested that Ca^{2+} and Mg^{2+} ions are also involved in control of its activity. The conductivity is also dependent on the previous history of the mitochondria with regard to exposure to oxygen or anaerobic conditions.

We have found that the effect of oxygen levels on the conductivity is mediated by changes in the electrical potential across the inner membrane and that under these conditions there is little change in either intramitochondrial pH or total Mg^{2+}.

Our data indicate that under normal physiological conditions the major factor controlling anion conductivity is the inner membrane potential but matrix pH could exert an effect under conditions of high Ca^{2+} loading. It is probable that cytosolic factors also modulate the conductivity of this channel.

Tu-Pos379

THEORETICAL AND EXPERIMENTAL INVESTIGATION OF THE COMPLEMENTARY CURE OF EPILEPSY USING MAGNETIC FIELDS.

A.Adamopoulos and P.A.Anninos

One of the many ways that we can study structure and function of the human brain is through computer simulation studies. In our previous work we have constructed a neural net model which exhibits epileptic behavior. The proposed neural theory not only explains the appearance of such behavior but also shows how to netralize them, using time varying magnetic fields. The general features of the above theory are consistent with several experimental measurements using SQUID Technology.

Tu-Pos380

A PRACTICAL APPROXIMATION TO THE DWELL TIME OMISSION PROBLEM

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Distortion of the dwell-time distributions of single channel currents in their open or closed states, due to the limited bandwidth of the recording device, has already been adressed by many authors. Roux and Sauve (*Biophys. J.*, 48:149-158) have shown how to calculate these distributions, as modified by a fixed dead time, but their general solution is difficult to apply in practice. An alternative approach by Blatz and Magleby (*Biophys. J.*, 49:967-980) yields the correct mean dwell times for simple kinetic schemes, but the application to coupled schemes involves further approximations.

Our approach is to introduce "virtual states" into the kinetic scheme, as suggested by Blatz and Magleby; the transitions into and out of the virtual states correspond to transitions that are undetected experimentally. To avoid the difficulty of assigning virtual states in the case of complicated, coupled schemes we use the recent results of Kienker (*Proc. R. Soc. Lond.*, 236:269-309) to allow any scheme to be transformed for the simple introduction of virtual states. The only approximation in our solution is that we treat the missed dwell times as if they were dwells in single kinetic states and therefore have exponential probability distributions; the true distributions are exponentials that are truncated at the dead-time. This approximation allows the observable dwell-time distributions to be computed by standard matrix techniques with errors that are expected to be negligible in practice.

Tu-Pos381

TOPOCHEMICAL PROOF FOR THE EXPRESSION OF A PORIN IN THE CYTOPLASMIC MEMBRANE OF HUMAN B-LYMPHOCYTES AND EVALUATION OF ITS PORE-FORMING PROPERTIES. F.P. Thinnes[a], H. Götz[a], R. Benz[b], and N. Hilschmann[a] (Intro. by C.A. Mannella). [a]Max-Planck-Inst., Göttingen; [b]Lehrstuhl für Biotechnologie, Univ. Würzburg, (F.R.G.).

We describe for the first time the existence of a porin in the cytoplasmic membrane of a eukaryotic cell line. This type of porin has so far only been observed in the outer membrane of mitochondria. The protein was isolated and purified to homogeneity by passing a total membrane fraction of the B-cell line through CM- and DEAE-Cellulose ion exchange columns. Rabbit xenoantisera were raised against the pure protein and against the free and acetylated 19 amino-acid N-terminus. Expression of the porin in the cytoplasmic membrane of transformed human B-lymphocytes was demonstrated by cytotoxicity and by indirect immunofluorescence with living and fixed cells. The purified protein formed in lipid bilayers slightly anion-selective, voltage-dependent pores with a single-channel conductance of 0.5 nS in 0.1 M KCl, very similar to those of mitochondrial porins.

Tu-Pos382

LOCALIZATION OF POSITIVE RESIDUES ON THE MOUTH OF PORIN PORE. V. De Pinto, A. Jalal and F. Palmieri (Intro. by C-H. Chen), Dept. Pharmaco-Biology, Lab of Biochemistry, University of Bari, Italy.

The mitochondrial porin or VDAC represents the protein structure responsible for the outer mitochondrial membrane permeability pathway. The pore in the o.m.m. allows the passage of molecules of M.W. up to 3000 dalton; it is slightly anion selective and voltage-dependent. It has been suggested that positive charges of aminoacidic residues are involved in the phenomena of anion selectivity and voltage-dependence. Positive charges are located on the mouth or on the exterior portion of the pre as demonstrated by the interaction of porin purified in LDAO with cation-exchangers. By using the model system constituted of porin purified in LDAO and cation-exchanger, we have investigated the interaction of a number of chemical modifiers with the functionally active pore unit. This experimental approach shows that it is possible to individuate in the primary structure positive charged residues located on the mouth or on the exterior part of the pore.