Th-Pos119
INTERRUPTED TRIPLE-HELICAL PEPTIDES.((Cynthia Gwynne Long[1], Emory H. Braswell[2],Ming-Hua Li[3], Jean Baum[3], and Barbara Brodsky[1])) [1]UMDNJ-Robert Wood Johnson Medical School and [3]Rutgers University, Piscataway, N.J.; [2] National Analytical Ultracentrifugation Facility at the University of Connecticut, Storrs, CT.

The triple-helical domains of collagens which form D-periodic fibrils have a precise Gly-X-Y repeating tripeptide pattern. In contrast, the triple-helices of non-fibrillar collagens and proteins such as C1q and mannose binding protein contain some breaks in the repeating Gly-X-Y pattern. Investigations are in progress to examine the effect of different kinds of interruptions in the Gly-X-Y repeat on triple-helix properties using a model peptide approach. The peptide $(Pro\text{-}Hyp\text{-}Gly)_{10}$ forms a stable triple-helix which melts at 60°C in aqueous solution. Four variants of this peptide were synthesized, introducing a single interruption in the center of the peptide chain: (1) a Gly -> Ala substitution; (2) a deletion of Hyp; (3) a deletion of Gly; and (4)an insertion of an Ala residue. Equilibrium ultracentrifugation showed that the Gly-> Ala, Hyp deletion, and Ala insertion peptides had a high degree of association, and that a monomer to trimer model best fit the data for the Gly -> Ala and Hyp deletion peptides. The Gly deletion peptide was largely in a monomer form, with little association. Circular dichroism and NMR studies indicated a substantial amount of triple-helical conformation had been disrupted by the interruptions, and helical stability was markedly decreased. A model which includes beta bends at the interruption site is proposed. This model helps explain why a Gly deletion may cause a greater destabilization of the triple-helix than other kinds of interruptions.

SARCOPLASMIC RETICULUM II

Th-Pos120
MODULATION BY INORGANIC PHOSPHATE OF RYANODINE BINDING TO SKELETAL MUSCLE SARCOPLASMIC RETICULUM. ((B.R. Fruen, J.M. Mickelson and C.F. Louis.)) Dept. of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

Sustained or repeated contraction of skeletal muscle is associated with a marked increase in myoplasmic levels of inorganic phosphate (P_i). We have examined the effect of P_i on the sarcoplasmic reticulum (SR) Ca^{2+} release channel using [^{3}H]ryanodine binding as a measure of channel activity. At pH 7.0, ryanodine binding to skeletal muscle heavy SR vesicles was increased when P_i was present in the medium. In the presence of 10 to 30 mM P_i and optimally activating levels of Ca^{2+} (6 μM) an approximately two-fold increase in ryanodine binding was observed. This increased binding was associated with an increase in the affinity of ryanodine for the channel (K_d=107 nM in the presence, versus 204 nM in the absence of 10 mM P_i) with little affect on maximal binding (B_{max}=14.5 pmol/mg in the presence, versus 12.1 pmol/mg in the absence of 10 mM P_i). In contrast to its affect on ryanodine binding to skeletal muscle preparations, P_i did not increase ryanodine binding to cardiac SR preparations, indicating that the effect is specific to the skeletal muscle isoform of the SR Ca^{2+} release channel. Our findings suggest that physiologic changes in P_i levels may modulate the activity of the skeletal muscle SR Ca^{2+} release channel. Such an effect could have important implications for understanding the role of P_i in muscle fatigue. Supported by NIH grant GM 31382 and the Minnesota Affiliate of the American Heart Association.

Th-Pos121
ALTERED RYANODINE RECEPTOR EXPRESSION IN CROOKED NECK DWARF MUTANT CHICKENS. 1. BIOCHEMICAL STUDIES. ((J.A. Airey, M. Baring, C.F. Beck[@], Y. Chelliah, T.J. Deerinck*, M.H. Ellisman*, L. Houenou*, D. McKemy, R. Oppenheim*, J.L. Sutko, J. Talvenheimo[@])) U. Nevada, Reno, NV 89557; [@]U. Idaho, Moscow, ID 83843; *U. Calif. San Diego, La Jolla, CA 92093; *Wake Forest U., Winston-Salem, NC 27157; [@]Amgen Corp., Thousand Oaks, CA 91329.

The recessive lethal crooked neck dwarf (cn) mutation in chickens results in a muscle dysgenesis, a failure to maintain skeletal muscle and connective tissue, and the degenerative loss of all embryonic skeletal muscle. Using immunoprecipitation and Western analyses we are unable to detect normal α ryanodine receptor (RyR) protein in cn mutant skeletal muscle at days E10, E15, and E18 of embryonic development, but do find Ca^{2+}-ATPase, calsequestrin, α_1-dihydropyridine receptor proteins and [^{3}H]PN200-110 binding. At day E12, RyR mRNA levels are reduced in cn mutant muscle. At day E18, βRyR protein can be detected at extremely low levels. The RyR isoform found in cardiac muscle is expressed at normal levels in cn mutant heart. These data suggest that a failure to produce normal αRyR is closely associated with the cn mutation.

Th-Pos122
ALTERED RYANODINE RECEPTOR EXPRESSION IN CROOKED NECK DWARF MUTANT CHICKENS. 2. MICROSCOPIC STUDIES. ((J.A. Airey, M. Baring, T.J. Deerinck*, M.H. Ellisman*, L. Houenou*, D. McKemy, J.L. Sutko)) U. Nevada, Reno, NV 89557; *U. Calif. San Diego, La Jolla, CA 92093; *Wake Forest U., Winston-Salem, NC 27157.

We have investigated ryanodine receptor (RyR) expression in crooked neck dwarf (cn) mutant embryos in immuno-localization studies using RyR isoform-specific antibodies. In cn skeletal muscle, αRyR immunoreactivity can be detected at extremely low levels in foci which exhibit a different cellular distribution than observed for this protein in normal muscle. We cannot detect αRyR in day E18 cn cerebellum, whereas this protein is abundant in E18 normal cerebellum. In day E18 mutant muscle, βRyR having a normal distribution is observed in <10% of the muscle fibers at ~20% of normal levels. Day E18 mutant skeletal muscles show marked degeneration, whereas cerebella from the same animals appear normal. Ultrastructural studies of cn mutant muscles have been conducted to determine the morphological changes associated with the cn mutation. These results are consistent with the failure to make normal αRyR in cn mutant tissues.

Th-Pos123
ALTERED RYANODINE RECEPTOR EXPRESSION IN CROOKED NECK DWARF MUTANT CHICKENS. 3. CELL CULTURE STUDIES. ((J.A. Airey, T.J. Deerinck*, M.H. Ellisman*, L. Houenou*, A. Ivanenko, J.L. Kenyon, D. McKemy, J.L. Sutko[@])) U. Nevada, Reno, NV 89557; *U. Calif. San Diego, La Jolla, CA 92093; *Wake Forest U., Winston-Salem, NC 27157.

We have investigated the properties of crooked neck dwarf (cn) mutant chicken skeletal muscle cells maintained in primary culture. When cultured at low densities under standard conditions, cn muscle cells proliferate and fuse to form myotubes in a manner indistinguishable from normal muscle cells. We cannot detect α ryanodine receptor (RyR) in these cells, but find βRyR at levels comparable to those in normal muscle cells. The βRyR in cn muscle cells appears to be functional as a SR calcium channel, since electrical depolarization and acetylcholine can stimulate Ca^{2+} transients. Cn mutant cells also exhibit normal L- and T-type calcium currents. Cn mutant and normal cells express comparable levels of α_1-dihydropyridine receptor subunit, Ca^{2+}-ATPase and calsequestrin proteins. Cultured cn muscle cells are not normal as they exhibit morphological changes. These results are consistent with a failure to make normal αRyR in cn mutant muscle.

Th-Pos124

TEMPORAL CORRELATIONS IN Ca^{2+} RELEASE CHANNELS REVEAL MODAL GATING. ((P. Vélez, R. Armisén, J. Sierralta, A. Ocampo, J. Vergara* and B. A. Suárez-Isla)) Depto. Fisiología y Biofísica, Fac. de Medicina, Universidad de Chile, Casilla 70005 & *UCLA School of Medicine, Los Angeles, CA 90024.

Steady state records of single Ca^{2+} release channels present in native membranes from muscle sarcoplasmic reticulum (SR) and hyppocampal endoplasmic reticulum incorporated in planar bilayers (Ca^{2+}, Ba^{2+} or Cs^{+} as current carriers), show complex burst kinetics. Dwell time distributions of open and closed times fitted by maximum likelihood criteria, exhibit not less than three exponential components. Burst analysis of records (120 s, interburst duration $\geq$ 25x predominant closed τ) exhibiting moderate overall P_o (0.2-0.5), indicated that distributions of burst durations and intraburst fractional open times (P_o) were bimodal. Records displayed sequences of bursts of low P_o (LP_o) interspersed with sequences of bursts with very high intraburst P_o (HP_o, near 1.0, called "gearshifts"). Average P_o was used to classify bursts as of low or high P_o, and the predominant burst time constant to classify them as short or long bursts. The random occurrence (null hypothesis) of low and high P_o bursts (and short and long bursts) was tested studying the statistical correlations between P_o values (or durations) of pairs of consecutive bursts. The observed 2x2 contingency tables constructed with LP_o-LP_o, LP_o-HP_o, HP_o-LP_o and HP_o-HP_o pairs (and duration pairs), had a probability of less than 0.01 of being generated by random occurrence (two-tailed Fisher's exact test). The number of HP_o-HP_o pairs exceeded most the expected overall occurrence. According to observations by Gyorki & Fill (this meeting), temporal correlations may be explained by a modal gating hypothesis that considers slow transitions between states of low and high probability of opening. Supported by FONDECYT #91-1294 and D.T.I. B-3199.

Th-Pos125

"ADAPTING" Ca^{2+} ACTIVATION: A MOLECULAR CONTROL MECHANISM OF Ca^{2+}-INDUCED Ca^{2+} RELEASE IN HEART. ((S. Gyorke and M. Fill)), University of Texas Medical Branch, Department of Physiology, Galveston, TX 77555-0641. (Sponsored by A. K. Ritchie)

To define the molecular basis of Ca^{2+}-induced Ca^{2+} release (CICR), the caged-Ca^{2+} methodology was applied to single cardiac ryanodine receptor (RyR) channels incorporated in lipid bilayers (BLMs). Solutions contained (in mM): 250 $CsCH_3SO_3$, 20 HEPES (ph 7.4), 3 DM-nitrophen, 2 $CaCl_2$, 2 glutathione, 0.0001 free Ca^{2+}. UV flashes from a Nd:Yag Laser were applied via a single fiber optic positioned perpendicular to the BLM. RyR Ca^{2+} sensitivity was $\approx$10 fold higher when Ca^{2+} was applied step-wise compared to steady state Ca^{2+} measurements. During a Ca^{2+} step, channel activity peaked then spontaneously decayed to a lower value corresponding to the steady state Ca^{2+} level. The spontaneous decay was not due to a conventional "desensitization" mechanism since a second incremental Ca^{2+} step activated a second burst of channel activity. These results suggest that RyR exhibit a novel "adapting" ligand-gating mechanism in which the RyR Ca^{2+} sensitivity shifts in response to sustained Ca^{2+} stimuli. This adapting behavior may represent a molecular mechanism for smoothly graded CICR in heart and may be an integral feature of channels, including the IP_3 receptor, which are involved in intracellular Ca^{2+} signalling in many cell types. Supported by NIH AR41197 (MF) and AHA (SG).

Th-Pos126

DANTROLENE ACTIVATES, THEN BLOCKS THE RYANODINE-SENSITIVE CALCIUM RELEASE CHANNEL IN A PLANAR LIPID BILAYER ((T.E. Nelson and M. Lin)) Dept. Anesthesia, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1009.

Dantrolene, a direct-acting skeletal muscle relaxant is efficacious for treating or preventing the anesthetic-induced malignant hyperthermia (MH) syndrome. The exact site of action for dantrolene is disputed, but it is commonly believed to block calcium release within skeletal muscle. After incorporation of normal and MH pig ryanodine-sensitive calcium release channels (RYR) into planar lipid bilayer (POPC:POPE, 70:30), control records were obtained in cesium methylsulfonate (250 mM CIS, 50 mM TRANS), HEPES, 20 mM (pH 7.4) and pCa=4.5. Holding potential was 0 mV and channels were activated by a voltage pulse protocol. Following control records, dantrolene was added in 5 μM increments to the CIS chamber to a final concentration of 30 μM. Two effects of dantrolene on the RYR were observed: activation followed by inactivation. Activation by dantrolene was characterized by increased open state probability and dwell times, and although a dantrolene concentration dependency for activation was observed, a time dependency precludes definition. Activation by dantrolene was followed by inactivation as evidenced by complete block of channel events. Increasing the activating pulse voltage could reverse the dantrolene block in some, but not all channels. These effects of dantrolene will be discussed in relation to its pharmacologic action in skeletal muscle.

Th-Pos127

ROLE OF INTRACELLULAR CALCIUM STORES IN THE CONTRACTIONS INDUCED BY AGONISTS IN TRACHEAL SMOOTH MUSCLE. ((M.P. NAVARRO-HUERTA. J.R. VALLE-AGUILERA & R. ESPINOSA-TANGUMA)) DEPT. OF PHYSIOL & PHARMACOL, MEDICAL SCHOOL, UASLP, S.L.P., S.L.P., MEXICO, 78230, AND DEPT. OF PHYSIOL & BIOPHYS, U.H.S./CHICAGO MEDICAL SCHOOL, N. CHICAGO, IL. 60064.

The role of caffeine and ryanodine-sensitive intracellular calcium stores (ICS) in the contractions induced by KCl (80mM), Carbachol, Carb, ($5x10^{-6}$M), and Histamine, Hist, ($1x10^{-5}$M) were studied in strips of tracheal smooth muscle of guinea-pig. Caffeine induced a concentration-dependent transient contraction whereas ryanodine at low doses ($\leq 5x10^{-5}$M) produced a concentration-dependent steady contraction and at higher doses ($\geq 1x10^{-4}$M) induced concentration-independent transient contraction. Pre-incubation (5 min) with caffeine (30 mM) delayed the onset of the contractions produced by KCl, Carb and Hist. In Hist induced contractions, caffeine pre-incubation reduced maximal contraction. Pre-incubation with ryanodine ($1x10^{-5}$M) did not result in any of the observations described above. Application of caffeine at the peak of the agonist-stimulated contraction produced a small additional transient contraction followed by relaxation. A similar application of ryanodine produced a dose-dependent partial relaxation. Our data suggest that KCl, Carb and Hist induce Ca release from caffeine and ryanodine-sensitive ICS.

Th-Pos128

TYPE 1 INOSITOL 1,4,5-TRISPHOSPHATE (IP_3) RECEPTOR IS CONCENTRATED IN PURKINJE MYOCYTES OF THE HEART CONDUCTION SYSTEM. ((L. Gorza, S. Schiaffino and P. Volpe)) Centro CNR Biol. Fisiopatol. Muscol., Dipartimento di Scienze Biomediche Sperimentali, Università di Padova, Italy.

IP_3 is one of the second messengers capable of releasing Ca^{2+} from sarcoplasmic reticulum (SR)/endoplasmic reticulum subcompartments. The mRNA encoding the IP_3 receptor/Ca^{2+} channel has been detected in the heart by Northern blot analysis. The myocardium, however, is a heterogeneous tissue composed of working myocytes and conduction system cells. In the present study, the cellular distribution of the heart IP_3 receptor has been investigated. [^{3}H]IP_3 binding experiments, Western blot analysis and immunofluorescence, with antibodies specific for the IP_3 receptor, indicated that the majority of Purkinje myocytes (the ventricular conduction system) express much higher IP_3 receptor levels than atrial and ventricular myocardium. In situ hybridization to a riboprobe generated from the brain type 1 IP_3 receptor cDNA, showed increased accumulation of IP_3 receptor mRNA in the heart conduction system. Our findings provide a molecular basis for the hypothesis that Ca^{2+} release from IP_3-sensitive Ca^{2+} stores evoked by α_1-adrenergic stimulation is responsible for the increase in automaticity of Purkinje myocytes (del Balzo et al. *Circ. Res.* 67,1535, 1990).

Th-Pos129

DOUBLE-BARRELED Cl CHANNEL OF LARGE CONDUCTANCE IN ROUGH ENDOPLASMIC RETICULUM MEMBRANE FROM RAT HEPATOCYTES. ((N. Morier and R. Sauvé)) Groupe de recherche en transport membranaire, Département de physiologie, Université de Montréal, Canada H3C 3J7.

The presence of anionic channels in stripped rough endoplamic reticulum (SRER) membrane from rat hepatocytes was investigated by fusion of SRER microsomes with a lipid planar bilayer. These experiments led to the identification of a chloride permeable ($P_{Cl}/P_K > 7.2$) channel of 160 $\pm$ 2 pS in asymmetrical 450 mM/50 mM KCl conditions. The channel activity was characterized by a strong voltage dependent bursting behaviour in which channel fluctuations occurred among three different current levels S_c (baseline level), S_{o1} and S_{o2} with S_{o2} being associated to a unitary conductance twice that of S_{o1} (160 pS). It was also found that current jumps could occur directly from baseline (S_c level) to the maximum current level S_{o2}, and vice versa, without any detectable transition to S_{o1}. A binomial analysis of the current amplitude distribution within current bursts revealed in addition that the fluctuations observed were compatible with a system of two independent conducting units, each unit characterized by a voltage dependency corresponding to a displacement of 1.55 electronic charge moving across the membrane upon channel opening. It was concluded on the basis of these results and from the channel open time interval distribution that the observed fluctuation patterns could be described in terms of two independent conducting units controlled by a common gate.

Th-Pos130

IMMORTAL HYPOTHALAMIC NEURONS EXPRESS A NOVEL VOLTAGE-GATED CALCIUM CHANNEL. ((A.D. Shcherbatko[1], W.C. Wetsel[2], A. Negro-Vilar[2] and D.L. Armstrong[1])) Labs. [1]Cellular & Molecular Pharmacology, [2]Molecular & Integrative Neuroscience, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 USA.

We have used patch clamp techniques to characterize Ca^{2+} channel diversity in an immortalized line (GT1-7) of mouse hypothalamic neurons (Shcherbatko *et al* 1992 *Biophys. J.* 61, A398), which secrete leutinizing hormone releasing hormone (LHRH) in a Ca^{2+}-dependent manner. Although these cells express L, N and T type channels, more than half of the high-threshold calcium current in most metabolically intact cells is resistant to maximal concentrations of dihydropyridines and ω-conotoxin-GVIA. We now report that this voltage-gated current is carried by a novel channel of approximately 12 pS conductance in 90 mM Ba^{2+}. The novel channels are also insensitive to 200 nM ω-agatoxin-IVA, but they are blocked completely by 10 μM La^{3+} or by 100 μM Cd^{2+}. Although these channels inactivate very slowly in cell-attached patches ($\tau_{1/2}$ > 1 s at 0 mV), their activity runs down rapidly in cell-free patches. Unlike L-type channels, however, the novel channels are not stimulated by cyclic AMP in intact cells. In contrast, 10 nM phorbol 12-myristate 13-acetate rapidly increased the frequency of novel channel openings in cell-attached patches. LHRH secretion from these cells is also stimulated much more effectively by phorbol esters than by forskolin. Furthermore, both the novel channels and potassium-stimulated secretion were inhibited by baclofen, an agonist at $GABA_B$ receptors. Therefore, these novel voltage-gated calcium channels could play a potentially important role in the synaptic regulation of neuropeptide release from the hypothalamus.

Th-Pos131

4-AMINOPYRIDINE BLOCKS THE CARDIAC SARCOPLASMIC RETICULUM POTASSIUM CHANNEL. ((Q-Y Liu, Q-X Liu, and H.C. Strauss)). Departments of Pharmacology and Medicine, Duke University, Durham, N.C. 27710

4-Aminopyridine (4-AP) is widely used to block sarcolemmal K^+ channels. We have employed the reconstituted bilayer technique to assess whether 4-AP blocks cardiac sarcoplasmic reticulum potassium SR-K^+ channels and to identify the characteristics of putative binding site(s). We found that 4-AP blocked the SR-K^+ channel by reducing channel current (data were filtered with 100 Hz 8-pole Bessel). 4-AP blocks the cardiac SR-K^+ channel from both *cis* (cytoplasmic) and *trans* (luminal) sides in a voltage-dependent manner. Blockade by 4-AP was competitive with K^+ ions, insensitive to pH (from 7.0 to 8.5), and consistent with a two-binding-site model of an impermeant blocker (Liu and Strauss, 1991, Biophys. J. 60:198-203). In 100 mM symmetric K^+, the apparent zero-voltage K_D for both *cis* and *trans* blockade was on the order of 10 mM. The effective electrical distances for the two binding sites were - 0.19 and -0.26 from the *cis* and *trans* sides, respectively, similar to the locations of Ca^{2+} binding sites described previously. Because 4-AP may freely cross the cell membrane in its neutral form, these results suggest that changes in SR Ca^{2+} release may occur when 4-AP is used to block sarcolemmal K^+ channels in cardiac muscle, because K^+ current through the SR-K^+ channel is an important counter-current for Ca^{2+} release. (Supported by NIH grant HL-19216).

Th-Pos132

CYTOPLASMIC DISULFIDE BRIDGES OF SKELETAL MUSCLE TRIADIN. ((H.R.Fan and A.H.Caswell)), Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33136

Triadin (M, 95K) is an intrinsic terminal cisterna protein that binds directly to the dihydropyridine receptor in the T-tubule and junctional foot protein in the SR. This protein can be detected by two mAb in Western blot and ELISA. mAb GE4.90 and AE8.91 recognize cytoplasmic regions of triadin but bind to different epitopes. Treatment of triadic vesicles with trypsin leads to the release of water soluble fragments of triadin into the medium which are recognized by GE4.90 but not AE8.91. The major fragments identified by immunoelectrophoresis under reducing conditions have M_r of 18K and 11K while a minor component has M_r of 13K. Intact triadin consists of a ladder of multimers formed by disulfide bonds from the 95kDa monomer. Two dimensional electrophoresis immunoblots with the first dimension non-reducing and the second reducing have been employed to study the disulfide bonds of these fragments. Spots corresponding to the monomers are observed lying along the diagonal of the gel. In addition spots are observed lying off the diagonal indicating the presence of disulfide bridged peptides. Each of the spots is interpretable as originating from dimers formed from the combination of each permutation of the three fragments. There is no evidence for the existence of higher polymers. This indicates that one of the intermolecular disulfide bonds is present in these fragments and that the bond is formed between identical domains of the protein. The presence of the epitope for GE4.90 in the fragments indicates that the disulfide bond is cytoplasmic and probably in the N terminal region of the molecule. (Supported by AHA)

Th-Pos133

IDENTIFICATION AND LOCALIZATION OF TRIADIN IN RAT VENTRICULAR MUSCLE. **((NR Brandt, AH Caswell, HK Motoike, SA Lewis-Carl*, DG Ferguson*, TN Brandt, JP Brunschwig, & AL Bassett))**, Dept. Molec.& Cell. Pharm, U. Miami Sch. Med., Miami, Fl and *Dept. Physiol. & Biophys., U. Cincinnati Col. Med., Cincinnati, OH.

Monoclonal antibodies against triadin, the protein in skeletal muscle which links the ryanodine receptor (Rr) to the dihydropyridine receptor (DHPr) recognize on Western blots a protein of M_r 95K in rat ventricular muscle heavy microsomes. The epitope comigrates with the subunit M_r of skeletal muscle triadin. Rat cardiac heavy microsomes were shown to contain dyads by their enrichment for Rr and DHPr, markers for junctional sarcoplasmic reticulum (SR) and T-tubules, respectively. The distribution of the triadin epitope matched the distribution pattern for DHPr and Rr when total ventricular muscle microsomes were centrifuged on isopycnic sucrose gradients and remained with the junctional SR after disruption of the dyads by French press treatment. A junctional location was also suggested by immunofluorescence microscopy. Cardiac triadin was not solubilized by 1M NaCl or TritonX-100, indicating that it is an integral protein of the junction. The native state of cardiac triadin is monomeric in contrast to the multiple oligomeric forms found in skeletal muscle microsomes isolated in the presence of 100mM iodoacetamide to prevent disulfide bridge formation. Rat dyads were dissolved in 1MKCl/0.03M KP_i/2%CHAPS detergent and chromatographed on hydroxylapatite. Triadin remained bound in 0.18M KP_i and co-eluted with the Rr and DHPr upon addition of 0.82M KCl/0.18M KP_i, suggesting the presence of a complex of these proteins. (supported by NIH, AHA, & AHA (FL Affil.)).

Th-Pos134

EVIDENCE FOR A JUNCTIONAL COMPLEX BETWEEN TRIADIN, THE JUNCTIONAL FOOT PROTEIN AND DIHYDROPYRIDINE RECEPTOR OF SKELETAL MUSCLE. ((H.K. Motoike[1], A.H. Caswell[1], N.R. Brandt[1], J-P. Brunschwig[1] and H. Smilowitz[2])) [1]Department of Mol. and Cell. Pharmacology, University of Miami Sch. Med., Miami, FL 33136; [2]Department of Pharmacology, University Conn. Health Cent., Farmington Ct 06032.

We have proposed a model of the triad junction consisting of a complex between the dihydropyridine receptor (DHPr), triadin and the junctional foot protein (JFP). We have separated by hydroxylapatite chromatography a complex of triadin, DHPr and JFP which elutes in 180 mM KP_i and 820 mM KCl from free DHPr and JFP which elutes in 180 mM KP_i/0 KCl. Further tests for junctional complexes include 1. The fractions were crosslinked with a photoactivable agent and centrifuged on rate zonal gradients. In the high salt fraction the DHPr, JFP and triadin moved as a high molecular weight complex while crosslinking had no effect on the low salt fraction. 2. All three proteins from the high salt fraction were retained both by a heparin column and also after prolonged incubation with a WGA column. On the other hand, on short term incubation with the WGA column most of the JFP and triadin as well as a significant portion of the DHPr were not retained. 3. Copper chelate chromatography of the high salt fraction selectively enriches for the triadin-DHPr complex. These results strengthen our hypothesis for the existence of a junctional complex containing triadin. (Supported by AHA)

Th-Pos135

MICROPROBE ANALYSIS OF SARCOPLASMIC RETICULUM IN PORCINE CORONARY ARTERY AND RABBIT AORTIC SMOOTH MUSCLE. ((J.A. Maloney and E.S. Wheeler-Clark)) School of Pharmacy, University of Wisconsin, Madison, WI, 53706.

The elemental content of sarcoplasmic reticulum (SR) was measured in freeze-dried sections of porcine coronary artery (PCA) and rabbit aorta (RA) using microprobe analysis. Structures that visually resembled SR also had a high P:K content ratio and low S:Cl ratio. In relaxed RA, the Ca content of SR (mean±SEM = 9.3±1.3 mmol Ca/kg dry wt, n=37) was higher than the Ca content of cytosol (2.5±0.8 mmol Ca/kg dry wt, n=19; $P < 0.001$). The RA SR data had a bimodal distribution: Ca was lower in SR located within 50 nm of nuclei (NSR) or mitochondria (MSR) than in junctional (JSR), peripheral (PSR) and central SR (CSR) unassociated with these structures (mean ± SEM for the MSR+NSR subgroup = 5.2±1.4 mmol Ca/kg dry wt, n=17; and mean ± SEM for the JSR+PSR+CSR subgroup = 12.7±1.7 mmol Ca/kg dry wt, n=20). The SR Ca content of both subgroups was decreased by nearly 60% in aorta frozen during a 1 μM phenylephrine contraction, consistent with the suggestion that SR releases Ca^{2+} in response to agonists that generate inositol 1,4,5-trisphosphate (IP_3). In contrast to RA, the calcium content of SR in relaxed PCA was reduced (mean ± SEM for PCA SR = 6.0±0.7 mmol Ca/kg dry wt, n=59) and the individual data were more normally distributed. In PCA frozen during contraction with 1 μM acetylcholine (which also releases IP_3), the Ca content of SR was further decreased but this change was quantitatively smaller than that observed in aorta. Based on these data, we suggest that PCA SR may sequester less Ca^{2+} at rest than RA SR, which may partly explain the greater dependence of coronary arteries on extracellular Ca^{2+} influx for agonist-induced contractions. Supported by AHA-WI affiliate.

Th-Pos136

SUBCELLULAR Ca^{2+} REGULATION IN CARDIOMYOPATHIC HAMSTER HEARTS.

((E. KELLER, C.S. MORAVEC, M. BOND)) Dept. of Cardiovascular Biology, Cleveland Clinic Foundation, Cleveland OH 44195

Development of cardiac dysfunction in cardiomyopathic hamster (CM) hearts may be linked to abnormal cellular Ca^{2+} regulation. Using electron probe microanalysis (EPMA), we previously measured mitochondrial (MT) and A-band (AB) Ca^{2+} in CM and normal hamster hearts rapidly frozen *in vivo* (Bond et al, Circ Res, 1989) and showed no generalized elevation in MT or AB Ca^{2+} under baseline conditions. We have now extended these studies to determine (1) the effect of an increased Ca^{2+} load on subcellular Ca^{2+} distribution and (2) whether Ca^{2+} regulation by the sarcoplasmic reticulum (SR) in CM is altered as compared with normal hearts. Experiments were performed on papillary muscles from 100-110 day CM and normal hamsters, using Bay K 8644 (10 μM) to increase Ca^{2+} influx. MT, AB and junctional SR Ca^{2+} were measured by EPMA in cryosections from the surface of papillary muscles rapidly frozen at peak contraction. We observed no increase in either MT or AB Ca^{2+} in Bay K treated or untreated muscles from CM hearts, as compared to treated or untreated normal muscles. In contrast, there was a decrease in junctional SR Ca^{2+} content in Bay K treated (4.1 $\pm$ 0.4 (SEM) mmol/kg dry wt) and untreated (4.5 $\pm$ 0.5) CM papillary muscles, as compared with treated (7.1 $\pm$ 0.6) or untreated (6.5 $\pm$ 0.8) normals. The observed reduction of SR Ca^{2+} in CM muscles, indicates either a decrease in SR Ca^{2+} stores or, alternatively, increased SR Ca^{2+} release during contraction.

Th-Pos137

THE ROLE OF SARCOPLASMIC RETICULUM FUNCTION AND PHOSPHOLAMBAN IN THE DEVELOPING RABBIT HEART.

((G. Szymanska, I. Grupp and E.G. Kranias)) Dept. Pharmacology & Cell Biophysics, Un. Cincinnati, Cinti., OH 45267.

Developmental changes in cardiac sarcoplasmic reticulum (SR) function and its regulation by phospholamban (PLB) were assessed in vitro and in vivo, using hearts from fetal, newborn, 4-day old, 21-day old and adult rabbits. The V_{max} of the SR Ca^{2+}-pump increased with age and it was the highest in the 4-day old rabbit hearts. Conversely, the affinity of the SR Ca^{2+}-pump for Ca^{2+}, which is regulated by PLB, decreased with age and it was the lowest in the 4-day old rabbit hearts. The 21-day old and adult rabbit hearts exhibited similar V_{max} and Ca^{2+}-affinity values to the 4-day old hearts. The rates of contraction (+dP/dt) and relaxation (-dP/dt) also increased with age and they reached maximal levels in the 4-day old hearts. Perfusion of the hearts with increasing concentrations of isoproterenol resulted in significant increases in both +dP/dt and -dP/dt, and the ED_{50} for isoproterenol stimulation increased progressively with age. Isoproterenol stimulation was also associated with increases in the affinity of the SR Ca^{2+}-pump for Ca^{2+}, due to PLB phosphorylation. These increases were mostly pronounced in the 4-day old, 21-day old and adult rabbit hearts. These findings indicate that the activity of the SR Ca^{2+}-pump and its regulation by PLB are developmentally regulated, and such regulation may reflect alterations in cardiac contractile parameters.

Th-Pos138

TIME RESOLVED X-RAY DIFFRACTION STUDIES OF THE EFFECT OF CA^{2+} BINDING ON THE PROFILE STRUCTURE OF THE SARCOPLASMIC RETICULUM MEMBRANE.

((L. J. DeLong and J. K. Blasie)) Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104. (Spon. by James M. Pachence)

A number of studies have indicated that the Ca^{2+}-ATPase of the sarcoplasmic reticulum membrane undergoes some structural change upon Ca^{2+} binding in the absence of enzyme phosphorylation. We have investigated the effect of such Ca^{2+} binding on the profile structure of the sarcoplasmic reticulum membrane, utilizing time-resolved x-ray diffraction and flash-photolysis of the calcium cage, DM-nitrophen. Critical control experiments were devised to exclude possible artifacts arising from sample heating, organic photolysis products, etc. The changes in the membrane profile structure induced by Ca^{2+} binding, for Ca^{2+}/Ca^{2+}-ATPase mole ratios of 2-3, are localized exclusively to the neighborhood of each of the lanthanide (Tb^{3+} and La^{3+}) binding sites in the membrane profile independently identified by resonance x-ray diffraction experiments. These changes can be attributed in part to the added electron density of the Ca^{2+} bound at these discrete sites, but they also require structural changes in the cylindrically-averaged profile structure of the Ca^{2+}-ATPase localized to the neighborhood (+/- 13Å) of these sites.

Th-Pos139

ONE-STEP HPLC RESOLUTION OF AGE-RELATED ALTERATIONS IN PHOSPHOLIPID COMPOSITION OF SKELETAL MUSCLE SARCOPLASMIC RETICULUM MEMBRANES.

Krainev, A.G., Bigelow, D.J., and Squier, T.C., Dept. of Biochemistry, University of Kansas, Lawrence, KS 66045.

Lipids have been extracted from sarcoplasmic reticulum (SR) membranes isolated from both young (5 mo) and aged (28 mo) Fischer strain 344 rats in order to examine the effects of aging on the lipid composition of skeletal SR. These lipids have been separated on a silica gel HPLC column using hexane/2-propanol (50/50) mixture with water gradient (from 4 to 8%) as a mobile phase. This solvent system has the advantage that it provides single step resolution of phospholipid species with good reproducibility. We find age-related changes in phospholipids that include an increase in total cholesterol and cardiolipin content (2.5- and 1.1-fold, respectively) and a decrease in phosphatidic acid, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol content (2.1-, 1.1-, 1.5- and 3.1-fold, respectively). Spin-label EPR measurements of lipid and protein rotational dynamics detect no changes in SR membrane structure. The results suggest that there are compensatory changes in lipid composition associated with age, which maintain an optimal Ca-ATPase activity.

Th-Pos140

FUNCTIONAL AND BIOPHYSICAL CHARACTERIZATION OF PORCINE CARDIAC SARCOPLASMIC RETICULUM MEMBRANES.

Linda Chen, and D. J. Bigelow, Dept. of Biochemistry, University of Kansas, Lawrence, KS 66045

We have developed a procedure to isolate native sarcoplasmic reticulum membranes from porcine hearts. The resulting preparation yields 0.6 mg SR/g heart, 30% of which is the Ca-ATPase, as assessed by a band on SDS-PAGE that migrates with an apparent molecular weight of 95-100 kilodaltons. These membranes exhibit efficient calcium-dependent ATPase activity (1.1 μmol phosphate/min/mg SR protein at 22°C), and minimal contamination by mitochondrial and sarcolemmal membranes as indicated by low calcium-*independent* ATPase activity. Moreover, the functional properties of this preparation are stable for several days on ice and for several months at -70°C. We find that the K_m for calcium of the cardiac Ca-ATPase is higher (K_m = 0.63 μM) than that of the skeletal muscle Ca-ATPase (K_m = 0.40 μM). However, when phospholamban is phosphorylated by cAMP-dependent protein kinase the calcium affinity of the cardiac enzyme increases so that it is identical to that of the skeletal enzyme. Specific and progressive derivatization of the cardiac Ca-ATPase with FITC results in an inhibition of enzymatic activity that is first order. These results are in contrast to the second order inactivation observed with the skeletal Ca-ATPase (Squier, et. al. (1988) *J. Biol. Chem.* 263, 9162.)

Th-Pos141

PROTEIN CONFORMATIONAL CHANGES INDUCED BY SURFACTANTS AS STUDIED BY FT-IR.

((J.L.R. Arrondo, A. Prado, I. Echabe and F.M. Goñi)) Departamento de Bioquímica. Universidad del Pais Vasco. Spain.

Surfactants are widely used in the study of soluble and membrane proteins. They maintain the hydrophobic milieu when the lipid is removed from a membrane protein and are used to disrupt the quaternary structure of a protein. Sarcoplasmic reticulum is a membraneous structure rich in a Ca^{2+}-ATPase, used as a model for intrinsic membrane proteins. Polioxyethylenic surfactants are known to affect the activity of this protein. Low concentrations of surfactant increase the activity of sarcoplasmic reticulum ATPase without major changes in protein conformation. At higher surfactant concentrations enzyme activity is inhibited and secondary structure changes. Quantitative studies give results similar to those reported for the interaction of the soluble protein ß-galactosidase with SDS where the presence of surfactants, at concentrations at which the enzymatic activity is maintained, do not produce appreciable changes in secondary structure, while surfactant concentrations that produce complete enzyme inactivation also produce small but significant changes in secondary structure.

Th-Pos142

INTERACTIONS OF PHOTORECEPTOR G PROTEIN, TRANSDUCIN, WITH GUANINE NUCLEOTIDE ANALOGUES ((Evelyn Zera, Theodore G. Wensel, David P. Molloy, Jagannath B. Lamture, Joseph K. Angleson, and Justine A. Malinski*)) Verna & Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas.

We have found that the interactions of several guanine nucleotides and analogues with transducin (G_t) are different from those previously described for G_t or other G proteins. In equilibrium binding experiments with bovine rod outer segments, GTPγS was found to bind the α subunit of transducin ($G_{t\alpha}$) with a K_d of 50 pM (not 50 nM), and transient competition experiments with GTP suggest its affinity for $G_{t\alpha}$ is comparable to that of GTPγS. The affinity of GDP for $G_{t\alpha}$ was at least 300-fold lower than that of GTPγS. The affinity of GDPβS for $G_{t\alpha}$ was at least 1500-fold lower than that of GTPγS, and no inhibition of transducin activation was observed at any GDPβS concentration tested. Commercial GDPβS contained a contaminant that activated transducin, and the presence of this activity was correlated with the ability of high concentrations of GDPβS to inhibit GTPγS binding. Several analogues of GTP modified at the 5' or 3' positions were tested, including "mantGTP", and found to interact with transducin weakly if at all. Traces of GTP were found to be produced rapidly from these analogues under the conditions used to assay transducin, and at high concentrations of analogues this contamination was sufficient to activate transducin and inhibit GTPγS binding.

Th-Pos143

EFFECTS OF THE γ SUBUNIT OF ROD cGMP-PHOSPHODIESTERASE ON THE KINETICS OF INTERACTION OF FLUORESCENTLY LABELED GUANINE NUCLEOTIDES WITH TRANSDUCIN. ((J.S.Mills, N.O. Artemyev, N.P.Skiba and H.E. Hamm)) Univ. of Illinois at Chicago, Chicago, IL ,60612.

Transducin is the retinal G protein which couples the light activation of rhodopsin to the hydrolysis of cGMP by activating phosphodiesterase. We have investigated the effects of the inhibitory subunit of bovine retinal cGMP phosphodiesterase(Pγ) on the interaction of guanine nucleotides with the retinal G-protein transducin using fluorescently labeled guanine nucleotides and a fluorescently labeled fusion protein of Pγ labeled at its single cysteine with lucifer yellow vinyl sulfone. Pγ stimulates the GTPase activitiy of transducin as measured by following the fluorescence of dGTP labeled with methylanthranylate(MANTdGTP), but the effect is due to a decrease in K_m with only a slight effect on K_{cat}. Similar effects were observed with the peptides Pγ 24-46 and Pγ 46–87. Pγ also affected the dissociation of GTPγS labeled with methylanthranylate (MANTGTPγS) from transducin. The rate of dissociation was 0.023 /sec in the absence of Pγ but decreased to essentially undetectable levels at 2 μM Pγ. ($K_{1/2}$=37 nM). Pγ 46-87 produced a similar effect half-maximally at 1.5 μM while Pγ 24-46 had essentially no effect on MANTGTPγS dissociation but increased the effectiveness of Pγ 46-87 7-fold. Thus, since Pγ selectively interacts with the GTP form of transducin, it increases the affinity of transducin for GTP (and GTPγS) as would be expected based on free energy considerations. Morever, since Pγ 24-46 increases the effectiveness of Pγ 46–87, the two interactions are free energy linked, indicating that they both produce complementary conformational changes upon binding to transducin and thus enhance each others' binding.

Th-Pos144

SITES OF INTERACTION BETWEEN ROD G-PROTEIN α-SUBUNIT AND cGMP-PHOSPHODIESTERASE γ-SUBUNIT. ((N.O. Artemyev, H.M. Rarick, J.S. Mills, N.P. Skiba and H.E. Hamm)) Univ. of Illinois at Chicago, Chicago IL 60612

In photoreceptor cells of vertebrates light activates a series of protein-protein interactions resulting in activation of cGMP-phosphodiesterase (PDE). Interaction between the GTPγS-bound form of rod G-protein α-subunit (α_t) and PDE inhibitory γ-subunit (Pγ) is a key event for effector enzyme activation. This interaction has been studied using Pγ labeled with the fluorescent probe, lucifer-yellow vinyl sulfone, at Cys68 (PγLY). Addition of α_tGTPγS to PγLY produced a 3.2-fold increase in the fluorescence of PγLY. The K_d for α_tGTPγS-PγLY interaction was 36 nM. α_tGTPγS enhanced the fluorescence of a C-terminal Pγ fragment, PγLY-46-87, as well (K_d=1.5 μM). We demonstrate that an α_t peptide, α_t-293-314, which activated PDE (Rarick et al., (1992) *Science 256*, 1031), mediates PDE activation by interacting with the Pγ-46-87 region. Peptide α_t-293-314 bound to PγLY ($K_{d,2}$= 1.2 μM) as well as C-terminal Pγ fragment, PγLY-46-87 ($K_{d,1}$=1.7 μM) as measured by fluorescence increase, while other α_t peptides had no effect. A peptide, Pγ-24-46, blocked the interaction between α_tGTPγS and PγLY, but had no effect on α_t-293-314 interaction with PγLY. The K_d for α_tGTPγS - Pγ-24-46 interaction was 0.7 μM. Our data suggest that there are at least two distinct sites of interaction between α_tGTPγS and Pγ. The interaction between α_t-293-314 and Pγ-46-87 is important for PDE activation. A second site of interaction involves the Pγ-24-46 region and an as yet unknown region on the α_t. We suggest that the initial step of PDE activation by α_t includes the binding of α_t to the C-terminal region of Pγ with the formation of an intermediate complex, α_t-Pγ-Pαβ. An α_t-induced conformational change in the Pγ-24-46 region may result in a decreased affinity of Pγ for Pαβ and an increased affinity for α_t.

Th-Pos145

A PERTUSSIS AND CHOLERA TOXIN-INSENSITIVE G PROTEIN MEDIATES α_1-ADRENERGIC MODULATION OF THE L-TYPE CALCIUM CURRENT IN NEONATAL RAT VENTRICULAR MYOCYTES. ((Q-Y. Liu, E. Karpinski, P.K.T. Pang)) Dept. Physiology, Univ. Alberta, Edmonton, Canada. (Spon. by M.J. Poznansky)

It has been demonstrated that in neonatal rat ventricular cells phenylephrine induces an increase in the L-type Ca^{2+} channel current. This effect is mediated by α_1-adrenoceptors. In order to investigate the involvement of G proteins in this effect, non-hydrolysable GTP and GDP analogues, GTPγS and GDPβS, were applied intracellularly. The L-type Ca^{2+} current was recorded using the whole cell version of the patch clamp technique. GTPγS is a G protein activator while GDPβS is a G protein inhibitor. In addition, two toxins, pertussin toxin (PTX) and cholera toxin (CTX), were used to identify the G proteins involved. PTX ADP-ribosylates G_i and G_o, whereas CTX ADP-ribosylates G_s. The results show that GTPγS (100 μM) potentiated the phenylephrine-induced increase in the L-type current. GDPβS (1 mM) completely blocked the phenylephrine-induced increase in the L-type current. Pretreatment of cells with PTX (200 ng/ml) and CTX (1 μg/ml) for 12 to 14 hr did not abolish the effect of phenylephrine on the L-type current. Therefore, the results obtained from these experiments suggest that a G protein is involved in the α_1-adrenergic modulation of the L-type Ca^{2+} channel current in neonatal rat ventricular cells and that this G protein is a pertussis and cholera toxin-insensitive G protein.

Th-Pos146

GTPγS STIMULATES EXOCYTOSIS IN PATCH CLAMPED RAT MELANOTROPHS. ((Keiju Okano, Jonathan R. Monck and Julio M. Fernandez)) Dept. of Physiol. and Biophys., Mayo Clinic, Rochester, MN 55905.

Rat melanotrophs, α-MSH-secreting cells from the intermediate lobe of the pituitary, are excitable cells that exhibit Ca^{2+}-dependent exocytosis in response to membrane depolarization. We have investigated the possible role of GTP-binding proteins in regulation of exocytosis by measuring the cells membrane capacitance in patch clamped cells while simultaneously measuring membrane currents and Ca^{2+} concentration. Depolarization from a holding potential of -60 mV to +20 mV causes opening of Ca^{2+} channels and an increase in membrane capacitance indicating exocytosis. The exocytotic response exhibits properties of classical Ca^{2+}-dependent exocytosis such as facilitation. Ca^{2+}-dependent exocytosis could also be induced by including high Ca^{2+} buffers in the pipette solutions. The hydrolysis-resistant GTP analog, GTPγS, also stimulated exocytosis in these cells, whereas GTP had no effect. This stimulation occurred in solutions where the intracellular Ca^{2+} concentration was clamped below 100nM and in the absence of measurable ion channel activity indicating that an elevated Ca^{2+} concentration is not necessary for the response. The response to GTPγS is abolished by GTP and GDPβS. The inhibition by GTP suggests that a sustained activation of a GTP-binding protein is necessary for exocytosis and that GTP is hydrolyzed before exocytosis can be activated. This behavior is similar to the stimulation of exocytosis by guanine nucleotides in mast cells and other non-excitable cells. The response to elevated Ca^{2+} is partially inhibited by GDPβS suggesting that GTP-binding proteins also play a role in Ca^{2+}-dependent exocytosis in melanotrophs.

Th-Pos147

EXOCYTOTIC FUSION IS ACTIVATED BY RAB3A PEPTIDES. ((Andres F. Oberhauser, Jonathan R. Monck, William E. Balch* and Julio M. Fernandez)) Mayo Clinic, Rochester MN 55905, and *Scripps Institute, La Jolla, CA 92037.

Studies of intracellular traffic in yeast and mammalian systems have implicated members of the rab family of small GTP-binding proteins as regulators of membrane fusion. We have used the patch clamp technique to directly measure exocytotic fusion and investigate the role of GTP-binding proteins in regulating exocytosis in mast cells. Intracellular perfusion of mast cells with GTPγS is sufficient to trigger complete exocytotic degranulation. The response to GTPγS does not washout, since release of GTPγS by UV photolysis of caged GTPγS is still able to induce complete degranulation after ~30 min of intracellular perfusion. Therefore, the GTP-binding protein activated by GTPγS must be close to, or part of, the exocytotic fusion pore machinery and not a soluble component of the cytosol. The exocytotic response to GTPγS is inhibited by GTP suggesting that GTP and GTPγS compete for binding to the GTP-binding protein and that GTP is hydrolyzed before membrane fusion is activated. Thus, a sustained activation of the GTP-binding protein seems necessary for stimulation of membrane fusion. We have found that synthetic peptides, corresponding to the part of the effector domain of rab3a, stimulate complete exocytotic degranulation. The response is selective for rab3a sequence and is strictly dependent on MgATP. The peptide response can be accelerated by GDPβS suggesting that rab3a peptides compete with endogenous rab3a proteins for a binding site on a target effector, which upon activation causes fusion. These results suggest that a sustained activation of a rab3 protein is sufficient to cause exocytosis in mast cells.

Th-Pos148

SINGLE TURNOVER STUDIES OF THE INTERACTION OF WILD TYPE P21ras AND THE GLY12→PRO MUTANT WITH GAP AND NEUROFIBROMIN ((K.J.M. Moore, P.N. Lowe and J.F. Eccleston)) NIMR, Mill Hill, London NW7 1AA, U.K. and Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, U.K.

The activation of the hydrolysis of p21ras.GTP by GAP and neurofibromin has been studied under single turnover conditions (excess GAP or neurofibromin over p21ras) using the fluorescent nucleotide analogue 2'(3')-O-(N-methyl)anthraniloyl GTP (mantGTP). It has been shown that with p21ras.mantGTP, a 10% decrease in fluorescence occurs concurrent with the cleavage step (Neal, S.E., Eccleston, J.F. and Webb, M.R., Proc. Natl. Acad. Sci. 1990 87 3562-3565). When the hydrolysis occurs with wild type protein in the presence of excess GAP, this process is accelerated. The first order rate constant shows a hyperbolic dependence on [GAP] which is interpreted as a rapid equilibrium binding of p21ras.mantGTP to GAP (K_D=17 μM) followed by a first order step of 13.9 s^{-1} (20 mM Tris HCl, pH 7.5, 1 mM $MgCl_2$, 0.1 mM DTT, 30°C). On increasing the ionic strength the initial binding step became weaker but the rate of the first order step remained constant. In identical experiments with neurofibromin, the rate constant of the fluorescence change is independent of [neurofibromin] over the range 2-8 μM. Thus the initial binding step is much tighter (< 1μM) and the first order step is 6.0 s^{-1}. Similar studies with the p21ras.mantGTP complexes of the Gly12→Pro (weakly oncogenic mutant) protein showed that the values of the binding constants were similar but that the first-order processes were reduced by factors of 730 and 65 for GAP and neurofibromin respectively. These results are discussed in terms of the *in vivo* regulation of p21ras. (Supported by the MRC and Human Frontiers Science Program).

SIGNAL TRANSDUCTION

Th-Pos149

MOLECULAR PROPERTIES OF HEMORRHAGIC SHOCK FACTOR ((John A. Evans, Gina Massoglia, Barbara Sutherland, Donald S. Gann)) Departments of Surgery, Biological Chemistry, and Physiology, Univ. Maryland at Baltimore, Baltimore, MD, 10201.

Restitution of blood volume after hemorrhage requires the shift of fluid from cells into the interstitium. Shock, accompanying hemorrhage >25% prevents or offsets this movement. An early event in this sequence is long duration cell depolarization by unknown mediators. Post-hemorrhage plasma contains hemorrhagic shock factor (HSF) a protein which induces long-term cell depolarization inhibitable by MgATP. HSF activity exists as a complex of peptides which remain strongly associated even in the presence of 6M urea. HSF is resolved by RP-HPLC and preparative SDS-PAGE into an active, 80kD peptide (csp80), and a regulatory fraction. After removal of denaturing agents, csp80 manifests depolarizing and ATPase activities; both activities have similar, acidic pH optima, and both are strongly potentiated by trivalent cations. The ATP concentration dependence suggests two components with respective K_M of 4 and 40μM. HSF actions may be regulated by ATP binding or hydrolysis. (NIH GM27946).

Th-Pos150

INSIGHTS INTO THE ACTIVATION OF Ca^{++} RESPONSES IN T CELLS BY BEAD-ATTACHED ANTI-T CELL RECEPTOR COMPLEX ANTIBODIES. ((B. Hashemi, J. Slattery, D. Holowka, B. Baird)) Cornell University, Ithaca, NY 14853.

Aggregation of the T cell receptor complex (TCR) triggers activation of T cells. One of the early signals in this process is the elevation of cytoplasmic free Ca^{++}. In some T cells, this can be triggered by soluble anti-TCR antibodies as well as by antibodies bound to surfaces and by peptide/MHC complexes on antigen presenting cells. To better understand the differences in the activation signals generated by receptor aggregation with soluble antibodies versus surface-associated ligands, we have developed a model system to measure cytoplasmic Ca^{++} levels of cells activated with anti-TCR antibodies attached to latex beads. A flow cytometric method has been devised that allows the simultaneous measurement and separate analysis of intracellular Ca^{++} responses of cells interacting with beads and non-interacting cells. Two argon ion lasers are used, one for excitation of Indo-1 using the 351.1 and 363.1 nm U.V. multiline, and another for the excitation of fluorescent beads at 514 nm. Gating techniques are used to separate the three populations of cell/bead couples, free cells, and free beads with less than 0.05% population overlaps. Cells triggered with bead-bound monoclonal antibodies against the ε and α/β subunits of TCR show a much larger Ca^{++} response compared to antibodies in solution. Furthermore, the rise in cytoplasmic Ca^{++} is sustained for a longer period of time with the bead-bound monoclonal antibodies. Analysis of calcium response patterns with bead-bound monoclonal antibodies and their Fab fragments provide insight into the mechanisms of receptor mediated activation and desensitization.

Th-Pos151

ASSOCIATION OF L-SELECTIN (LEU-8, LAM1) WITH THE T-CELL RECEPTOR.

((A. Aszalos, P.S. Pine, and G. Szabo, Jr.)) FDA, CDER, Division of Research and Testing, Washington, DC 20204. (Spon. by Joseph F. Reilly)

Accessory molecules, such as CD4 and CD2 were shown to play important roles in T4 cell activation (Haughn et al. Nature 358:328,1992; Hahn et al. Science 256:1805,1992). For the CD4 molecule, association with the T-cell receptor is a requirement for T4 cell activation. We report here that the L-selectin molecule is in the physical proximity (within 150 Å) of the T-cell receptor in unstimulated human peripheral blood lymphocytes (PBL). For this study we employed the photobleaching fluorescence resonance energy transfer (pFRET) method (Szabo et al. Biophysics J. 61:661,1992). Three FITC labeled monoclonal antibodies (mAb), αLam1-1, αLam1-3 and αLeu-8, were used as donor molecules and phycoerythrin (PE) labeled αCD3 mAb was the acceptor molecule. Alternatively, we have measured pFRET between αTCR-FITC and αLeu-8-PE. In each case, considerable energy transfer was measured (E_{CR} or E_{Tavg}= 8-14%). For negative control αTCR-FITC and αLeu-3a-PE were used (E_{CR} or E_{Tavg}= 1-2%). In agreement with results of the biophysical measurements, treatment of PBL cultures with Leu8 mAb suppressed the proliferation initiated through the T-cell receptor.

Th-Pos152

ASPARTATE RECEPTOR/METHYLTRANSFERASE INTERACTIONS; MEASUREMENT OF BINDING PARAMETERS BY TITRATION CALORIMETERY ((D. G. Long and R. M. Weis)) The Department of Chemistry and the Program in Molecular and Cellular Biology; University of Massachusetts, Amherst, MA 01003.

The specific binding of the *Escherichia coli* aspartate receptor cytoplasmic fragment (c-fragment) to the methyltransferase, which catalyzes covalent modification of the receptor, has been measured by titration calorimetry. An injection series of the methyltransferase into a solution of the wildtype c-fragment yielded a saturable binding curve, and was well-described by a simple single-site binding. Titrations were carried out at 27° C and at pH 7.0. Fits to the data produced an affinity constant of 3 x 10^5 M^{-1}, a binding enthalpy of 15 kcal/mole, and a binding ratio of 1 to 1. In addition to the wildtype form of the c-fragment, point mutations which appear to 'lock' the intact receptor into either a smooth-swimming or tumbling signaling state were titrated, but no significant differences in the binding properties of the various mutants were observed.

The observation that one methyltransferase binds to one c-fragment suggests that the sites of methylation, which are clustered into two separate groups in the primary structure of the receptor, must be accessible to methylation from a single binding site of the methyltransferase.

This work was supported by the NIH Grant GM42636.

Th-Pos153

INTERACTION OF GP75 AND GP140^{trk} IN THE HIGH AFFINITY NERVE GROWTH FACTOR RECEPTOR. ((D.E. Wolf*, C.A. McKinnon*, D. Kaplan+, R. Stephens+ and A.H. Ross*)) *Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545 and +ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702.

Nerve growth factor receptor (NGFR) exists in both high and low affinity forms. The high affinity form is required for biological responsiveness. Two NGFR's have been identified; gp75 and gp140^{trk}. The gp140^{trk} is required for high affinity binding and it has been hypothesized that the low affinity form consists of gp75, while the high affinity form is a complex of gp75 and gp140^{trk}. Using fluorescence recovery after photobleaching (FRAP), nonradiative fluorescence resonance energy transfer (FRET), and coimmunofluorescence localization we have examined the interaction of these tw-o proteins. The gp75 was found to be highly mobile on nonresponsive cells which do not express gp140^{trk}, but relatively immobilized on responsive gp140^{trk} positive cells. We have prepared bacculovirus vectors for both gp75 and gp140^{trk}. When gp75 alone was expressed in SF9 cells it was found to be highly mobile. Coexpression of gp75 and gp140^{trk} caused partial immobilization of gp75, demonstrating an interaction between these two proteins. Further evidence of interaction is provided by colocalization and FRET data.

Th-Pos154

THE EFFECT OF SUBCLONING A TUMOR MAST CELL LINE ON THE HETEROGENEITY OF THE Ca^{2+} RESPONSE TO ANTIGEN STIMULATION. ((J. Kuchtey, W. Weintraub, C. Fewtrell)) Department of Pharmacology, Cornell University, Ithaca, NY 14853

Changes in the level of intracellular free ionized calcium are associated with the secretion of mediators of immediate hypersensitivity by rat basophilic leukemia (RBL) cells, a mast cell line. Digital video fluorescence imaging of intracellular fura-2 has allowed us to study Ca^{2+} signals simultaneously in many individual cells with high temporal and spatial resolution. When RBL cells are stimulated with antigen, there is considerable variability in the Ca^{2+} response of individual cells within a single field of view of the microscope (Millard et al. 1989, J. Biol. Chem. 264:19730). This variability of Ca^{2+} responses may be seen in both the delay between stimulus and response and in the general type of response (ie. sustained, oscillatory or transient).
One possible explanation for the variability of the Ca^{2+} response could be that, due to genetic drift in culture, the RBL-2H3 line may consist of a heterogeneous population, giving rise to a variety of Ca^{2+} responses. We have subcloned cells from the RBL-2H3 line using the limited dilution technique to see whether it is possible to derive populations of cells with more homogeneous responses to antigen stimulation. The individual Ca^{2+} responses of cells from these subclones and the mean response of each population will be presented together with the secretory responses of the various clones.

Th-Pos155

FLUORESCENCE ANISOTROPY DETERMINATION OF ASSOCIATION/DISSOCIATION KINETICS OF EGF WITH THE EGF RECEPTOR IN A431 CELL MEMBRANES. ((D.L. Rousseau Jr., J.V. Staros, and J.M. Beechem)) Vanderbilt University, Nashville, TN 37232.

Epidermal growth factor (EGF) is a 6kD polypeptide hormone that exerts its mitogenic effects through specific binding to a membrane-bound EGF receptor. In order to study the relationship between EGF binding and receptor activation/oligomerization, we used changes in the fluorescence anisotropy of fluorescein-labeled mEGF (FITC-EGF) to monitor the real-time kinetics of association and dissociation of EGF with its receptor in A431 membrane vesicles. EGF and FITC-EGF have indistinguishable biological activities as assayed by competition binding, receptor kinase activation, and receptor dimerization. With T-format anisotropy detection following rapid mixing in a stop-flow instrument, we observed the binding and dissociation of FITC-EGF from its receptor. FITC-EGF concentration-dependent kinetics were simultaneously analyzed with a modified global analysis program, revealing at least two separate receptor populations in A431 membrane vesicles with different association and dissociation rate constants. We are currently assessing various models of receptor/ligand interactions (negative cooperativity, receptor heterogeneity, etc.) to determine the best model for the data. The kinetic constants derived from these fits can then be compared with those from spectroscopic equilibrium binding studies for a thorough characterization of EGF/EGF receptor interactions in A431 membrane vesicles. This FITC-EGF/EGF receptor system represents a case in which detailed on, off, and equilibrium experiments can be performed in real time, at concentrations close to physiological. Supported by NIH grants P01 CA43720. J.M.B. is a Lucille P. Markey Scholar.

Th-Pos156

ENDOTHELIN POTENTIATES Ca^{2+} TRANSIENTS EVOKED BY ATP IN ADULT RAT CARDIOMYOCYTES. ((Derek S. Damron and Meredith Bond)) Dept. of Cardiovascular Biology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Endothelin (ET) has a positive inotropic effect in cardiac muscle (Moravec and Bond, 1989), suggesting that ET increases Ca^{2+} influx or the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR). We used suspensions of adult rat ventricular myocytes loaded with fura-2/AM to investigate the effects of ET on Ca^{2+} transients evoked by exogenous ATP. It was previously shown that addition of exogenous ATP (25 μM) to cardiac myocytes triggers an increase in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) which is dependent on both Ca^{2+} influx and Ca^{2+} release from the SR (De Young and Scarpa, 1989). We recently showed that pretreatment of cardiac myocytes with arachidonic acid (AA, 50 μM) potentiated the amplitude of the ATP-triggered Ca^{2+} transient by 124% (50 μM), and this potentiation was blocked by 70 and 61% with staurosporine (10 nM) and sphingosine (0.5 μM), respectively. We now report that ET (500 nM) enhances the ATP-triggered Ca^{2+} transient by 113%, however this event is unaffected by similar concentrations of the PKC inhibitor staurosporine (10 nM). Also, the combination of ET and AA produced a synergistic effect on the amplitude of the Ca^{2+} transient induced by exogenous ATP, since pretreatment with AA plus ET increased peak $[Ca^{2+}]_i$ by as much as 300% over unstimulated controls. We conclude that ET may be involved in the modulation of inotropic activity in cardiac muscle via a mechanism which increases the Ca^{2+} load of the SR leading to a modification of SR Ca^{2+} release.

Th-Pos157

Depletion and refilling of intracellular Ca^{2+} stores induce oscillations of Ca^{2+} current. ((Vaca L & Kunze DL)) Department of Molecular Physiology, Baylor College of Medicine. Houston, 77030 TX.

An agonist-induced Ca^{2+} influx pathway in vascular endothelium and other non-excitable cells is closely aligned with the depletion of microsomal Ca^{2+} stores. The mechanism by which this occurs is unknown. In these studies 2,5-di-t-butylhydroquinone (BHQ), a specific inhibitor of the microsomal Ca^{2+} ATPase, and whole-cell patch-clamp recordings were used to evaluate the relationship of inward Ca^{2+} current to the depletion of intracellular Ca^{2+} stores. Na^+, Ca^{2+} and Ba^{2+} are all able to carry inward current (activated by BHQ) with a relative selectivity sequence of $Ca^{2+} > Ba^{2+}$. Partial depletion of intracellular Ca^{2+} stores by 1 μM BHQ induced a slowly activating Na^+ inward current (50±12 pA, n=3) that was inhibited by transient application of 10 mM Ca^{2+} to the extracellular solution which refills the previously depleted stores. A higher BHQ concentration (which induces a more complete depletion) induced a Na^+ current of greater amplitude (110±35 pA, n=3) that was only partially reduced by 10 mM Ca^{2+}. The results demonstrate that depletion and refilling of Ca^{2+} stores controls the amplitude of an electrogenic Ca^{2+} influx in vascular endothelium. Prominent fluctuations in Ca^{2+} current occur when there is an imbalance between depletion and refilling of the stores. This work was supported by a grant from the American Heart Association.

Th-Pos158

RECEPTOR IMMOBILIZATION AND MEMBRANE RIGIDIFICATION IN HUMAN ERYTHROCYTES INDUCED BY LIGAND BINDING: A MODEL FOR TRANSMEMBRANE SIGNALLING. ((D. Knowles*, M. Narla#, J. Chasis#, and E. Evans*)) *Univ. of British Columbia, Vancouver, Canada; #Lawrence Berkeley Labs., Calif.

We report experiments that expose a transmembrane signal process in human erythrocytes which leads to profound changes in membrane elasticity and receptor mobility. Specifically, binding monoclonal antibodies to glycophorin A in normal-intact red cells immobilizes the receptor and rigidifies the membrane. Using Fab fragments of antibodies and mutant red cells that lack the cytoplasmic domain of glycophorin A, we conclude that the locus of the immobilization/rigidification lies within the membrane skeletal structure and requires the cytoplasmic domain of glycophorin A. The unexpected discovery is that glycophorin A immobilization and membrane rigidification is accompanied by immobilization of band 3 (the anion transport channel) but glycophorin C (a companion integral protein) remains mobile. The evidence indicates cooperative coupling between liganded glycophorin A, band 3, and the membrane skeleton. The intriguing speculation is that this cooperativity may represent a general mechanism for cytoskeletal linkage and skeletal transformation initiated by receptors with short cytoplasmic sequences (e.g. integrins).

Th-Pos159

ENERGY TRANSFER BETWEEN A NOVEL MEMBRANE COMPONENT AND THE TYPE I FCE RECEPTOR ON MAST CELLS

((M. Kircheis[$], R. Schweitzer-Stenner[$] and I. Pecht[#])) [$] Institut für Experimentelle Physik, Universität Bremen, Germany, [#]Dept. of Chem. Immunology, The Weizmann Institute of Science, Israel

The cell line RBL-2H3 carry the type I Fc_ϵ receptors ($Fc_\epsilon RI$) for antibodies of the class E (IgE). Crosslinking of $Fc_\epsilon RI$-IgE complexes initiates a cascade of biochemical events culminating in secretion of inflammatory mediators. Binding of the recently raised monoclonal antibody (designated G63) that recognizes a glcoprotein, inhibits the $Fc_\epsilon RI$ mediated release (1). This finding and others suggest that the glycoprotein plays a role in the $Fc_\epsilon RI$ signal transduction and both these membrane components may come into proximity upon $Fc_\epsilon RI$ clustering. We employed the method of Tron et al. (3) to measure energy transfer efficiency E on single cells between probes attached to ligands to the above membrane components using a fluorescence activated cell sorter (FACS). Cell suspensions were incubated for 1 h on ice with fluorescein isothiocyanate (FITC) conjugated mAb G63 and monoclonal anti DNP specific tetramethylrhodamin isothiocyanate (TRITC) conjugated IgE-SPE-7 (2). At 0°C E was lower than 4 %, suggesting that the two membrane components are randomly distributed. Then, the cells were brought to 37°C, antigen was added and E was monitored as a function of time. We found that E increased with time both in the presence and absence of crosslinking antigen. Antigen induced crosslinking of IgE, however, caused E to increase (3 %). The time constant for the enhancement of E and its amplitude were found to increase with higher temperature. At T=0°C E was time independent. Inhibitors of respiration and glycolysis (NaN_3, deoxyglucose) caused a significant inhibition of the antigen induced contribution to E suggesting that an active process brings the two components together. (1) Ortega Soto, E. and Pecht I., J. Immunol. 141:4324 (1988); (2) Eshhar, Z. et al., J. Immunol., 124, 775 (1980); (3) Tron, L. et al., Biophys. J. 45:939 (1984)

Th-Pos160

Thermodynamic characterization of hapten binding to IgE class monoclonal antibodies.

((R. Schweitzer-Stenner[1], P. Engelmohr[1], A. Licht[2] and I. Pecht[2]),[1] Inst. of Exp. Physics, Univ. of Bremen, 2800 Bremen, Germany; [2] Dep. of Chem Immunology, The Weizmann Institute of Science, Rehovot, Israel; sponsored by: I. Steinberg

The interactions between dinitrophenyl (DNP)-haptens and three monoclonal, DNP-specific IgE-class antibodies, (A2, SPE7 and H1-26.82) was studied over a range of temperatures. Hapten binding was monitored either via the IgE intrinsic trytophan emission or that of the fluorescein isothiocyanat (FITC) covalently attached to the antibodies. The haptens employed were N^{ϵ}-2,4-DNP-lysin (DNP-lys), N^{γ}-2,4-DNP-aminobutyric acid (DNP-but) and N^{ϵ}-2,4-DNP-aminocaproic acid (DNP-cap). Fluorescence titrations of the above IgE with these haptens were performed. Their analysis showed that the binding affinity for all haptens increases in the order A2 (10^6-$10^7 M^{-1}$), SPE7 (2-$4\ 10^8 M^{-1}$) and H1-26.82 ($5\ 10^7$-$3\ 10^8 M^{-1}$), all at 25°C. The binding enthalpies to A2 are considerably larger (≈ -60KJ/Mol), but are partially compensated by negative reaction entropies (≈ 0.09 KJ/K Mol). The binding enthalpies to SPE7 and H26.82 are significantly lower (20-45 KJ/mol and 35-50 KJ/mol). The corresponding entropies have positive values for DNP-cap and DNP-lys (0.02-0.03 KJ/K Mol) and small negative values for DNP-but(-0.02-0.0 KJ/K Mol). The rates of the above haptens dissociation (k^-) and association (k^+) were also measured as a function of temperature employing a fluorescence technique recently developed by Goldstein et al. (Biophys. J, 56, 955, 1989) k^{-1} were found to be slow for both IgE, i.e.≈ 0.02-0.1 s^{-1} (SPE7) and 0.1s^{-1} for H1-26.8 (k^- for A2 is >1s^{-1}). Assuming a single step equilibrium k^+ was calculated and found to be low for SPE7 (≈2 $10^6 M^{-1}s^{-1}$) due to a large enthalpy barrier (80-100 KJ/Mol). The higher hapten affinity of H1-26.82 is mainly caused by a markedly lower enthalpic barrier for the association (≈10KJ/Mol), which give rise to association rate constants of ≈ $10^7 M^{-1}s^{-1}$. This suggests that hapten binding to H1-26-82 is diffusion controlled.

REGULATION OF ION CHANNELS

Th-Pos161

DUAL HORMONAL REGULATION OF PKA-DEPENDENT PHOSPHORYLATION IN K_{Ca} CHANNELS FROM UTERINE SMOOTH MUSCLE.

((G. Pérez, E. Stefani & L.Toro)) Dept. Mol. Physiol. & Biophys. Baylor College of Medicine. Houston, TX 77030

Uterine smooth muscle undergoes large changes in contractility and excitability during the estrus cycle and pregnancy. Contrary to other smooth muscles, K_{Ca} channels from nonpregnant human myometrium were inhibited upon PKA-dependent phosphorylation. Thus, we hypothesized that K_{Ca} channels from uterine smooth muscle could be differentially regulated by PKA-dependent phosphorylation in pregnant (PR) vs. nonpregnant (NPR) myometrium. K_{Ca} channel activity was recorded after reconstitution into lipid bilayers. Addition of 20 nM PKA (catalytic subunit) in the presence of MgATP (500 μM) induced activation of the majority of K_{Ca} channels from PR rat myometrium (18-19 days) (8 activated, 3 inhibited); while it inhibited most of the channels when they belonged to NPR tissue (estrus) (6 inhibited, 1 activated). This suggests that hormones preferentially modulate the expression of a type or modulatory subunit of K_{Ca} channels. In both cases, PR and NPR myometrium, the activity of K_{Ca} channels was restored near control values after the addition of 40 U/ml of alkaline phosphatase. The dual mode of action of phosphorylation occurred on K_{Ca} channels with similar characteristics. Either kinetics or unitary conductance (≈ 260 pS), as well as charybdotoxin sensitivity, were similar in K_{Ca} channels from NPR and PR myometrium. At +20 mV and pCa 5 channel Po was 0.60 ± 0.11 in NPR and 0.52 ± 0.23 in PR myometrium. The mean open times were 53 ± 11 ms and 44 ± 20 ms; while the mean closed times were 46 ± 23 ms and 64 ± 53 ms in NPR and PR myometrium, respectively. Our findings reveal that the regulation of K_{Ca} channels in myometrium is also target of the changes during pregnancy, suggesting a role of these channels in the excitation and contraction of the uterine smooth muscle. Supported by HD25616, HL47382.

Th-Pos162

INDEPENDENT ACTION OF G PROTEIN AND PKA-DEPENDENT PHOSPHORYLATION UNDERLIE β-ADRENOCEPTOR ACTIVATION OF K_{Ca} CHANNELS.

((F.S. Scornik*, J. Codina†, L. Birnbaumer*† & L. Toro*)) *Mol. Physiol. & Biophys. & †Cell Biol. BCM, Houston, TX 77030.

K_{Ca} channels from coronary smooth muscle were studied using the bilayer technique. Addition of 10 μM isoproterenol to the external side of the channel, in the presence of 0.5 μM internal GTP, increased K_{Ca} channel activity in 30% of the channels studied (n=13). This effect could be reversed after addition of the β-adrenoceptor antagonist, propranolol (100 μM). Because β-adrenoceptors are known to activate the G_s/adenylyl cyclase complex to increase intracellular cAMP, at least two mechanisms of action are feasible: 1) cAMP-dependent phosphorylation, and 2) direct G protein-mediated activation. We demonstrate that both mechanisms may occur independently. Addition of the catalytic subunit of the protein kinase A (PKA), in the presence of MgATP (0.5 mM), increased K_{Ca} channel open probability (Po) 3.4 ± 0.6 fold (n=3). This channel activation was prevented by the PKA inhibitor (PKI) (n=3). On the other hand, GTPγS and the activated α subunit of G_s (α_s*) induce K_{Ca} channel activation in the absence of exogenous ATP. Activation was observed after the inhibition of an hypothetical endogenous phosphorylation with PKI (Po increased 2.2 ± 0.4 times with GTPγS, n=2, and 4.1 ± 1.3 times with α_s*, n=3) and other inhibitors of PKA-activation (Rp-cAMP[S]; 100 μM, n=3), or phosphorylation (AMP-PNP; 100 μM, n=6). GTPγS increased channel Po 3.7 ± 1.2 times and 2.7 ± 0.4 times, respectively. Moreover, GTPγS increased channel Po 5.8 ± 3.8 times in the presence of 10 μM internal cAMP (n=3). Thus, β-adrenoceptor induced K_{Ca} channel activation seems to be the result of at least two independent effects: PKA-mediated phosphorylation, and direct activation by G_s. Supported by AHA-National Center (900963 to LT) and NIH (HL47382 to LT, DK19318 to LB).

Th-Pos163

SODIUMNITROPRUSSIDE (SNP) ACTIVATES CALCIUM-ACTIVATED K^+ CURRENT IN COLONIC MYOCYTES.

((J.D. Campbell and K.M.Sanders)) University of Nevada School of Medicine, Reno, Nevada 89557-0352.

Exogenous nitric oxide (NO) treatment results in both a transient hyperpolarization and a mechanical relaxation in intact canine colon (AJP 260:G789-G792, 1991). NO has also recently been proposed to be the primary inhibitory neurotransmitter released by non-adrenergic, non-cholinergic (NANC) nerves in the gut wall (AJP 261:G553-G557, 1991). In this study, the whole cell patch clamp technique was used to investigate the conductances responsible for NO mediated changes in membrane potential seen in intact colon.

In isolated colonic myocytes, NO (~10 μM) elicited up to a 30 mV transient hyperpolarization using current clamp mode. To ascertain if a nitric oxide donor could elicit a similar effect on resting membrane potential, membrane voltage was monitored upon the addition of 100 μM SNP and found to hyperpolarize by up to 20 mV (n=3) in a sustained fashion. Voltage clamp studies designed to investigate the effects of SNP on K^+ current utilized 5 mM 4-aminopyridine to block voltage dependent K^+ channels and more specifically isolate the calcium-activated potassium current. Ramp protocols (-80 mV to +80 mV for 4 secs) run before and after the addition of 10 μM SNP under these conditions increased outward current above +30 mV potentials (n=4). We conclude that NO and NO donors may elicit hyperpolarization in colonic tissue by the activation of calcium-activated K^+ current. (Supported by DK08811)

Th-Pos164

ISOPROTERENOL AND PROTEIN KINASE A INCREASE ACTIVITY OF Ca^{2+}-ACTIVATED K^+ CHANNELS IN BASILAR ARTERY SMOOTH MUSCLE.

((Yumin Song, Spencer Dunn, J. Marc Simard)) University of Texas Medical Branch Galveston TX 77550

B-adrenergic stimulation and cAMP-dependent mechanisms are well known to cause relaxation of cerebral vascular smooth muscle. We investigated membrane mechanisms that might contribute to this effect by recording outward currents due to large conductance Ca^{2+}-activated K^+ channels in smooth muscle cells from the basilar artery of the guinea pig. Most experiments were carried out using the whole cell configuration of the patch clamp technique, with physiological intra- and extracellular solutions. Isoproterenol (0.4-0.8 μM, n=16) caused a reversible increase by 50-60% of the macroscopic current, and this effect was completely blocked by 4 MM propranolol. A comparable increase in current was observed with dibutyryl-cAMP (100 μM, n=3), 8-bromo-cAMP (100 μM, n=3) and forskolin (1 μM, n=5). Single channel currents were recorded using the inside-out patch configuration. The outward current due to the 260 pS channel was highly sensitive to Ca^{2+} on the cytoplasmic side. In the presence of 0.1-0.5 μM Ca^{2+}, addition of the catalytic subunit of protein kinase A caused a 5-8 fold increase in the probability of channel opening. Our data indicate that cAMP-dependent mechanisms responsible for vasorelaxation act in part by hyperpolarization of smooth muscle cells.

Th-Pos165

REGULATION OF VOLTAGE-DEPENDENT CURRENT IN MOUSE NEUROBLASTOMA CELLS. ((J.K. Hirsh and F.N. Quandt)) Rush Med. Col., Dept. of Physiology & Multiple Sclerosis Center, Chicago, IL 60612.

We are testing the hypothesis that the number and type of voltage dependent ion channels expressed in neurons are subject to control by membrane potential and internal transmitters. Whole cell currents were recorded from isolated, round N1E-115 neuroblastoma cells which were 25 to 35 μm diameter and lacked processes. These cells were grown in tissue culture in the presence of 1.5% DMSO to arrest cell division. The neurons contain two types of voltage-gated K channels: one which rapidly activates and inactivates following a step depolarization (K_f), and one with much slower kinetics (K_s, F.N. Quandt, *J. Physiol.* 395, 401-418, 1988.). The proportion of each type can be separated by fitting the time course of the outward current during a 700 ms depolarization to +70 mV by a function having two exponential decaying processes which are summed. We have found that the proportion of K_f is modulated by growing the cells under conditions expected to alter membrane potential and Ca content. When cells were grown in medium containing elevated K (50 mM) for 3 days, the ratio $[K_f/(K_f + K_s)]$ was 41 ± 8% (n=6). This value was a factor of 2 larger than that for cells grown in control medium (5 mM K). If the cells are grown in the presence of 1 μM A23187, the ratio was reduced to one-third of that for cells grown in medium containing elevated K. In addition, the Na current was reduced or eliminated when medium contained A23187 when compared to control conditions. We are currently studying whether transcriptional or translational regulation is responsible for these changes. Sponsored by the National Multiple Sclerosis Society.

Th-Pos166

METAL IONS MODULATE GATING OF TRANSIENT OUTWARD CURRENT IN HIPPOCAMPAL NEURONS.

((G. Talukder and N.L. Harrison)) The Department of Anesthesia and Critical Care, The University of Chicago, IL 60637. (Spon. by M. Villereal)

Zn^{2+} modulates the gating of transient outward K^+ currents in rat hippocampal neurons, shifting both activation and inactivation curves to the right by 30-50mV (Harrison et al, 1992). There are several plausible mechanisms for the modulation of channel gating, as described by Hille (1992). Two such mechanisms are: binding to specific sites on channel proteins and the screening of negative charge on the membrane surface. To distinguish between these hypotheses, we studied the effects of a range of metal ions on gating of transient outward current (TOC) and also investigated whether lowering the ionic strength of extracellular solutions affects modulation of gating by Zn^{2+}.

Whole cell recordings were made from single neurons at 25°C, using intracellular solutions based on K gluconate and continuous extracellular perfusion with HEPES-buffered saline containing 0.2mM Ca^{2+}, 3mM K^+, 50mM TEA^+, 5mM Cs^+, and 500nM TTX. To lower ionic strength of extracellular solutions, all NaCl was replaced with sucrose. Solutions of the metal ions were applied by bath perfusion.

Fe^{3+}, Cu^{2+}, and Ni^{2+} had no effect on TOC gating at 100μM. Pb^{2+}(≥500nM), Cd^{2+}(≥200μM), and La^{3+}(≥5μM) shifted both the activation and inactivation curves for TOC to the right. Lowering the ionic strength of the extracellular solution had no significant effect on TOC gating, or on its modulation by Zn^{2+}. Taken together, these results suggest that modulation of gating of TOC is via a specific metal binding site, rather than by the screening of surface negative charge.

We thank the Brain Research Foundation of Chicago for financial support.

References

Harrison, N.L. et al (1992) Biophys. J., 61, 2186.
Hille, B. (1992) *Ionic Channels of Excitable Membranes*, Ch.17, Sinauer: Sunderland, MA

Th-Pos167

DEPOLARIZATION-ACTIVATED K CHANNELS IN A PLANT MOTOR CELL ARE REGULATED BY PHOSPHORYLATION.

((Nava Moran)) Dept. Neurobiology, Weizmann Inst. Rehovot 76100 Israel.

The efflux of K^+ from cells in a motor organ of the plant Samanea, during their shrinking phase, occurs via the delayed-rectifier-like K channels (K_D channels) in their plasma membrane. The patch-clamp technique, in a whole-cell configuration, was used to investigate the role of phosphorylation in the regulation of these channels. The omission of ATP from the patch-pipette solution shortened the K_D channel viability period from the usual 1-2 h to <30 min. H7 (20 uM), a kinase inhibitor, reversibly blocked K_D channel activity. These results indicate that phosphorylation is needed to maintain the K_D channel. ATP-γ-S (350 uM), which leads to hydrolysis-resistant phosphorylation, also inhibited the K_D channel. Okadaic acid (OKac), a protein phosphatase inhibitor, decreased the K_D channel activity irreversibly. The dose-dependence of the inhibition rate, fitted with a Michaelis-Menten relationship, yielded an apparent K_a for the action of OKac (assuming that the inhibition of phosphatase by OKac is the rate limiting reaction) of ~100 nM, comparable to its effective blocking concentration (C_{50}=10-200 nM) of the serine\threonine protein phosphatase 1 in biochemical assays in animal preparations. It is therefore likely that a similar phosphatase regulates the activity of this plant potassium channel, by dephosphorylating a Ser or Thr residue.

Th-Pos168

ACTIVATION OF A POTASSIUM CURRENT IN HUMAN CIRCULAR SMOOTH MUSCLE CELLS BY CARBON MONOXIDE.

((G. Farrugia, J. L. Rae, M. G. Sarr and J.H. Szurszewski)) Mayo Foundation, Rochester, MN 55905.

Carbon monoxide (CO) is a low molecular weight oxide with a similar structure to nitric oxide, a putative neurotransmittor in the human small intestine. A possible physiologic role for CO in smooth muscle has been suggested but there has been no direct evidence for its action to date. The effects of 1% CO on isolated normal human circular smooth muscle cells were studied using the perforated patch clamp technique. The whole cell outward current in human jejunal circular smooth muscle consists of a highly potassium selective, voltage dependant current and a non selective ohmic current that reverses at 0 mV, i.e. a leak current. CO increased the whole cell current in 19 of 21 cells tested (mean ± SD = 175 ± 181%). Activation of the potassium current was accompanied by membrane hyperpolarization (mean ± SD = 15.6 ± 14mV). The leak current was also increased by CO (mean ± SD = 72 ± 70%). In 15 cells the increase in the potassium current was greater than the increase in leak current resulting in membrane hyperpolarization while in 4 cells the membrane depolarized and the increase in potassium current was less than the increase in leak current. The increase in the whole cell current was delayed (mean ± SD = 13.6 ± 7. 8 sec.) and was transient. Prolonged recordings from 3 cells revealed further cyclic increases and decreases in the whole cell current. The data suggest that in isolated human circular smooth muscle cells carbon monoxide may not only be a byproduct of cellular metabolism but may also have a physiologic role. Supported by DK17238, EY06005, and EY03282.

Th-Pos169

MODULATION OF SINGLE CHANNEL K^+ CURRENTS OF FROG SKELETAL MUSCLE BY G PROTEINS. M. Vazquez and J. A. Sanchez. Department of Pharmacology CINVESTAV-IPN, A. Postal 14-740, Mexico D.F. 07000 and Department of Physiology UNAM, Mexico, City D.F. Mexico.

Modulation of K^+ channels of frog muscle was investigated in isolated vesicles formed from the sarcolemma of twitch skeletal muscle (Rana montezumae). Methods: Patch clamp recordings in the inside-out patch configuration in vesicles formed as described by Standen et al, (J. Physiol. (1985) 364:339-358). Solutions (mM): Pipette contained KCl=120, $CaCl_2$=2, HEPES=5. Bath contained KCl=120, $MgCl_2$=2, EGTA=5, HEPES=5, ATP=2. pH=7.2 and T=22-25°C. Results: Single channel K^+ currents were identified by their sensitivity to TEA and by reversal potential measurements in asymmetrical solutions. The single channel conductance was ca. 60 pS. Continuous recordings for 50 s revealed the presence of bursts followed by silent periods of ca. 5 s. Substitution of Cl^- by F^- greatly increases the duration of the silent periods in a concentration dependent manner. In $[F^-]_i \geq 30$ mM the single channel activity virtually disappears. The effects are reversible. No significant changes in the amplitude of single channel currents were observed. GTPγS (100 μM) had similar effect and GDPβS prevented the effects of both F^- and GTPγS.

Supported by CONACyT grant #0287N.

Th-Pos170

EFFECTS OF CA-BUFFERS AND TEMPERATURE ON REGULATION OF I_{Ks} BY ß-ADRENERGIC AGONISTS IN GUINEA PIG VENTRICULAR MYOCYTES.

((A. E. Busch and J. Maylie)) Department of Obstetrics and Gynecology, Oregon Health Sciences University, Portland, OR 97201

ß-receptor mediated regulation of I_K in guinea pig ventricular myocytes has been shown to be temperature dependent. However, these experiments were performed under conditions that use high concentrations of EGTA in the whole cell recording pipette to buffer intracellular Ca^{2+} to ~1 nM. These recording conditions may affect receptor mediated regulation of I_K, since Ca^{2+} has been shown to modulate I_K. Furthermore, recent studies have demonstrated that I_K in guinea pig ventricular myocytes is composed of two distinct components, a rapidly activating I_{Kr} and a slowly activating I_{Ks} in which only I_{Ks} appears to be the target for ß-receptor mediated regulation. In this study we re-examine the effects of temperature on ß-receptor mediated regulation of whole cell I_K, specifically of the slow component I_{Ks} in guinea pig cardiocytes and the possible involvement of $[Ca^{2+}]_i$ on such regulation. Temperature increase from 21 to 31°C dramatically accelerated activation of I_{Ks}. With low concentrations of Ca^{2+}-buffer (0.1 mM EGTA or BAPTA) in the recording pipette isoproterenol increased I_{Ks} 2 to 3 fold at both temperatures. With high internal Ca-buffer concentrations (EGTA or BAPTA ≥ 10 mM) I_{Ks} was regulated by isoproterenol at 31 °C but not at 21 °C. We conclude that ß-receptor regulation of I_{Ks} is dependent on the $[Ca^{2+}]_i$ buffering capacity, and that temperature can override this dependence. A mechanism consistent with the results is that Ca^{2+} and temperature increase the availability of subunits for channel formation and regulation by the ß-adrenergic pathway.

Th-Pos171

β-ADRENERGIC MODULATION OF THE INWARDLY-RECTIFYING K^+ CHANNEL IN GUINEA PIG VENTRICULAR MYOCYTES. ((S. Koumi, R. E. Ten Eick and J. A. Wasserstrom)) Departments of Medicine and Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

β-adrenergic stimulation of second messenger-mediated phosphorylation by catecholamines modulates several ionic current systems in mammalian heart. However, β-adrenergic modulation of the cardiac inwardly-rectifying K^+ channel (I_{K1}) has not been reported. Using the patch-clamp technique in the whole-cell voltage-clamp condition, we have found that (in the presence of 2 mM Co^{2+} or 0.1 mM 9-anthracenecarboxylic acid plus 1 μM nifedipine in the external solution) bath application of isoproterenol (ISO) can reversibly inhibit I_{K1} in a concentration-dependent fashion with half-maximal inhibition at 0.049 μM. This effect was mimicked by forskolin and dibutyryl cAMP. ISO-induced inhibition was prevented by inclusion of an inhibitor of cAMP-dependent protein kinase (PKA) in the internal solution, suggesting that inhibition of I_{K1} induced by ISO is mediated by PKA. In single channel recordings from cell-attached patches, ISO, forskolin and dibutyryl cAMP could completely suppress channel activity by decreasing the probability of burst appearance without affecting the open-close kinetics of channel events occurring within an individual burst. Channel activity recovered following washout of ISO from the bath. In the inside-out patch configuration, application of the purified catalytic subunit of PKA to the bath solution caused complete inhibition of channel activity in a manner similar to that observed in cell-attached recordings. We conclude that I_{K1} channels can be inhibited by PKA-mediated phosphorylation of the I_{K1} channel or an associated protein in response to stimulation of β-adrenergic receptors in guinea pig ventricular myocytes.

Th-Pos172

PROPERTIES OF MUSCARINIC K^+ CURRENT DESENSITIZATION. ((C.F. Lo and G.E. Breitwieser)) Dept. of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Desensitization of the muscarinic K^+ current ($I_{K[ACh]}$) was studied in isolated bullfrog atrial myocytes by whole cell patch clamp. Low concentrations of ACh produced a slowly activating steady state $I_{K[ACh]}$, whereas high [ACh] produced an instantaneous peak which decayed exponentially to a quasi-steady state. The K_d values for activation of steady state and peak currents were 157 and 813 nM respectively. Predesensitization of peak $I_{K[ACh]}$ could be produced by exposure of the cell to low [ACh] with a K_d of 68 nM. Recovery from desensitization was studied with 2 applications of ACh separated by a variable length washout period. The steady state current after a 1 minute ACh exposure required 3 minutes to fully recover, whereas the peak current did not show a significant decrease even at very short washout periods (< 30 seconds). To test whether phosphorylation is involved in desensitization of $I_{K[ACh]}$, we used pipet solutions containing 10 mM 5'-adenylylimidodiphosphate (AMP-PNP), which blocked isoproterenol-mediated Ca^{2+} channel activation but did not alter $I_{K[ACh]}$ desensitization induced by 1 μM ACh. Hence, phosphorylation is not responsible for desensitization of $I_{K[ACh]}$. Activation of peak $I_{K[ACh]}$ could, however, be blocked by 10 μM ETYA, a blocker of arachidonic acid metabolism. We propose that lipid metabolism is responsible for some of the properties of $I_{K[ACh]}$ desensitization. NIH HL41972.

Th-Pos173

CARDIAC MUSCARINIC K CHANNEL FUNCTION: MODULATION BY Mg-ATP IN THE PRESENCE AND ABSENCE OF ACETYLCHOLINE. (D. Kim) Chicago Medical School, North Chicago, IL 60061

Recent studies have shown that the atrial muscarinic-gated K channel activity ($i_{K,ACh}$) is modulated by both GTP and ATP. In the agonist-free or agonist-bound state, cytosolic GTP enhances $i_{K,ACh}$ by its effects on G_K, and ATP activates $i_{K,ACh}$ via nucleoside diphosphate (NDP) kinase in the absence of GTP (Heidbuchel et al., 1992). In our previous studies in neonatal rat atrial cells, it was found that Mg-ATP increased the open probability of $i_{K,ACh}$ by ~4-6 fold by prolonging the duration of the open state of the K channel. We studied further the effects of ATP on $i_{K,ACh}$ in adult rat and guinea pig atrial cells. In the agonist-free state, 1 mM ATP to the cytosol caused a slow activation of $i_{K,ACh}$; further addition of 0.1 mM GTP first increased, then decreased, and then slowly increased the K channel activity. The mean open time (τ_o) of $i_{K,ACh}$ was ~5 ms in all patches treated with ATP, compared to ~1 ms with GTP only. In the presence of ACh, GTP activated channels with a τ_o of ~1 ms; Mg-ATP (50 μM-5 mM), but not AMPPNP or Mg-free ATP, prolonged it to 4-6 ms, increasing the K channel activity several fold. The observed activation of $i_{K,ACh}$ by ATP can be explained by two separate effects of ATP on the K channel: (1) activation of G_K via NDP kinase and (2) activation by increasing the open probability of $i_{K,ACh}$ via phosphorylation.

Th-Pos174

ACTIVATION OF CARDIAC MUSCARINIC K+ CHANNELS BY TRANSDUCIN βγ SUBUNITS. ((Mitsuhiko Yamada[1], Yee-Kin Ho[3], Toshiaki Katada [4], and Yoshihisa Kurachi[1,2])) [1] Division of Cardiovascular Diseases, Departments of Internal Medicine and [2]Pharmacology, Mayo Clinic, Rochester, MN 55905, [3]Departments of Biochemistry and Ophthalmology, University of Illinois at Chicago, Chicago, IL 60612., [4]Tokyo Institute of Technology, Tokyo, Japan. (Spon. by R. Weinshilboum).

G protein βγ subunits ($G_{\beta\gamma}$) activate the cardiac muscarinic K+ channel (K_{ACh}). To gain a further insight into the molecular mechanism of this response, we examined the effect of transducin βγ subunits ($T_{\beta\gamma}$) on K_{ACh} in guinea pig atrial myocytes. K_{ACh} currents were measured by the patch clamp method in the I-O configuration. Preparation of hydrophilic $T_{\beta\gamma}$ did not contain detergent. $T_{\beta\gamma}$ (100 nM- 3 μM) applied to the internal side of the patch membrane promptly activated K_{ACh} in a concentration-dependent fashion with EC_{50} of ~1 μM, which was much higher than EC_{50} for the brain $G_{\beta\gamma}$ (5 nM). The effect of $T_{\beta\gamma}$ on K_{ACh} was rapidly reversed on washout, which is in contrast with the irreversible activation of the channels by brain $G_{\beta\gamma}$. $T_{\beta\gamma}$ (1 nM-1 μM) had no effect on K_{ACh} currents induced by GTPγS (10 μM) and brain $G_{\beta\gamma}$ (10 nM). $T_{\beta\gamma}$ did not affect the GTP-induced activation of K_{ACh} in the presence of ACh (0.5 μM), although it was previously reported that $T_{\beta\gamma}$ reversed this K_{ACh} activation as a proof of physiological $G_{K\alpha}$-activation of K_{ACh}. In conclusion, (1) G protein βγ subunits may activate K_{ACh} through a common pathway between retinal and nonretinal G protein βγ subunits. (2) Brain $G_{\beta\gamma}$ possesses a property, which $T_{\beta\gamma}$ lacks and is responsible for its increased affinity for K_{ACh}. (3) There is no evidence that $G_{K\alpha}$ activates K_{ACh} physiologically. Supported by NIH RO1 HL47360-01.

Th-Pos175

EXAMINATION OF POSSIBLE INTERACTION BETWEEN G PROTEIN α AND βγ SUBUNITS IN REGULATION OF CARDIAC MUSCARINIC K^+ CHANNEL. ((Mitsuhiko Yamada[1], Toshiaki Katada[3], Yoshihisa Kurachi[1,2])) [1]Division of Cardiovascular Diseases, Departments of Internal Medicine and [2]Pharmacology, Mayo Clinic, Rochester MN 55905, [3]Tokyo Institute of Technology, Tokyo, Japan.(Spon. by Win K. Shen).

G protein βγ subunits ($G_{\beta\gamma}$) enhance GTPγS-bound $G_{s\alpha}$ ($G_{s\alpha}$*)-stimulated type II and IV adenylyl cyclase activity, suggesting that G_α* and $G_{\beta\gamma}$ can synergically regulate certain effectors. We examined whether PTX-sensitive G_α* and $G_{\beta\gamma}$, purified from bovine brain can interact in regulating muscarinic K^+ channel (K_{ACh}) in guinea pig atrial cells. K_{ACh} currents in response to $G_{i\ or\ o\alpha}$*s and $G_{\beta\gamma}$ were measured under inside-out patch clamp configuration. $G_{\beta\gamma}$ was chromatographically subdivided into two fractions termed $G_{\beta\gamma I}$ and $G_{\beta\gamma II}$, which contained 5-kDa and 7-/6-kDa G_γ, respectively. G_βs in these fractions were the same ($G_{\beta 36}$ and $G_{\beta 35}$). $G_{\beta\gamma I}$ and $G_{\beta\gamma II}$, by themselves, activated K_{ACh} in a concentration-dependent fashion. $G_{\beta\gamma I}$ was slightly more potent than $G_{\beta\gamma II}$: EC_{50} of $G_{\beta\gamma I}$ and $G_{\beta\gamma II}$ was ~3 and ~8 nM, respectively. In both cases, the relationship between the concentration of the subunit and K_{ACh} activity fit the Hill equation with a Hill coefficient of ~3. Any of $G_{i\text{-}1\alpha}$*, $G_{i\text{-}2\alpha}$*, $G_{i\text{-}3\alpha}$*, and $G_{o\alpha}$* at 300 pM rarely, if at all, activated K_{ACh}. Pretreatment of the patches by G_α*s did not affect the properties of $G_{\beta\gamma}$-activation of K_{ACh}. Inversely, preapplication of $G_{\beta\gamma}$s to the patches did not affect the effects of $G_{i\ or\ o\alpha}$*s on K_{ACh}. These results indicate that there is no interaction between G_α* and $G_{\beta\gamma}$ in regulating K_{ACh}, and suggest that PTX-sensitive G proteins activate K_{ACh} mainly through their βγ subunits. Supported by NIH RO1 HL47360-01.

Th-Pos176

AMYLIN MODULATES EXCITATION-SECRETION COUPLING IN NORMAL, BUT NOT DIABETIC PANCREATIC BETA CELLS. ((P.K. Wagoner, C.Chen, and G.S. Oxford)) Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27599 and Glaxo Research Institute, Research Triangle Park, NC 27709.

The actions of amylin, a 37 amino acid peptide co-secreted from pancreatic β-cells with insulin, were studied in β-cells isolated from control (+/db) and diabetic (db/db) mice. Measurements of membrane potential and current were performed with the perforated patch technique, while insulin secretion was monitored using a sequential cell immunoblot assay and digital image analysis to resolve secretion from individual cells. Elevations in glucose (2 to 8mM) induced depolarization and phasic action potentials in +/db cells, while db/db cells exhibited depolarized resting potentials and spontaneous action potentials even in 2mM glucose. Under voltage clamp both cell types exhibited sulfonylurea sensitive K_{ATP} currents, but this component was larger in +/db cells and was reduced by 8mM glucose, whereas this small component in db/db cells was insensitive to glucose. Addition of amylin (0.1-500nM) repolarized and inhibited action potential activity of +/db cells (n=25/32) in 8mM glucose, whereas amylin failed to inhibit db/db cells (n=16). In voltage clamp amylin stimulated a glibenclamide-sensitive K current in +/db, but not db/db cells. Insulin secretion from single +/db cells was stimulated by glucose and significantly inhibited by amylin. Although basal (2mM glucose) insulin secretion was greater in db/db cells, amylin did not reduce secretion at any glucose concentration. Electrical and secretory assays were also performed on normal rat β-cells and the glucose-resistant RIN-38 cell line, which gave responses similar to +/db and db/db cells, respectively. We conclude that amylin interferes with the signal transduction system involved in glucose sensing and may play an important local negative feedback role in insulin secretion.

Th-Pos177

REGULATION OF Kx-CURRENT BY A DIFFUSIBLE SUBSTANCE IN ROD INNER SEGMENTS.

((D.E. Kurenny and S. Barnes)) Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Voltage-dependent non-inactivating potassium current (I_{Kx}) in rod inner segments of tiger salamander was studied using ruptured-patch and perforated-patch whole-cell techniques. The kinetic properties of I_{Kx} bear strong similarity to those of M-current, and a voltage-clamp paradigm, consisting of long hyperpolarising steps to different potentials from a holding potential of -30mV, was used. When studied with the ruptured-patch technique the activation curve shifted up to 50 mV in the negative direction and tail relaxations at -30 mV became up to 10 times faster after 10-50 min of dialysis in bright light. In the dark, both parameters were stable for up to 40 min until bright light was applied. When nystatin was used to permeabilise the patch, both parameters were stable for up to 60 min of recording, even in bright light. Superfusion of nitroprusside caused a reversible decrease of the tail current time constant but this was not associated with the negative shift of the activation curve. These results suggest that I_{Kx} is regulated by a diffusible, light-modulated intracellular substance, possibly cGMP.

Supported by Alberta Heritage Foundation for Medical Research and Medical Research Council of Canada.

Th-Pos178

CRITERIA FOR PERFORATED-PATCH RECORDINGS: ION CURRENTS VS DYE PERMEATION.

((L.C. Schlichter and I. Chung)) Playfair Neuroscience Unit, Toronto Western Hospital, Canada M5T 2S8.

The recent development by Horn and Marty (1988) of the perforated - patch recording (PPR) configuration has added a valuable new version of the patch-clamp technique. Many studies have now employed nystatin in the patch pipette in an attempt to keep the intracellular environment relatively undisturbed. Criteria that have been cited for distinguishing a PPR from a conventional whole-cell recording (WCR) include the slow, spontaneous development of a capacitance current whose magnitude is consistent with the cell's surface area and capacitance (Korn et al., 1991), the concomitant appearance of macroscopic currents, and differences in some property of these currents from those seen in conventional WCR. We have compared voltage-dependent K^+ currents in human T lymphocytes in WCR with PPR. Although there were pronounced differences in the kinetics, we discovered that PPR recordings were not "intact". In every case, coinciding with the gradual capacitance transient increase and decrease in access resistance, we observed large ($\geq$ 1,000 MW) fluorescent dyes enter the cell from the pipette. Similarly, in macroscopic fluorescence studies, nystatin caused dye loss from preloaded cells. These results suggest caution in studying the regulation of ion currents in PPR without another means of confirming that the nystatin-containing patch is intact.

Th-Pos179

THE CALCIUM-ACTIVATED CONDUCTANCE, ITO2, IN CANINE VENTRICLE IS A CHLORIDE CURRENT.

((Andrew C. Zygmunt)) Masonic Medical Research Lab., Utica, NY 13501. (Spon. by Joseph B. Patlak)

Previous studies by others have shown that two transient outward currents are present in canine myocytes. Tseng and Hoffman (1989) described a voltage-dependent potassium current, Ito1, that is blocked by 4-aminopyridine (4AP). A small, calcium-dependent transient outward current, Ito2, remained in the presence of 4AP. The aim of the present study was to determine the contribution of chloride, potassium, and nonspecific cation current to Ito2 in single canine ventricular myocytes dissociated from the epi- and mid-myocardium of adult dogs. Whole cell currents were measured under conditions that inhibited sodium current, Ito1, and sodium-calcium exchange current. In cells dialysed by low concentrations of egta, calcium-dependent outward tail currents were eliminated by lowering extracellular chloride, even though a large driving force for potassium existed in these cells. These data suggest that calcium-activated potassium and nonspecific cation currents do not contribute to Ito2. Ito2 is blocked by 10 mM caffeine, ryanodine, and anion transport blockers, suggesting its similarity to the calcium-activated chloride current, $I_{Cl(Ca)}$, reported by Zygmunt and Gibbons (1991, 1992) in rabbit atrial and ventricular myocytes.

Th-Pos180

HYPOSMOLARITY POTENTIATES CHLORIDE CURRENTS IN *XENOPUS* OOCYTES

((J.G. Chen, Y. Chen, S.A. Kempson and L. Yu)) Departments of Physiology & Biophysics and Medical & Molecular Genetics, Indiana University School of Medicine, IN 46202. (spon. C.S. Hui)

Xenopus oocytes experience hyposmotic shock following their ovulation into pond water. Mechanisms of osmoregulation in oocytes are largely unknown. To examine the effects of hyposmolarity on endogenous ion channels, oocytes were shifted from iso-(220mOsm) to hypo-(120mOsm) osmotic solutions and the membrane currents were recorded by two-electrode voltage clamp. The resting membrane conductance did not change significantly by hyposmotic action. But the Ca^{2+}-dependent, depolarization-activated Cl^- current was increased by twofold. This potentiation was not due to an enhanced entry of Ca^{2+} because the Ba^{2+} current was not increased in hyposmotic medium, suggesting a volume regulation of Ca^{2+}-dependent Cl^- channels. Hyposmotic medium also stimulated the hyperpolarization-activated Cl^- currents both of the transient and the stable type. Our results are consistent with the hypothesis that increases in mechanical stress in the cell cortex may stimulate Cl^- channels during oocyte cell division. Hyposmolarity potentiation of Ca^{2+}-dependent Cl^- currents may also be related to the fertilization-induced depolarization in *Xenopus* oocytes.

Th-Pos181

PHOSPHOLEMMAN, A UNIQUE CHLORIDE-SELECTIVE ION CHANNEL, EXHIBITS WIDESPREAD TISSUE DISTRIBUTION & DISCRETE SUBCELLULAR LOCALIZATION.

((Cathy J. Palmer and Larry R. Jones)) Krannert Institute of Cardiology, Dept. of Medicine, Indiana Univ. School of Medicine, Indpls, IN 46202.

Phospholemman (PLM), the major sarcolemmal substrate for cAMP-dependent protein kinase and protein kinase C in myocardium, is a 72 amino acid protein with a single transmembrane domain. Expression of PLM in *Xenopus* oocytes induces a hyperpolarization-activated chloride current, $I_{Cl(PLM)}$. Prior studies utilizing high stringency Northern blots probed with the canine cardiac full-length antisense mRNA indicated significant levels of cardiac PLM mRNA in all muscle types and in liver but only negligible amounts in kidney and brain. In the work described here, Western blot analyses were performed to further delineate the physical characteristics and the tissue and species distribution of PLM. Immunoblots of purified plasma membrane preparations from various tissues were probed with affinity-purified antibodies (Ab) to synthetic peptides corresponding to the N-terminus (residues 1-15) and C-terminus (residues 58-72) of canine cardiac PLM. Using C-terminal Ab, we detected high levels of PLM in heart, liver, and kidney plasma membranes. Interestingly, in liver and kidney, PLM was localized to basolateral membranes but not canalicular or brush border membranes, respectively. C-terminal Ab also detected PLM in membranes purified from brain. In contrast, when N-terminal Ab was used, tissue and species variability was noted in detecting PLM. Sequencing of a mouse cardiac PLM cDNA clone revealed several amino acid substitutions within the N-terminus of the protein, providing an explanation for species differences in reactivity of the N-terminal Ab. The existence of tissue isoforms is also suggested. Our results indicate that PLM has a widespread tissue and species distribution. Furthermore, in certain tissues such as liver and kidney, the protein is localized to discrete regions of the plasma membrane.

Th-Pos182

EFFECT OF TETRAMETHYLAMMONIUM ON THE ISOPROTERENOL ACTIVATED CHLORIDE CURRENT IN CARDIAC MYOCYTES

((S.I. Zakharov and R.D. Harvey)) Department of Physiology and Biophysics Case Western Reserve University, Cleveland, OH 44106 (Spon. by G.R. Dubyak)

Replacement of extracellular Na^+ (Na^+_o) with tetramethylammonium (TMA) reduces the magnitude of the autonomically regulated Cl^- current (I_{Cl}) in isolated guinea-pig ventricular myocytes. The mechanism underlying this effect was investigated using the whole cell configuration of the patch clamp technique. Following activation of I_{Cl} with 1 μM isoproterenol (ISO), subsequent exposure to ISO in Na^+-free solution significantly attenuates the magnitude of the Cl^- conductance. This suggests that regulation of I_{Cl} depends on the concentration of Na^+ in the extracellular solution. In order to characterize the Na^+ concentration dependence of this effect, cells were exposed to solutions in which Na^+_o was varied by equimolar replacement with TMA. However, maximal reduction of I_{Cl} was obtained with replacement of less than 30 mM Na^+, suggesting that the response to Na^+_o replacement was due to the addition of TMA rather than the removal of Na^+. This conclusion was supported by the observation that I_{Cl} could be inhibited by exposing cells to solution containing 30 mM TMA without reduction of Na^+_o. Furthermore, the effect 30 mM TMA was blocked by 1 μM atropine, indicating that the action of TMA had been due to activation of muscarinic receptors. However, atropine did not reverse the effect of complete replacement of Na^+_o with TMA, suggesting that in addition to activation of muscarinic receptors, there may also be a true Na^+ dependence of the ISO-activated current. This is supported by the observation that I_{Cl} was inhibited by Na^+-free solution containing TRIS, but addition of TRIS without reduction of Na^+_o had no effect.

Th-Pos183

BLOCK OF NEURONAL FAST CHLORIDE CHANNELS BY CELLULAR EXTRACTS. ((M.L. Phillips and A.L. Blatz)) Department of Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

The fast Cl channel from acutely dissociated rat cortical neurons was studied using the patch clamp technique. This channel exhibits a delayed activation following excision into the inside-out configuration at room temperature. This activation delay could be due to the release of an intracellular molecule that when bound, keeps the channel in an non-conducting configuration. We found that the supernatant fraction of a high salt solution of hypothalamus acetone powder (Sigma) blocks the fast Cl channel from the intracellular surface. Upon application of the hypothalamus extract, the channel displays a fast, flickery type block as well as a reduction in amplitude. The inhibitory molecule is not a protein, as boiling the extract does not abolish the blocking activity. Pepsin digestion also does not reverse the blocking activity, suggesting that the blocker is not a peptide. Several known channel blockers were added to the channel's intracellular surface. Both ATP and GTP blocked the channel at mM concentrations, while NAD blocked the channel in the μM range. Kunzelmann et. al. (1991) and Krick et. al. (1991) report that an epithelial Cl channel is also activated by patch excision. The epithelial channel is also inhibited by the addition of a cytosolic extract to the intracellular surface. Supported by NIH (GM-39731 & HL-07360).

Th-Pos184

PROTEIN KINASE A-ACTIVATED CHLORIDE CHANNEL IS INHIBITED BY Ca^{2+}/CALMODULIN COMPLEX IN CARDIAC SARCOPLASMIC RETICULUM. ((S. Kawano and M. Hiraoka)) Department of Cardiovascular Disease, Medical Research Institute, Tokyo Medical and Dental University. Tokyo, Japan

Cardiac sarcoplasmic reticulum (SR) has several chloride (Cl) channels, which may neutralize the charge across SR membrane generated by Ca^{2+} movement. We recently reported a novel 116 pS Cl^- channel (Ch_{cl}), which is activated by cytosolic protein kinase A in cardiac SR. Ch_{Cl} may serve as a novel target protein in the receptor-dependent regulation of cardiac excitation-contraction coupling. To understand further regulatory mechanisms, the effects of Ca^{2+} on Ch_{Cl} were studied using the planar lipid bilayer-vesicle fusion technique. Ca^{2+} (1 nM-1 mM) added to cis did not affect Ch_{Cl} activity. However, in the presence of calmodulin (CaM, 0.,1 μM/μg SR vesicles), Ca^{2+} (2 nM-1 mM) reduced the channel openings in a concentration-dependent fashion, while CaM alone did not affect the channel. One millimolar Ca^{2+} with CaM completely inhibited the channel activity. This inhibitory effect of Ca^{2+}/CaM was prevented by CaM inhibitors (W7 and calmidazolium) but not CaM kinase II inhibitor (KN62). These results suggest that Ca^{2+}/CaM itself, but not CaM kinase II, is involved in this channel inhibition. Thus, Ch_{Cl} is regulated not only by PKA-dependent phosphorylation but also by cytosolic Ca^{2+}/CaM complex. This is a novel second messenger-mediated regulation of Cl^- channel in cardiac SR membrane.

Th-Pos185

SEROTONIN MODULATES VOLTAGE-ACTIVATED CALCIUM CURRENTS IN *NECTURUS* TASTE RECEPTOR CELLS ((R. J. Delay, S.C. Kinnamon and S. D. Roper)) Dept. of Anatomy & Neurobiology, Colorado State University, Ft. Collins CO 80523 and the Rocky Mountain Taste & Smell Center, UCHSC Denver CO 80623

Taste buds in *Necturus* contain 50-100 taste receptor cells, many of which are innervated by afferent nerve fibers. In addition, Merkel-like cells in the base of the taste bud form chemical synapses with receptor cells and with nerve fibers. Recent studies have shown that Merkel-like basal cells contain serotonin (5-HT) and may release 5-HT in response to taste stimulation. Using whole-cell voltage clamp, we examined the responses of voltage-activated Ca currents (I_{Ca}) in isolated receptor cells to 5-HT. Two different effects of 5-HT were observed. Approximately 45% of receptor cells responded to 100 μM 5-HT with an increase in the peak I_{Ca} (mean increase = 160%). The increase was sustained during application of 5-HT. Serotonin increased Ca currents in responsive cells at concentrations as low as 1 μM. The increase in I_{Ca} was mimicked by 8cpt-cAMP (2 mM). In other cells (55%), 100 μM 5-HT caused a sustained but reversible decrease in the peak I_{Ca} (mean decrease = 40%). Addition of methysergide (0.1 μM) completely abolished both responses. Thus, the results suggest that serotonin may modulate taste receptor cell function, such as transmitter release during taste stimulation. Currently, experiments are underway to determine the subtype(s) of serotonin receptors involved in these responses and the intracellular second messenger pathway that mediate these effects.
Supported by NIH grants DC 00374 and DC 00244.

Th-Pos186

THE NEUROTROPHIC AGENT, ACETYL-L-CARNITINE ARGINYL AMIDE, INCREASES Ca^{2+} CHANNEL EXPRESSION IN PC12 CELLS. ((Yuan Bo Peng, Kirti Tewari, Spencer Dunn, J. Regino Perez-Polo, J. Marc Simard)) Univ of Texas Medical Branch Galveston TX 77550

Acetyl-L-carnitine arginyl amide (ALCAA) stimulates neurite outgrowth and choline acetyl transferase in PC12 cells (Taglialatela et al., Pharmacol Res 25:81, 1992). We examined the effect of ALCAA on expression of dihydropyridine-sensitive Ca^{2+} channels. PC12 cells were cultured for 6 days under 3 conditions: [1] control, [2] in the presence of nerve growth factor (NGF, 10 ng/ml), [3] in the presence of ALCAA (1 mM). Ca^{2+} channels were recorded in a cell-attached patch configuration using 40 mM Ba^{2+} as the charge carrier. The open-channel slope conductance (20-25 pS) was not affected by culture condition, but the probability of opening ($N \cdot P_o$) was significantly increased by ALCAA compared to control or NGF-treated cells: at E_m=-10 mV, $N \cdot P_o$ (mean±S.D.) was 0.049±0.042 (n=4), 0.067±0.041 (n=5) and 0.173±0.094 (n=7) for conditions [1], [2] and [3], respectively. The voltage dependence of $N \cdot P_o$ was examined by fitting pooled data obtained between -40 and +20 mV to a standard Boltzmann function. For all three conditions, the current was half-activated near 0 mV; the maximum value of $N \cdot P_o$ was similar for control and NGF-treated cells, but was larger by a factor of 2 for ALCAA-treated cells. Our data indicate that in PC12 cells, promotion of differentiation by ALCAA is accompanied by stimulation of Ca^{2+} channel expression.

Th-Pos187

THE ANTIGEN RECEPTOR OF T LYMPHOCYTES ACTIVATES CALCIUM INFLUX THROUGH DEPLETION OF INTRACELLULAR CALCIUM STORES. ((Adam Zweifach and Richard S. Lewis)) Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305. (Spon. by D. Friel)

Stimulated influx of extracellular Ca^{2+} is an essential triggering signal for the activation of T lymphocytes by antigen. There is currently debate over whether Ca^{2+} influx is activated by the direct action of inositol 1,4,5-trisphosphate (IP_3) or by a signal produced by the depletion of intracellular Ca^{2+} stores. To resolve this issue, we have used perforated-patch recording from the human Jurkat T-cell line to compare the properties of Ca^{2+} currents activated by phytohemagglutinin (PHA; 10 μg/ml), which cross links the T-cell receptor and evokes IP_3 generation, and thapsigargin (TG; 1 μM), a Ca^{2+}-ATPase inhibitor which depletes intracellular stores. PHA- and TG-stimulated currents show a number of similarities. Both currents are dependent on Ca^{2+}_o, are not voltage-gated, are blocked by 5 mM Ni^{2+}, and show slight inward rectification. Substitution of Ba^{2+} or Sr^{2+} for Ca^{2+}_o reduces the amplitude of both currents by 50%. From fluctuation analysis we estimate the unitary current amplitude at -80 mV to be ~1.5 fA for both PHA- and TG-stimulated currents in 2 mM Ca^{2+}, and ~3.6 fA in 110 mM Ca^{2+}. These results indicate that PHA and TG activate the same Ca^{2+} conductance. Because TG does so without elevating IP_3 levels (Gouy, H., et al., *Eur. J. Imm.*· 20: 2269-75 [1990]), the results further imply that Ca^{2+} influx during T-cell activation is initiated by the depletion of intracellular Ca^{2+} stores rather than by the direct action of IP_3 on Ca^{2+} channels in the plasma membrane. Supported by NIH postdoctoral fellowship AI08568 (AZ) and grant GM45374 (RSL).

Th-Pos188

REGULATION OF THE DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS IN SKELETAL MUSCLE BY THE CATALYTIC SUBUNIT OF PROTEIN PHOSPHATASE TYPE 1.
((Xiao-lan Zhao, Chan Fong Chang, Luis M. Gutierrez and M. Marlene Hosey)) Dept. of Pharmacology, Northwestern University Med. School, Chicago, IL 60611.

Dihydropyridine(DHP)-sensitive calcium channels from skeletal muscle are multi-subunit proteins. Evidence from biochemical and electrophysiological studies has shown that these channels are regulated by protein phosphorylation, but little is known about the dephosphorylation of the channels. Here we studied the regulation of these channels by the catalytic subunit of protein phosphatase type-1 (PP-1c), which is one of the major serine-threonine protein phosphatases in skeletal muscle. The results demonstrated that PP-1c can dephosphorylate calcium channels that previously were phosphorylated by protein kinase A (PKA) or protein kinase C (PKC). Both the α_1 and β subunits of the channels were substrates for PP-1c, but they were dephosphorylated to different extents. Functional studies using reconstituted channels showed that the dephosphorylation by PP-1c can reverse the activation of the channels by either PKA or PKC phosphorylation. Two-dimensional tryptic phosphopeptide maps suggested that PP-1c preferentially dephosphorylated certain phosphopeptides of α_1 subunit.

Th-Pos189

FURTHER EVIDENCE IN FAVOUR OF THE "SUPERFICIAL BUFFER BARRIER HYPOTHESIS" IN RABBIT CORONARY MYOCYTES ((N. Leblanc, and X. Wan)) Dept. Physiol., Univ. Manitoba, Winnipeg, Manitoba, CANADA R2H 2A6.

The purpose of these experiments was to test the hypothesis that a superficial pool of Ca^{2+} exists and controls the activity of membrane ion channels in rabbit coronary smooth muscle cells. Membrane current was recorded using the standard whole-cell variant of the patch clamp technique. In some experiments, $[Ca^{2+}]_i$ was simultaneously monitored using the salt form of Indo-1 (50 μM). Under control conditions, quasi steady-state I-V curves were generated using 5 sec voltage clamp depolarizing ramps from -80 to +60 mV (1 ramp/min). Membrane current was plotted against ramp voltage in control and after exposure to 1-10 μM Nifedipine (NIF). NIF reduced the amplitude of the outward current between ~ -20 and +60 mV; below -20 mV, NIF caused an outward displacement which was accompanied with a negative shift of the 0-current potential, indicative of cell hyperpolarization. The bell shape voltage-dependence of the NIF-sensitive outward current seen above -20 mV was almost a mirror image of the one predicted for Ca^{2+} channels ($I_{Ca,L}$). Below -20 mV, NF unmasked a steady-state component of $I_{Ca,L}$. Application of 1 μM BAY K 8644 enhanced outward ramp current from ~ -70 mV to +60 mV with a peak near 0 mV. These effects were inhibited by dialysing the cells with 5 mM EGTA or by bath application of 1 mM TEA. The dihydropyridines-induced changes in ramp current were abolished by preincubating the cells with 5 mM Caffeine. In contrast, varying $[Ca]_o$ revealed an outwardly rectifying K^+ current that lacked the bell shape voltage-dependence observed with the dihydropyridines. Simultaneous measurements of $[Ca^{2+}]_i$ and membrane current revealed a poor correlation between changes in "bulk" free $[Ca^{2+}]_i$ (Indo-1) and Ca^{2+}-activated K^+ current (K_{Ca}; localized $[Ca^{2+}]_i$). These results suggest that a subsarcolemmal pool of Ca^{2+} exists and regulates K_{Ca}. This restricted pool appears to tightly depend on the activity of resting L-type Ca^{2+} channels and SR and is consistent with the proposed existence of the "Superficial Buffer Barrier" in vascular smooth muscle. This may provide a very sensitive regulatory mechanism for controlling RMP, resting Ca^{2+} influx through Ca^{2+} channels, and coronary arterial tone.

Th-Pos190

EFFECTS OF PROTEIN PHOSPHATASE INHIBITORS ON MUSCARINIC INHIBITION OF CARDIAC CA^{2+} CHANNELS.
((S. Herzig and A. Meier)) Dept. of Pharmacology, University of Kiel (W-2300), Germany.

Muscarinic receptor activation inhibits the stimulation of cardiac L-type Ca^{2+} current exerted by ß-adrenergic agonists like isoproterenol (ISP). Besides a reduction in the rate of cAMP-dependent phosphorylation, modulation of protein phosphatase activity could also underly this response.

The effects of acetylcholine (ACH, 10^{-5} mol/l) upon the ISP-stimulated (10^{-8} mol/l) whole-cell Ca^{2+} currents (steps from -40 mV to +10-20 mV, 0.1 Hz) were studied in isolated guinea-pig ventricular myocytes. Compared to the inhibition seen in control cells (64+3 %, n=14), the ACH-effect was significantly reduced by extracellular fluoride ions ($3x10^{-3}$ mol/l, to 2.5+3.9%, n=5), or by okadaic acid ($9x10^{-6}$ mol/l, to 20+5 %, n=6) or trifluoperazine (10^{-5} mol/l, to 17+ 10 %, n=6) added to the recording pipette solution. Intracellular dialysis with calyculin A (10^{-6} mol/l) had no influence on the ACH-response (82+11 %, n=5), but the basal current was elevated and the ISP-effect was reduced by calyculin A, as with okadaic acid. Phosphatase inhibitor 2 (1000 U/ml, no effect on basal current or ISP-effect) added to the pipette solution did not significantly influence the ACH-response (52+12 %, n=15).

The activity of a protein phosphatase (possibly PP-2B) seems crucial for the ACH-induced inhibition of the cardiac Ca^{2+} current.

Th-Pos191

BRADYKININ-INDUCED $[Ca^{2+}]_i$ RESPONSE AND ION CHANNELS IN CORONARY VENULAR ENDOTHELIAL CELLS. ((J.B. Song, M.J. Davis)) Dept. of Medical Physiology, Texas A&M Univ. Health Science Center, College Station, TX 77843.

The vasodilator bradykinin (BK) stimulates biphasic $[Ca^{2+}]_i$ increases in endothelial cells. The transient phase of the Ca^{2+} increase is mainly due to intracellular release while the plateau phase is due to Ca^{2+} influx. Although an ion channel mechanism has been postulated to explain the Ca^{2+} influx pathway (JBC 264:12838,1990; Biochem J 284:521,1992; AJP 262:H942,1992), this issue has not been completely resolved. To test this hypothesis, we measured $[Ca^{2+}]_i$ using fura-2 microfluorimetry in single, voltage-clamped (or current-clamped) endothelial cells cultured from bovine coronary venules (CVEC). Perforated-patch pipettes were used to record membrane potential (E_m) or whole-cell current simultaneously with $[Ca^{2+}]_i$. In unstimulated cells, $[Ca^{2+}]_i$ varied inversely with holding potential. Under current-clamp, the resting potential (E_r) of the unstimulated cells was bimodally distributed (-70±9mV, n=26; -15±8mV, n=30). In all healthy cells, BK (10nM) application from a pipette evoked a biphasic $[Ca^{2+}]_i$ increase simultaneously with a change in E_m. When E_r was more negative than -50mV, an initial 41±4mV depolarization was observed. During the time when $[Ca^{2+}]_i$ was elevated, a transient repolarization paralleled the $[Ca^{2+}]_i$ increase in some cells, but most cells (12/15) subsequently depolarized. When E_r was more positive than -30mV, hyperpolarizations were typically observed. With E_m clamped at -70mV, a biphasic $[Ca^{2+}]_i$ increase was still elicited by BK and an inward current (up to 160pA), that could be blocked by either La^{3+} (0.2mM) or Ni^{2+} (2mM), was recorded. This inward current was present when the cells were bathed in Ca^{2+}-free or Na^+-free solutions, except that the duration of the response in Ca^{2+}-free bath was shortened. When holding potential was ramped from -120 to +60mV, the BK-induced current reversed at -3mV. An outward current recorded at positive holding potentials is likely to be a K_{Ca} current that is directly or indirectly activated by BK. The data suggest that in CVEC, BK activates a non-selective, receptor-gated channel which is permeable to Ca^{2+}. Supported by NIH HL-46502, AHA Established Investigator.

Th-Pos192

AGONIST-INDUCED CHANGES IN $[Ca^{2+}]_i$ AND MEMBRANE CURRENTS IN BOVINE AORTIC ENDOTHELIAL CELLS (BAEC). ((H.M. Himmel and H.C. Strauss)) Duke University, Durham, N.C. 27710

Major currents in BAEC are the inward rectifier K^+ current, I_{K1}, and the Ca^{2+}-activated K^+ current, $I_{K(Ca)}$. A nonselective cation current, I_{NS}, and a Ca^{2+}-activated Cl^- current, $I_{Cl(Ca)}$, have been proposed; however, their contribution to the current response in agonist-stimulated cells remains to be established. Single BAEC were loaded with K_5-Fura2 (50μM) via the patch pipette, and $[Ca^{2+}]_i$ (ratio 340/350 nm) was measured simultaneously with whole-cell currents (ramps -120 to +60 mV) at 22°C. Experiments with bradykinin (2μM) or ionomycin (100 nM) were done in physiological (PSS) or K^+-free (Cs^+) salt solution. Agonist exposure caused a rapid $[Ca^{2+}]_i$ increase, and a pronounced increase in outward and a small increase in inward current, which could be due to the activation of $I_{K(Ca)}$ and/or $I_{Cl(Ca)}$. In K^+-free solution, current changes were considerably smaller than in PSS; the reversal potential of -0 mV is consistent with the activation of I_{NS}. Chelation of intracellular Ca^{2+} with BAPTA attenuated both agonist-induced increase in $[Ca^{2+}]_i$ and current, while inhibition of cyclo-oxygenase (acetylsalicylic acid, ibuprofen) reduced mainly the current response. We conclude that $I_{K(Ca)}$, $I_{Cl(Ca)}$, and I_{NS} contribute to the agonist-induced whole-cell current response. Current activation depends on increased $[Ca^{2+}]_i$; however, products of the cyclooxygenase pathway may also play a modulatory role. Supported by Hi 475/1-1 (DFG) and HL-45132 (NIH).

Th-Pos193

DIFFERENTIAL EFFECTS OF PHOSPHOLIPIDS ON TRANSPORTER AND CHANNEL CURRENTS IN GIANT EXCISED PATCHES OF CARDIAC SARCOLEMMA. ((A. Collins and D.W. Hilgemann)) Dept. of Physiology, UT Southwestern, Dallas, TX 75235.

In giant excised cardiac sarcolemmal patches from ventricular myocytes, Na-Ca exchange (I_{NaCa}), Na pump, Na channel (I_{Na}) and K channel (I_{to}; rat ventricle) currents can be routinely measured. The influence of phospholipids on I_{NaCa} and I_{Na} (guinea-pig ventricle) has been studied by applying purified phospholipids in a tocopherol/hexane vehicle to the side of patch electrode tips during patch-clamp recording. In excised patches, both I_{NaCa} and I_{Na} showed time-dependent changes in their kinetics. Following activation by application of cytoplasmic Na, outward I_{NaCa} underwent a decay phase (inactivation) which usually became more prominent with time, while the voltage dependence of Na channel availability (h_{inf}) shifted in the negative direction. Phosphatidylserine (PS), applied to the tip of patch electrodes, stimulated I_{NaCa} by alleviating the inactivation phase, while phosphatidylcholine reversed this effect. Under conditions allowing measurement of both currents in the same patch, h_{inf} still shifted at a rate similar to control patches (-1 mV/min), despite stimulation of I_{NaCa} by PS. Thus, the secondary modulatory properties of I_{NaCa} are sensitive to the phospholipid environment, while those of I_{Na} are not.

Th-Pos194

DOES PROTEIN KINASE-C MODULATE MAGNESIUM BLOCKADE IN NMDA RECEPTOR CLONES?
((D.A. Wagner and J.P. Leonard) University of Illinois at Chicago, Chicago, Illinois 60607)

The N-methyl-D-aspartate (NMDA) receptors are a subclass of receptors for the excitatory neurotransmitter, glutamate, that are permeable to Ca^{2+} and demonstrate a characteristic voltage-dependent Mg^{2+} blockade. Modulation of the NMDA receptor may be involved in a wide range of processes including neuronal development, excitotoxicity, and associative forms of synaptic modification. We have shown that PKC activation enhances NMDA-activated currents in Xenopus oocytes injected with total rat brain mRNA. It has been shown in trigeminal neurons that the mechanism of this enhancement may involve a reduction of the Mg^{2+}-block (Chen&Huang,1992) However, we have observed the effect in oocytes using Mg^{2+}-free bathing medium. In order to resolve this discrepancy we expressed clones of the NMDA- NR1 receptor (rat) or the homologous ζ-1 (mouse) in oocytes. The Mg^{2+} dose-response curve was recorded before and after PKC activation by phorbol ester. We noted an increase (~2- fold) in NMDA currents but no significant shift in Mg^{2+}-K_i. This indicates that the PKC- induced enhancement is not mediated by a reduction of the Mg^{2+}-block in the NR1 or ζ-1 homomeric channel. We are now using clones of the NMDA epsilon subunits (ε-1, ε-2, ε-3) in order to determine whether PKC activation affects the Mg^{2+}- blockade in any of the heteromeric subtypes of the NMDA channel.

Th-Pos195

MODULATION OF NMDA RECEPTOR CHANNELS BY CALCIUM-CALMODULIN DEPENDENT PROTEIN KINASE II AND PHOSPHATASE INHIBITORS.

S. Sombati, Coulter, D.A., and DeLorenzo, R.J. Department of Neurology, Medical College of Virginia, Richmond, Va.

Glutamatergic neurotransmission in the CNS mediates an increase in intracellular Ca^{2+} of postsynaptic neurons by Ca^{2+} influx in part through activated NMDA receptor channels. Calcium-calmodulin protein kinase II (CaM kinase II) is activated by a rise in intracellular Ca^{2+}. Despite NMDA receptor and CaM kinase II involvement in processes such as learning and memory and excitotoxicity, not much is known about how CaM kinase II affects the NMDA receptor. We were interested in the possible role of CaM kinase II-mediated phosphorylation on NMDA receptor function.

"Perforated patch" whole-cell voltage clamp techniques (Amphotericin B) were employed to record NMDA currents from rat hippocampal neurons in culture. Pharmocological agents were applied to the neurons being recorded using a microperfusion system. NMDA (100-200 uM with 2 uM glycine) was applied for 10 seconds at 2 min intervals to prevent accumulated effects of receptor desensitization. The current did not exhibit "run down" after 1 hour of recording. Application of KN-62, a specific CaM kinase II inhibitor, resulted in a reduction of NMDA current amplitude. KN-62 had no effect on either kainate or GABA receptor currents. The inactive control drug KN-04 had no effect on NMDA current amplitude. Application of the phosphatase inhibitor okadaic acid resulted in an increase in NMDA current amplitude.

These results suggest that CaM kinase II-mediated phosphorylation may modulate NMDA receptor function. Supported by a NINDS Jacob Javits award (R01-NS23350) to RJD and the Sophie and Nathan Gumnick Neuroscience and Alzheimer Research Fund.

Th-Pos196

NMDA RECEPTOR MODULATION BY PROTEIN KINASE-C: PUTATIVE REGULATORY PHOSPHORYLATION SITES.

((S.M. Logan and J.P. Leonard)) University of Illinois at Chicago, Chicago IL 60607.

The NMDA receptor, a subtype of glutamate receptor, has been implicated in a wide variety of neuronal processes including synaptogenesis, neurotoxicity and LTP. Evidence now supports the hypothesis phosphorylation plays a role in modulating the NMDA response. Work in our lab has established that NMDA receptors expressed in oocytes from total rat brain mRNA are selectively enhanced 2.4 fold by the addition of the phorbol ester dibutyrate (PDBu), a PKC activator. Recently, we found a similar two fold enhancement by PDBu when NMDA clones isolated from rat (NR1) or mouse (ζ1) were expressed in oocytes. To test our hypothesis that PKC is acting directly on the receptor, we are using site directed mutagenesis to make conserved a.a. changes (Ser-Ala or Thr-Val) at putative PKC phosphorylation sites found in both NR1 and the highly homologous ζ1 clones. One of these sites located between the TM3 and TM4 domains of NR1 is a strong candidate site based on PKC artificial substrate sequence. However, in preliminary results, expression of a mutant constructed at this site gave currents that were still enhanced by PKC activation. Four other potential PKC sites are located in this cytoplasmic loop while one other is found between TM1 and TM2. Single, double and triple mutants located within the cytoplasmic loop have been constructed and are currently under investigation. Supported by NIH NS-26432.

Th-Pos197

DESENSITIZATION OF THE 5-HT_3 RECEPTOR IS ALTERED BY A SINGLE AMINO ACID SUBSTITUTION.

((J.L. Yakel, A. Lagrutta, J.P. Adelman, R.A North)) Vollum Institute, Oregon Health Sciences University, Portland, OR. 97201

The cloned 5-HT_3 receptor (Maricq et al., Science: 254, 432, 1991) was expressed in Xenopus oocytes to study the relation between primary structure and the kinetics of desensitization. 5-HT (300 nM to 100 μM) evoked an inward current at -60 mV, which became larger with hyperpolarization and reversed near 0 mV. The current decayed by 50% during the continual application of 5-HT (30 μM) in 47 ± 3 sec (mean ±s.e.m.; n=13). The site-directed mutant L286F, whereby the leucine at position 286 was replaced by phenylalanine, desensitized much faster (the current decayed by 50% in 1.2 ± 0.1 sec [n=9]), while the L286T mutant (a leucine-to-threonine substitution) desensitized much slower (the current decayed by 19 ± 4% [n=9] during a 1 min application of 5-HT). The concentration of 5-HT that produced a half-maximal current was not greatly altered by these mutations. The direction of these changes in desensitization kinetics is similar to that observed for the neuronal nicotinic receptor expressed in Xenopus oocytes (Revah et al., Nature: 353, 846, 1991), in which smaller, more polar amino acid substitutions at the equivalent position reduced the kinetics of desensitization.

Th-Pos198

PROPERTIES OF THE 5-HT_3R-A RECEPTOR EXPRESSED IN HEK 293 CELLS: EFFECTS OF SULFHYDRYL REDUCTION.

((Q. Zhou and D.M. Lovinger)) Dept. Mol. Physiol./Biophys., Vanderbilt Med. School, Nashville, TN 37232.

5-HT3R-A is a cloned subunit of the 5-HT_3 ligand-gated ion channel (Mariq et al., Science 254, p. 432, 1991). We have successfully transfected the 5-HT_3R-A clone into Hek 293 cells using Ca^{2+}-phosphate precipitation. Functional receptor expression is transient and shown by activation of current using the agonists serotonin (5-HT, 1-20μM), and Cl-Phenylbiguanide (5μM), and inhibition by the 5-HT_3 receptor antagonists MDL-72222 (100nM) and zacopride (200nM). The potency for 5-HT activation of current in transfected cells (EC_{50}=4.8μM) is close to its potency in NCB-20 cells (EC_{50}=6.0μM) from which the clone was originally derived. Furthermore, the activation of this receptor by 5-HT is inhibited by mM concentrations of Mg^{2+} and μM concentrations of Zn^{2+} in both Hek 293 and NCB-20 cells.

Inhibition of 5-HT activated current by the reducing agent dithiothreitol (DTT) has been observed in both transfected Hek 293 cells and NCB-20 cells. Application of 2mM DTT for 2 minutes reduced the peak current activated by 20μM 5-HT to 63.9±6.8% of control in NCB-20 cells and 45.4±8.1% in transfected Hek 293 cells. We also found a very potent inhibitory effect of Hg^{2+} ion which interacts with free sulfhydryl groups. Application of 2μM Hg^{2+} for 2 min reduced peak current to 48.7±8.3% of control in NCB-20 cells and to 44.3±21% of control in Hek 293 cells. DTT did not alter the potency with which 5-HT activated the receptor. These effects of DTT and Hg^{2+} might result from actions on the N-terminal Cys-Cys loop (disulfide bond) which interfere with activation of the 5-HT_3 receptor/channel. This work was supported in part by the Alcoholic Beverage Medical Research Foundation.

Th-Pos199

cAMP MODULATES THE ACTIVITY OF SINGLE ION CHANNELS IN HUMAN PLATELETS.

((V. Bolotina*#, V.Ilyin*, S.R. Alonso and A. Sanchez)) Valladolid University, Valladolid, Spain, *Cardiology Research Center, Moscow, Russia and #Boston University Medical School, Boston, USA.

Single ion channels were investigated in freshly isolated human platelets using the patch-clamp technique. In inside-out membrane patches single channels with 18 pS conductance were found in symmetrical NaCl (140mM) with ADP in the pipette. The current-voltage relation was linear from -80 to +80 mV. In some intact platelets inward single channel currents of 18 pS were also recorded in cell-attached configuration with either NaCl (140mM) or $BaCl_2$ (90mM) in the pipette. Application of forskolin (50μM) and IBMX (50μM) led to complete block of channels activity. It also inhibited ADP-evoked Ba^{2+} influx in platelets as shown with FURA-2 fluorescence measurements. Such treatment is known to elevate significantly the intracellular level of cAMP. To check whether cAMP can directly modulate channel activity we applied 200μ M cAMP from the inside of isolated membrane patches with 1-4 active channels. In all cases after about 3 min most of the channels closed completely while the activity of the remaining fell dramatically. Protein-kinase A and Mg-ATP were not required for this effect. Ion channel, described in this work, can be responsible for mono- and divalent cation influx in the platelets during their activation with ADP. cAMP can directly modulate the activity of this channel being one of the second messengers controlling cation influx.

Th-Pos200

IONIC BASES OF THE MEMBRANE POTENTIAL CHANGES INDUCED BY SPERACT IN SEA URCHIN SPERM.

((*E.Reynaud, +L. de De la Torre, *A.Liévano and *+A.Darszon)) *Biochemistry Dept., Instituto de Biotecnología, UNAM, Apdo.Postal 510-3, Cuernavaca, MO, México; +Centro de Investigación y de Estudios Avanzados del IPN, México, D.F.

Speract is a peptide found in the outer layer of *Strongylocentrotus purpuratus* sea urchin eggs. In sperm this peptide activates respiration, induces a K^+ dependent hyperpolaryzation, and increases cyclic nucleotides, intracellular pH (pHi) and Ca^{2+} (Ca^{2+}_i) (Garbers, Ann. Rev. Biochem. 58, 719). Although not shown yet, speract could be a chimoatractant. Sea urchin sperm can be swollen and patch clamped retaining their responses to speract. The initial hyperpolarization is K^+ dependent and mediated by K^+ channels (Babcock *et al*, Proc. Natl. Acad. Sci. USA 89:6001). Here we report that a valinomycin induced hyperpolarization activates an increase in pHi. Ionic substitution experiments indicate that the repolarization triggered by nM speract depends on external Na^+, suggesting Na^+ influx through an unidentified channel. The repolarization is required for the speract induced change in pHi and the opening by nM speract of Zn^{2+} sensitive Ca^{2+} channels. This work was partially supported by CONACYT, WHO, DGAPA, Miguel Alemán Foundation; A. Darszon is an international scholar of the Howard Hughes Medical Institute.

Th-Pos201

IMPROVEMENTS IN THE TWO-ELECTRODE VOLTAGE CLAMP TECHNIQUE OF *XENOPUS LAEVIS* OOCYTES FOR MODULATION STUDIES.

((W. Schreibmayer*⊕, N. Dascal*⊕, N. Davidson*, H. Lester*)) *: California Institute of Technology, Division of Biology, Pasadena, CA 91125; ⊕: permanent address: Institute for Medical Physics and Biophysics, University of Graz, Harrachgasse 21/4, A-8010 Graz, AUSTRIA; ⊕: permanent address: Department of Physiology and Pharmacology, University of Tel Aviv, Ramat Aviv 69978, ISRAEL.

Heterologous expression of ion channels, coexpression of modulatory proteins and application of electrophysiological techniques in *Xenopus laevis* oocytes has become routine in the electrophysiology laboratory. Inherent properties of the oocyte system and expressed channels i.e., input capacitance of > 200 nF and membrane currents of up to tens of μA in the ms range, require low resistance micro electrodes (< 1 MΩ), to achieve good membrane potential control during a voltage clamp experiment. KCl leaking out of such electrodes, however, leads to a decrease in input resistance of the oocyte and to damage of the preparation after longer exposure, complicating modulation studies. Here we report the fabrication of glass micro electrodes with tip diameters 2 - 30 μm. To prevent KCl leakage, the tips are back-filled with a cushion of 1% agarose in 3 M KCl. Resistance of these electrodes (*'Agar cushion electrodes (ACE)'*) ranges between 50 kΩ and 800 kΩ, depending on tip diameter and geometry. Typical measured input resistance of oocytes ranged between 1- 4 MΩ and was usually stable for up to 2 hours after ACE insertion. Furthermore, we describe a simple electronic circuit (*'Charging Compensator(CC)'*) that speeds up voltage clamp considerably, allowing optimal utilization of ACE's with conventional amplifiers. By using ACE's in connection with CC, the capacitance of *Xenopus laevis* oocytes can be charged with a time constant < 300 μs by rectangular voltage pulses. Speed of voltage clamp and stability of input resistance of the preparation make the ACE/CC system the system of choice for channel modulation studies requiring intact oocytes.

Supported by the following grants: Austrian Research Foundation FWF/S4504B (WS), Muscular Dystrophy Association (ND) , NIH (HL, ND) and the USA-Israel Binational Science Foundation (ND, HL).

CARDIAC POTASSIUM CHANNELS

Th-Pos202

CHARACTERIZATION OF SINGLE-CHANNEL PROPERTIES OF A CLONED CARDIAC TRANSIENT OUTWARD TYPE K^+ CHANNEL (RHK1). ((M. Wakamori and A. Yatani)) Department of Pharmacology & Cell Biophysics, University of Cincinnati, Cincinnati, OH 45267

The transient outward K^+ current (I_{to}) is an important modulator of repolarization of the cardiac action potential. Little information is available on single channel properties of I_{to}, due to the low density of channels and the presence of overlapping voltage-gated K^+ channels in native cells. To characterize the channel properties, a K^+ channel cloned from rat heart (RHK1) was expressed in Xenopus oocytes and single channel currents were recorded from cell-attached patches. Single channel currents were activated by depolarizing pulses from negative holding potentials (<-80 mV). Channel openings appeared at the beginning of each pulse and the ensemble currents at +60 mV produced activation (peaked at ~20 ms) and inactivation (tau of ~60 ms). The I-V relationship of the channel (between +20 and +80 mV with K_o=5.4 mM) was linear with a slope conductance of 10 pS. Open time histograms fit to a single exponential function (τ_o=2.5 ± 0.3 ms) and closed time histograms fit to double exponential (τ_{c1}=0.7 ± 0.1 ms and τ_{c2}=17 ± 0.2 ms). Both 4-amino pyridine and quinidine, reduced single channel open time in a concentration-dependent manner, while single channel amplitude, first latency and closed time (τ_{c1}) were not affected, indicating open channel block. The high density of the channel and the stable activity make this a useful system for studying the pharmacology of the cardiac K^+ channel at the molecular level.

Th-Pos203

THREE TYPES OF DELAYED RECTIFIER POTASSIUM CURRENTS IN HUMAN ATRIAL CARDIOMYOCYTES.

((S. Nattel, Z. Wang, B. Fermini)) Montreal Heart Institute, Montreal, Quebec, Canada H1T 1C8.

In previous voltage clamp studies of human atrial myocytes, a large transient outward current (I_{to}) was noted, and the delayed rectifier current (I_K) was felt to be absent or very small. To examine the potential role of I_K at physiologic temperatures, myocytes isolated from human right atrial appendages were studied with the whole-cell patch clamp technique. Of 126 cells studied at 36°C, 90 (71%) possessed a slowly developing outward current upon depolarization from -70 to +20 mV, and a tail current upon repolarizing to the holding potential. The time constant for current activation averaged 348±61 (M±SE) ms at -20 mV, and decreased at more positive potentials to 129±25 ms at +60 mV; while I_{ss}, at the end of a 2-sec pulse increased from 65±4 pA to 331±26 pA over the same range. E-4031 (5 μM) separated I_K into two components: a rapid, E-4031 sensitive component (I_{Kr}) with an activation τ at +20 mV of 92±9 ms, strong inward rectification, and more negative $V_{½}$; and a slower, E-4031 resistant component (I_{Ks}) with a τ of 525±45 ms, a smooth I-V relation, and more positive activation potential. TEA (10 mM) strongly inhibited I_K (by 76±4%, $p<0.001$) without affecting I_{to}, and increased APD_{90} by 53±14% ($p<0.01$). A third type of delayed rectifier current was demonstrated at 25°C, when depolarizing test pulses were preceded by conditioning pulses to inactivate I_{to}. This current activated with τ decreasing from 18 ms at 0 mV to 2 ms at +60 mV. This "ultra-rapid I_K" (I_{Kur}) was unaffected by TEA (to 100 mM), and highly sensitive to 4-AP (IC_{50} of 50 μM). Selective block of I_{Kur} with 50 μM 4-AP increased APD_{90} by 66±11% ($p<0.01$). Conclusion: 1) Human atrial myocytes contain 3 kinetically - and pharmacologically - distinct delayed rectifier K^+ currents; 2) delayed rectifier currents appear to play an important role in human atrial repolarization.

Th-Pos204

DEVELOPMENTAL DIFFERENCES IN THE TRANSIENT OUTWARD CURRENTS IN CANINE CARDIAC PURKINJE CELLS

((K.W. Hewett, P.S. Baek, and P.C. Gillette)) Medical University of South Carolina, Charleston, SC 29425

Standard whole-cell voltage clamp techniques were used to measure the transient outward currents of the Purkinje cells isolated from the hearts of dogs less than two weeks of age and from adults. A 4-aminopyridine sensitive current, I_{to1} and a 4-aminopyridine insensitive current, I_{to2} have been previously described in myocardial cells. Currents were normalized for cell size by dividing the current by the cell capacitance. The adult cells were found to have a smaller combined current (I_{to1} + I_{to2}) of 4.06 pA/pF (n=6) as compared with an average of 9.7 pA/pF (n=8) in the neonate. The threshold for activation of the combined current in the steady-state current-voltage relationship was more positive (-3.89 mV) in the neonatal cells as compared to a threshold of -19.7 mV in the adult. The mid-point ($V_{0.5}$) of the steady-state inactivation curves of the combined currents were similar between the age groups, -26 mV in the neonate and -22.5 mV in the adult. Forty-one percent of the total current in the neonate was found to be I_{to2} as compared to 20% in the adult. The phosphodiesterase inhibitor IBMX augmented I_{to2} suggesting that the current is modulated by intracellular calcium. I_{to2} was not affected by eliminating extracellular chloride or by chloride current blockers indicating that in canine cardiac Purkinje cells I_{to2} is not carried by chloride ions as has been shown in myocardial cells.

Th-Pos205

DIRECT EFFECTS OF H_2O_2 ON POTASSIUM CURRENTS IN FELINE VENTRICULAR MYOCYTES. ((Peggy L. Barrington)) Department of Pharmacology, Northwestern University, Chicago, IL 60611.

H_2O_2 can cause the amplitude of the plateau phase of the action potential to increase when intracellular Ca^{2+} is chelated with EGTA using patch-electrode techniques. The large increase in action potential duration and the afterdepolarizations recorded with conventional high-resistance microelectrodes are not seen. The potassium currents, I_{to}, I_{K1} and I_K, were examined using whole-cell voltage-clamp techniques to determine if H_2O_2 altered these currents in the absence of increased intracellular Ca^{2+}. Currents were recorded from enzymatically isolated feline myocytes using patch electrodes (0.8-1.5 MΩ) containing a standard internal solution with 5 mM EGTA and 5 mM ATP. Internal solution was allowed to equilibrate with the cytosol for 10-20 minutes after which a voltage clamp protocol using a holding potential of -40 mV and 10 mV steps was used to determine a current-voltage relationship. Subsequently, 100 μM H_2O_2 was added to the perfusion solution and the same voltage protocol was repeated at 5 minute intervals. The stability of each current over time was monitored in a separate series of cells not exposed to H_2O_2. Peak I_{to}, recorded in the presence of 0.5 mM Cd^{2+}, was very stable in control cells but gradually decreased in cells exposed to H_2O_2 for 30 minutes. The decay phase of I_{to} was fit with a single exponential and did not change over time nor during exposure to H_2O_2. I_{K1} and I_K were also stable over time and unaffected by the presence of H_2O_2. Thus, H_2O_2 appears to have a selective effect on only peak I_{to} while I_{K1} and I_K remain unaltered in the absence of changes in intracellular Ca^{2+}. The results suggest that changes in I_{to} contribute to the increased action potential plateau amplitude during exposure to H_2O_2.

Th-Pos206

TRANSIENT OUTWARD CURRENT INHIBITED BY ACTIVATION OF PROTEIN KINASE C BUT NOT BY A IN FELINE VENTRICULAR MYOCYTES

((Ke Zhang and R. E. Ten Eick))Northwestern University, Chicago, IL 60611

Transient outward current (I_{to}) flows briefly during the early phase (#1) of repolarization of cardiac action potentials. When feline ventricular myocytes are patched with a conventional internal solution containing 2-5 mM ATP using whole-cell-patch-clamp technique, I_{to} is robust (i.e., it neither runs down nor up). However, exposure of myocytes to phorbol 12-myristate 13-acetate (PMA) inhibited I_{to} in a dose dependent manner; the PMA-induced inhibitory effects could be prevented by intracellular dialysis with the protein kinase C (PK-C) inhibitor, staurosporine, suggesting that PMA-induced inhibition of I_{to} is mediated by the activation of PK-C. The time courses of the decay phases of I_{to} before and during exposure to PMA were identical, implying that the kinetic properties of I_{to} were not altered by activating PK-C. Activation of protein kinase A (PK-A) with either isoproterenol (ISO) or forskolin, however, had no effect on I_{to}. These findings suggest that I_{to} channels can be down-regulated by phosphorylating them via PK-C; that cAMP and PK-A do not play any role in I_{to} regulation. Exposure to the α-agonist, phenylephrine, also could decrease I_{to} and mimics the effects on I_{to} of activating PK-C, indicating that α-receptors also are involved with regulating I_{to} channels. Lack of an effect by ISO is consistent with the finding that cAMP and PK-A are not involved with I_{to} regulation. The modulation of I_{to} via PK-C may partially underlie changes in cardiac electrical activity during activation of the sympathetic nervous system.

Th-Pos207

EFFECTS OF Ca ANTAGONISTS AND ANTIARRYTHMIC AGENTS ON TRANSIENT AND DELAYED RECTIFIER POTASSIUM CURRENTS IN RABBIT ATRIAL CELLS.

((Y. Imaizumi, A. Kanai, K. Matsusita, M. Watanabe and W.R. Giles)) Dept. of Chemical Pharmacol., Nagoya City University, Nagoya, Japan and Dept. Medical Physiology, University of Calgary, Alberta, Canada T2N 4N1

Both Ca-independent transient K current (I_t) and delayed rectifier K current (I_K) in mammalian heart can be blocked by Ca antagonists and antiarrythmic agents. The specificity of some of these drugs was examined in the rabbit atrial cells at 35°C following block of both the sodium current, I_{Na} and the L-type Ca^{2+} current I_{Ca}. I_t was the major time- and voltage-dependent outward current when rabbit atrial myocytes were depolarized from holding potential of -80 to +20 mV for 100 msec in the frequency-range of 0.1 and 3 Hz. The amplitude of I_t depended strongly upon stimulation frequency; I_t was relatively small at 3 Hz (-200pA at the peak). I_K was studied after I_t was suppressed by using a 3 Hz stimulation rate and by application of 4 mM 4-aminopyridine (4-AP). I_K turned on slowly at +20 mV (T1/2= ~200 msec) and was less than 200 pA at the end of a 1 sec depolarization. When both I_t and I_K were activated by 100 msec depolarizations to +20 mV, 10 μM quinidine or 1 μM nicardipine blocked I_t much more strongly than I_K. Significant effects of these two drugs on I_K could be detected only after the transient outward K^+ current was blocked by inactivating it with relatively positive holding potentials in the presence of 4mM 4-AP. [Supported by Monbushyo International Society for Collaborative Research Program (Japan)]

Th-Pos208

DIFFERENTIAL INHIBITION OF K⁺ CURRENTS IN RAT VENTRICULAR MYOCYTES BY CLASS I ANTIARRHYTHMIC AGENTS.

((M. T. Slawsky and N. A. Castle)). *Cardiology Section,V.A. Medical Center; Anesthesia Research Labs, Brigham and Women's Hospital., Boston, MA*

The present study examined the effects of quinidine (Q), flecainide (F) and propafenone (P) on K^+ currents in isolated adult rat ventricular myocytes using whole cell patch-clamp. Outward currents activated by depolarization were comprised of an inactivating component (I_{TO}) and maintained component (I_K). Q, F and P produced both a reduction in peak current amplitude and an increase in the rate of inactivation of I_{TO}. The concentration-dependence of block yielded an apparent K_D of 3.9 μM, 3.7 μM and 3.3 μM for Q, F and P respectively. Q, F and P (10 μM) produced only minor hyperpolarizing shifts (≤ 3 mV) in the voltage-dependence of steady-state inactivation. F and P (10 μM) did not affect the rate of recovery from inactivation (τ~ 80 ms); however, in the presence of 10 μM Q, recovery exhibited an additional slow component (τ_{fast} = 55 ± 5 ms; τ_{slow} = 870 ± 80 ms, n=3). The relative magnitude of the slow component of recovery increased with Q concentration (0.36 ± 0.04 at 10 μM and 0.43 ± 0.02 at 30 μM). Q, F and P also inhibited I_K albeit with a lower potency than for I_{TO}. Apparent K_Ds were 18 μM, 38 μM and 7 μM for Q, F and P, respectively. In contrast to their effects on outward currents, Q, F and P produced little or no inhibition of the inward rectifier K^+ current even when present at 100 μM. The results show that at clinically relevant concentrations, Q, F and P produce similar degrees of block of I_{TO}. However, these agents exhibited different blocking affinities for I_K. The different sensitivities of K^+ currents to block by these agents in conjunction with regional variations in distribution of K^+ channels may result in regional changes in action potential propagation in the heart.

Th-Pos209

ALTERATIONS IN THE TRANSIENT OUTWARD POTASSIUM CURRENT IN CHAGAS' DISEASE.

((L.M. Pacioretty, S.C. Barr, and R.F. Gilmour, Jr.)) Departments of Physiology and Clinical Sciences, NYSCVM, Cornell University, Ithaca, NY 14853.

Chagas' disease is a prominent cause of heart failure in Latin America, resulting in an estimated 50,000 deaths per year. The disease is a result of infection by the parasite *Trypanosoma cruzi (T. cruzi)*. Electrocardiograms from Chagasic patients may show increased P-R interval, decreased QRS amplitude, and T wave changes. To investigate whether electrical abnormalities exist on the cellular level, whole cell voltage clamp recordings of the 4-aminopyridine sensitive component of the transient outward current (I_{to}) were obtained from canine epicardial myocytes following 21-25 days of infection with *T. cruzi*, and from age matched controls (22°C, 200 μM Cd^{2+}, 100 μM TTX). Data are expressed as mean±SEM; differences are significant at $p<0.05$ level. Peak I_{to} at +40 mV was decreased in Chagasic cells compared to control cells, 508.6±61.4 vs 1347.7±75.3 pA. Cell capacitance also was decreased in Chagasic cells compared to control cells, 95.8±9.9 vs 131.4±9.6 pF. When normalized for cell capacitance, the current densities were 5.6±0.7 and 10.5±0.7 pA/pF, for Chagasic and control cells, respectively. No differences were seen in the time constants of current decay (26.0±4.9 and 24.8±1.7 ms for Chagasic and control cells, respectively) nor in the steady-state inactivation parameters ($V_{1/2}$ = -38.9±2.5 and -32.3±1.8 mV and k=4.5±0.6, and 4.7±0.4 for Chagasic and control cells, respectively). The reduction in I_{to} was associated with an increase in phase 1 amplitude of the epicardial action potential. The existence of parasites within the cell may directly influence the channel protein or may alter cellular processes resulting in a secondary depression of I_{to}.

Th-Pos210

Open and closed state channel block of transient outward current in rat ventricular myocytes

Peter Klemm, Ulrich Jahnel, and Hermann Nawrath
From the Department of Pharmacology, University of Mainz,
Federal Republic of Germany

In various heart tissues, a transient outward current (I_{to}) is activated by depolarizing voltage clamp steps from -50 mV up to +40 mV. At +40 mV, I_{to} peaks after about 3 ms in ventricular myocytes from rat hearts; the time course of inactivation can be appropriately described by two time constants, τ_{fast} = 14 ms and τ_{slow} = 220 ms. Verapamil, nifedipine and quinidine in concentrations between 1 and 30 μmol/l accelerated the inactivation of I_{to}, whereas peak I_{to} was less affected. In contrast, 4-aminopyridine (4-AP) 1 mmol/l preferentially depressed peak I_{to}, whereas the time course of inactivation was rather slowed down. The efficacy of 4-AP was diminished at short and enhanced at long pulse intervals (reverse use-dependence). The time course of drug action at +40 mV was calculated by the fractional changes of I_{to}. Quinidine, nifedipine and verapamil produced a concentration-dependent block of I_{to} increasing during the depolarizing voltage clamp step. Conversely, the block by 4-AP of I_{to} decreased during the step. We conclude from our experiments that verapamil, nifedipine and quinidine bind to the I_{to} channel in the open state at positive potentials. In contrast, 4-AP obviously binds to the channel in the closed state at negative potentials and relief of block is observed at positive potentials.

Supported by the Deutsche Forschungsgemeinschaft

Th-Pos211

VOLTAGE-DEPENDENCE OF CARDIAC DELAYED RECTIFIER BLOCK BY CLASS III ANTIARRHYTHMIC DRUGS.

((D.S. Krafte and W.A. Volberg)) Dept. of Cardiovascular Pharmacology, Sterling Winthrop Pharmaceuticals Research Div., Rensselaer, NY 12144.

To investigate cardiac delayed rectifier current block by sotalol, semotilide, E-4031 and dofetilide, we have performed a series of voltage-clamp experiments in isolated guinea pig ventricular myocytes. These four drugs share a common methanesulfonamide group and are selective blockers of the rapidly activating component of the delayed rectifier current, I_{Kr}. This type of K channel blocker has been characterized by a phenomenon termed reverse-frequency dependence (i.e. smaller absolute changes in action potential duration and refractoriness as stimulation frequency is increased). To determine whether this phenomenon is attributable to the voltage-dependence of channel block we have compared the relative potency of I_{Kr} block over a 50 mV range. Our methodology consisted of collagenase dispersion of guinea pig ventricular myocytes and whole-cell patch clamp analysis of membrane currents. The recording chamber was perfused with (in mM): NaCl 145, KCl 4.5, $MgCl_2$ 1, $CaCl_2$ 0.1, $NiCl_2$ 2, HEPES 10, glucose 12, pH 7.5. Recording pipettes contained (in mM): NaCl 10, KCl 112, EGTA 10, HEPES 10, K-ATP 5, MgATP 5, pH 7.4. Membrane currents were recorded from holding potential of -40 mV at 33 -35 and cells were utilized with 10 hrs of isolation. IC_{50} values (μM) obtained for I_{Kr} block are illustrated in the table below.

	test potential (mV)						
	0	10	20	30	40	50	60
sotalol	195	105	68	91	118	112	79
semotilide	20	20	16	11	11	12	9
E-4031		.042	.029	.025	.025	.024	.020
dofetilide	.058	.048	.042	.034	.030	.027	.029

From the above data one can conclude that the voltage-dependence of I_{Kr} block can not be the cause of the phenomenon of reverse-frequency dependence. The data indicate sotalol, semotilide, E-4031 and dofetilide all block I_{Kr} more potently as membrane potential is made more positive. Other mechanisms, such as a greater contribution of I_{Kr} to total membrane current, might be responsible for the reverse-frequency dependence associated with sulfonamide-type potassium channel blockers.

Th-Pos212
MECHANISM OF β-STIMULATION OF $I_{K(ATP)}$ IN CAT CARDIOCYTES
((T.E. Schackow and R.E. Ten Eick))Northwestern University, Chicago, IL 60611

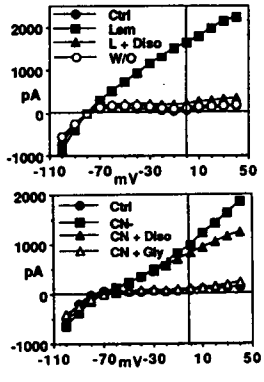
ATP-sensitive K current ($I_{K(ATP)}$) can be stimulated in heart by adenosine and β-agonists. Although adenosine modulates the channel through a direct G-protein interaction, it is unclear how the β-response is mediated. To investigate the mechanism, whole-cell ionic currents were recorded from adult cat cardiac ventricular myocytes using a conventional whole-cell-patch technique. A glyburide-sensitive (10 μM) $I_{K(ATP)}$ was allowed to develop slowly (~20 min) in cells perfused with an ATP-free pipette (intracellular) solution. Externally applied isoproterenol (ISO, 1 μM) caused a rapidly developing (< 60 s), sustained enhancement (~100-300 % increase) of $I_{K(ATP)}$ that could be blocked by either pretreatment with cholera toxin (10 μg/ml, >18 h) or internal application of GDP-β-S (5 mM). $I_{K(ATP)}$ remained elevated even after washout of ISO, and subsequent applications of the β-agonist did not cause further increases in $I_{K(ATP)}$. Neither pretreatment with pertussis toxin (2 μg/ml, >18 h) nor internal application of a protein kinase A inhibitor (PKI_{5-24}, 22.5 μM) prevented the ISO response, whereas internally applied GTP-γ-S (100 μM) or F^- (20 mM) caused $I_{K(ATP)}$ to rapidly increase in the absence of β-agonist. Application of forskolin (10 μM) elicited a more slowly developing increase in $I_{K(ATP)}$, but externally applied 8-(4-chlorophenylthio)-cAMP (200 μM) did not cause $I_{K(ATP)}$ to increase. Internal application of the adenylate cyclase inhibitor 2'-deoxyadenosine-3'-monophosphate (100 μM) caused a 70% inhibition in the ISO-induced $I_{K(ATP)}$. We conclude that β-receptor activation can stimulate $I_{K(ATP)}$ via a G_s- and adenylate cyclase-dependent, cAMP- and protein kinase A-independent pathway. Therefore, β-stimulation may act through G_s to stimulate adenylate cyclase which in turn causes sufficient further depletion of intracellular ATP to elicit the increase in $I_{K(ATP)}$. TE Schackow is a Howard Hughes Medical Institute Predoctoral Fellow.

Th-Pos213
CARDIAC ATP-SENSITIVE K⁺ CHANNEL MODIFICATION BY TRYPSIN.
((N. Deutsch, J.N. Weiss)) UCLA School of Medicine, Los Angeles, CA 90024.

We used inside-out membrane patches excised from isolated guinea-pig ventricular myocytes to study the sensitivity of ATP-sensitive K^+ (K.ATP) channels to closure by $[ATP]_i$ along with other properties of the K.ATP channel in the same membrane patches before and after trypsin treatment (2 mg/ml). In addition to increasing the half-maximal $[ATP]_i$ required to close K.ATP channels from 39 to 213 μM, trypsin treatment also prevented 10 μM glibenclamide from significantly blocking K.ATP channels (4/4 patches) and prevented 100 μM $[ADP]_i$ from decreasing the sensitivity of the channels to closure by $[ATP]_i$. Mg^{2+}-dependent inward rectification of the K.ATP current remained intact after trypsin treatment (7/7 patches) and single channel conductance was unaltered. The effects of trypsin on K.ATP channels occurred whether or not Mg^{2+} was present in the bath during trypsin exposure. To test whether occupancy of either the ATP- or ADP-binding site by their ligands could prevent modification of the channel by trypsin, we included either 15 mM $[ATP]_i$ or 1 mM $[ADP]_i$ during trypsin treatment. Neither ATP_i (7/7 patches) nor ADP_i (4/4 patches) prevented the modification of K.ATP channels by trypsin, indicating that the trypsin effect may occur at a site remote from the ATP or ADP-binding sites. Thus proteolytic digestion by trypsin modulates multiple regulatory aspects of K.ATP channel function.

Th-Pos214
EFFECT OF DISOPYRAMIDE ON $I_{K\text{-}ATP}$ IN METABOLIC INHIBITION
((F. de Lorenzi, T. Bridal and W. Spinelli)) Wyeth-Ayerst Research, Cardiovascular and Metabolic Disorders Division, Princeton, NJ 08543

It is known that class Ia antiarrhythmics prolong the cardiac action potential (AP) through block of voltage-gated K^+ channels. The ability of disopyramide (Diso) to block ATP-sensitive K^+ current ($I_{K\text{-}ATP}$) elicited by a K channel opener and metabolic inhibitors was investigated using whole cell current measurements in cat ventricular myocytes at 36°C. As shown, Diso (9μM) inhibited $I_{K\text{-}ATP}$ elicited by lemakalim (2.5 μM) but was less potent in blocking glyburide-sensitive currents of similar magnitude induced by CN^- (1mM) or CCCP (carbonylcyanide m-chloro-phenyl hydrazone). Diso had no significant effect on control steady-state currents. Our data suggest that I_{out} in metabolic inhibition has different properties from the lemakalim-induced I_{out} and that block of I_{out} by Diso may not play a major role in modulating the AP shortening and K^+ loss observed in metabolic inhibition.



Th-Pos215
DOES STRETCH ACTIVATION OF K-ATP CHANNELS ALTER CARDIAC CELL VOLUME?
((M.A. Suleymanian, N.M. Cohen, and C.M. Baumgarten))
Depts. of Physiology and Surgery, Medical College of Virginia, Richmond, VA and Dept. of Biophysics, Armenian Academy of Sciences, Yerevan, Armenia

It recently was suggested that cardiac K_{ATP} channels are mechanosensitive and strongly activated by stretch with physiological (5 mM) ATP levels (Van Wagoner and Russo, *Circulat.* 84:II-505, 1991; *Biophys. J.* 61:A251, 1992). Because K_{ATP} channel density is very high, their activation by stretch might alter cell volume. This hypothesis was tested in rabbit ventricular myocytes. Digital video microscopy was used to estimate changes in relative cell volume ($VOL_{test}/VOL_{control}$), and each cell served as its own control. Stretch was imposed osmotically with 0.6T (195 mosmol/l) Tyrode solution containing 2 mM K^+. A 10-min exposure to the K_{ATP} channel blocker glybenclamide (1 μM; GLYB) did not alter cell volume in 0.6T (1.00 ± 0.01; n = 8) or 1T (0.99 ± 0.01, n = 5). GLYB pretreatment also did not modify the change in cell volume induced by switching between 1T and 0.6T (CONTROL: 1.34 ± 0.01, n = 11; GLYB: 1.34 ± 0.02, n = 5). Similarly, a 10-min exposure to the K_{ATP} channel activator aprikalim (100 μM; RP52891; APRK) did not alter cell volume in 0.6T (1.00 ± 0.02; n = 6) or 1T (1.01 ± 0.01; n = 7). Further, APRK pretreatment also did not effect the volume change induced by switching between 1T and 0.6T (CONTROL: 1.34 ± 0.01, n = 11; APRK: 1.36 ± 0.02; n = 7). Analogous results were obtained in 0.6T and 1T solutions with 5 mM K^+. We conclude that modulation of K_{ATP} channels by stretch or drugs does not alter cardiac cell volume. After K_{ATP} channel activation, it is likely that K^+ efflux is small because E_m closely approaches E_K. Thus, these data do not challenge the idea that increases in cell volume open K_{ATP} channels. In contrast to GLYB and APRK, Gd^{3+} and 9-anthracene carboxylic acid, blockers of other, poorly selective stretch-activated cation and anion channels with reversal potentials far from E_m, alter cardiac cell volume in 1T and the volume attained in 0.6T solution (Clemo and Baumgarten, *Biophys. J.* 61:A442, 1992). (NIH grants HL46764 & HL08488)

CARDIAC SODIUM CHANNELS

Th-Pos216
SODIUM LACTATE ENHANCES SODIUM CURRENT IN ISOLATED GUINEA-PIG VENTRICULAR MYOCYTES.
((H. Guo, J.A. Wasserstrom and J.E. Rosenthal)) Department of Medicine (Cardiology), Northwestern University Medical School, Chicago, IL 60611.

Hypoxia results in anaerobic metabolism and in the production of lactate. To study the effect of excess lactate on the sodium current (I_{Na}), we used the whole-cell voltage clamp technique in enzymatically isolated guinea-pig ventricular myocytes. Experiments were performed at 17° C in external solution containing in mM: NaCl 4-6.7; $CaCl_2$ 1.8; $MgCl_2$ 1.2; CsCl 5.0; TMA-Cl 125; hepes 20; glucose 11. pH was 7.4. Na lactate did not alter pH. At all concentrations of Na lactate, $[Na^+]_o$ was maintained constant in control and test solutions. Na lactate (4.0-6.7 mM) increased I_{Na} in each of 6 experiments, for example, at -20 mV by 28±7 % (mean±SEM). Slope conductance was increased; reversal potential was unchanged. The voltage dependence of steady state availability (h_∞) was not shifted by Na lactate. Free $[Ca^{2+}]$, measured using a Ca-sensitive electrode, was not affected by Na lactate. In conclusion, our results suggest that Na lactate enhances I_{Na} in guinea-pig myocytes via a mechanism that does not involve chelation of Ca^{2+} by lactate or changes in pH, but appears to be a direct action on the Na^+ channel.

Th-Pos217
QUINIDINE BLOCK OF ANTHOPLEURIN-A--MODIFIED CARDIAC NA^+ CHANNELS IS NOT ONLY THE RESULT OF AN INTERACTION WITH THE OPEN CHANNEL.
((S.L. Eager, K.N. Liberty, J.E. Kelly and J.A. Wasserstrom)) Department of Medicine, Northwestern University Medical School, Chicago, IL. 60611. (Spon. by R. Tsushima)

Quinidine is thought to block Na^+ channels primarily by interacting with the open channels. We used Anthopleurin-A (AP-A) toxin to prolong channel openings in order to characterize quinidine block of cardiac Na^+ channels. Whole cell recordings were made of Na^+ current (I_{Na}) at internal and external Na^+ concentrations of 5 mM at 17°C. Internal Cs^+ and F^- blocked K^+ and Ca^{2+} currents, respectively. The rate of block development was assessed using a two-pulse protocol. Quinidine (10 μM) showed two phases of block development, the more rapid phase (τ_f = 1.5±0.34 msec, n=4) presumably indicating an interaction with open Na^+ channels. Channel modification by AP-A (50-100 nM) did not affect the rapid rate of block development by quinidine. In addition, quinidine did not alter the rate of decay of AP-A--modified I_{Na} during a depolarizing voltage step as would be expected for an agent that preferentially blocked open channels. Previous exposure to AP-A did, however, cause an increase in binding affinity for quinidine as evidenced by a decrease in apparent dissociation constant. Thus, despite evidence that quinidine blocks open Na^+ channels in heart (rapid onset of block and increase in binding affinity following channel modification by AP-A), our results suggest that an interaction with the open channel is probably not the primary mechanism for quinidine block of cardiac I_{Na}.

Th-Pos218

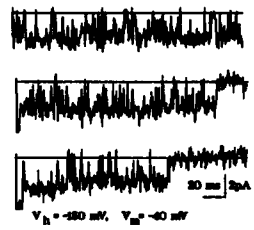
DUAL EFFECTS OF EXTRACELLULAR ATPγS ON I_{Na} IN CARDIAC CELLS.((B. Turan, F. Scamps, G. Vassort)) INSERM U-241, Orsay, France

The effects of purinergic stimulation were investigated on the sodium current (I_{Na}) of rat ventricular myocytes using the whole-cell patch-clamp technique. To ensure a reasonable voltage control, I_{Na} amplitude was reduced by the use of TTX or by the use of a low Na solution (10 mM). In both conditions, external application of ATPγS (100 μM) induced a marked reduction of I_{Na} elicited by depolarizing to -40 mV from a -80 mV holding potential every 2 s, consequent to a 6 mV leftward shift in the availability curve. If however the membrane was hyperpolarized to -130 mV for 150 ms before pulsing to -40 mV, ATPγS increased I_{Na} by 20%. TTX inhibition was unaffected. In a second set of experiments, Cd ions (10-300 μM) were added to the low Na solution to block the calcium current. Addition of Cd to the low Na solution induced a dose dependent decrease of I_{Na} amplitude. This inhibitory effect of Cd was antagonized by 100 μM ATPγS, so that I_{Na}-half inhibition by Cd occured at 60 μM instead of 120 μM in control condition. Thus, ATPγS affects I_{Na} amplitude by modifying both surface charges and blocking effects of Cd ions.

Th-Pos219

CYTOCHALASIN D MODIFIES CARDIAC Na CHANNEL GATING. ((A. I. Undrovinas and J. C. Makielski)) Cardiac Electrophysiology Labs, University of Chicago, Chicago Il, 60637.

Cardiac Na channels are anchored in the plasma membrane by the actin-spectrin based cytoskeleton. To test if this linkage plays a role in channel function, we studied the effect on Na channels of the actin filament disrupter cytochalasin D (Cyto D) in inside-out patches of rat and rabbit cardiomyocytes at 23 °C. Cyto D (5 μg/ml) was added to the cytoplasmic solution (in mM): 150 CsF, 10 EGTA, and 10 HEPES (pH 7.2). The pipette solution contained 280 mM Na and records were filtered at 5 kHz. In 7 patches Cyto D reduced average peak Na current by 60% within 3-10 min. In 4 of 7 patches Cyto D significantly slowed ensemble current decay. Individual sweeps revealed bursting in response to 200 ms step depolarizations from V_h= -150 mV to different membrane potentials. The figure shows three consecutive sweeps with Na channel bursts of 19.6 pS openings (20.7 pS in control) at -40 mV recorded in a rabbit. After clamp back to V_h the bursts terminated. These results suggest that cardiac Na channel gating depends upon the membrane-cytoskeleton environment and that this keeps the channel protein in specific conformations and promotes "normal" gating.



Th-Pos220

LIDOCAINE EFFECTS ON SODIUM CHANNELS FROM GUINEA PIG MYOCYTES AT 32°C.

((G. Maurice Briggs, P. Canniff and A. Ezrin)) Sterling Winthrop, Rensselaer, NY 12044

The effects of lidocaine have been extensively studied in cardiac preparations at 37°C using Vmax as an indicator of sodium channel availability and in isolated myocytes recording I_{Na} at reduced temperatures. Temperature is an important factor in drug channel interactions. We have determined the effects of lidocaine (LID) on I_{Na} at more physiological temperatures and compared the results to a single site binding model. Isolated myocytes were superfused with (mM) 70 NaCl, 70 TEACl, 1 $CaCl_2$, 1 $NiCl_2$, 1 $MgCl_2$, 10 HEPES, 12 dextrose, pH 7.4 at 32°C. Pipettes (200-800 kΩ) were filled with 80 NaCl, 35 CsCl, 10 EGTA, 10 HEPES, 5 Na_2ATP, 5 Mg ATP, pH 7.2. Cells were accepted for study when the IV curve had a graded smooth onset which reversed at the Nernst potential (-7 mV). Control inactivation curves were fit by the Boltzman equation with $V_{1/2}$ of -76±1 mV and a slope of 4.8±0.1 while activation curves were obtained similarly with a $V_{1/2}$ of -35±1 mV with a slope factor of 5.6±0.4 (13 cells). Tonic block developed in a concentration dependent, reversible manner which reduced peak obtainable I_{Na} and shifted the inactivation $V_{1/2}$ to -85±1; mV without a change in slope. Use dependent block (UDB) developed monoexponentially at stimulation frequencies faster than 1.4 Hz. The rate (per depolarization) of UDB development was inversely related to stimulation frequency (k_{on}=2.2, 7 Hz, k_{on}=3.6, 1.4 Hz) and was nonlinear. The level of steady state block was modulated on a beat to beat basis with 10 ms changes in recovery interval. Recovery from UDB was monoexponential (τ=220 ms). Channel reactivation was voltage dependent and rapid (τ<3 ms) which was slowed by drug binding (τ>50ms). The development and recovery from block were accurately modeled as a single binding site with a single rate constant from a constant recovery potential. However, the voltage dependence of drug unbinding could not be accounted for by the intrinsic channel potential sensitivity.

Th-Pos221

CARDIAC SODIUM CHANNEL KINETICS ARE NOT INFLUENCED BY ELEVATION OF EXTRACELLULAR POTASSIUM CONCENTRATION ((D.W. Whalley, D.J. Wendt, C.F. Starmer, A.O. Grant)) Duke University Medical Center, Durham, NC 27710.

A rise in extracellular K ([K]o) is believed to play a critical role in slowing conduction during myocardial ischemia. Studies in cardiac muscle have shown striking voltage-independent depression of Vmax in the presence of elevated [K]o. The non-linear relationship between Vmax and INa is exaggerated when membrane permeability to K is high as may occur with elevation of [K]o. Therefore we examined the effect of elevated [K]o on the kinetics of INa under voltage-clamp in cultured rabbit atrial myocytes using the amphotericin perforated patch technique. This technique obviates the time-dependent changes in INa amplitude and voltage-dependent gating seen during whole-cell recording. Superfusates contained (in mM): NaCl 150, MgCl2 1, CaCl2 1.5, Glucose 5, HEPES 10, CdCl2 0.05 and either 5 or 24 mM KCl. Experiments were performed at 22 degrees. In each experiment the I-V relationship, steady-state inactivation curve and kinetics of recovery from inactivation were determined under controlled conditions and after 10 min exposure to 24 mM [K]o. Elevation of [K]o from 5 to 24 mM caused no significant change in peak INa amplitude (4.5±1.7 vs 4.5±2.0 nA, mean ± SEM, n=4); the potential for half-inactivation (68.9±2.5 vs 69.9±1.9 mV) or the slope factor (6.4±0.3 vs 6.3±0.3 mV). The time constants of recovery from inactivation at -100 mV were 11.0±1.3 ms and 11.9±1.6 ms in 5 and 24 mM [K]o respectively.

We conclude that elevation of [K]o within the range observed in vivo during myocardial ischemia does not significantly influence the kinetics of INa. The voltage-independent depression of Vmax induced by elevated [K]o is therefore likely to reflect changes in membrane resistance and hence excitability.

CARDIAC SODIUM/CALCIUM EXCHANGE

Th-Pos222

SPECIES DIFFERENCE IN THE DENSITY OF Na^+-Ca^{2+} EXCHANGE CURRENT IN CARDIAC MYOCYTES. ((J.S.K. Sham, S.N. Hatem, and M. Morad)) Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104-6085.

In mammalian cardiac myocytes, the Na^+-Ca^{2+} exchanger is the major pathway for the trans-sarcolemmal Ca^{2+} efflux, competing with the sarcoplasmic Ca^{2+}-ATPase in removing Ca^{2+} from the cytosol,and contributing to cardiac relaxation. Previous studies have shown that there are species differences in cardiac excitation-contraction coupling (Morad and Goldman, 1973; Fabiato and Fabiato, 1979). The degree of development of sarcoplasmic reticulum and the Na^+-Ca^{2+} exchanger have been suggested to be responsible for such differences. We evaluated the contribution of the exchanger in different mammalian species by comparing the Na^+-Ca^{2+} exchange current ($I_{Na\text{-}Ca}$) activated by releases of Ca^{2+} induced either by rapid application of caffeine or by the voltage-gated Ca^{2+} channel in whole-cell clamped myocytes from rat, guinea-pig, hamster ventricles and human atria. Application of 5-10 mM caffeine triggered Ca^{2+} releases of similar magnitude in rat (355.1±29 nM, n=11), guinea-pig (356±36 nM, n=14), and hamster (412±39 nM, n=22) myocytes. The transient inward current activated by the caffeine-induced Ca^{2+} release (Callewaert et al., 1989), however, was larger in hamster (4.1±0.5 pA/pF), than guinea-pig (2.1±0.3 pA/pF) and rat (0.81±0.1 pA/pF) myocytes. Caffeine induced smaller Ca^{2+} releases (229±25 nM, n=4), and $I_{Na\text{-}Ca}$ (0.93±0.18 pA/pF) in human atrial myocytes. I_{Ca} also triggered Ca^{2+} releases in myocytes of all four species. The magnitudes of I_{Ca}-gated Ca^{2+} releases, unlike those produced by caffeine, varied significantly (351±36 nM in hamster; 228±43 nM in guinea-pig; 257±42 nM in rat; 136±37 nM in human). In myocytes where depolarizations induced Ca^{2+} releases of comparable magnitude, the tail currents measured at 30 ms following repolarization were in the order of hamster>guinea-pig≥human≥rat myocytes. The rate of relaxation of the Ca^{2+} transients in the presence of caffeine, followed the same order. These results support the idea that Na^+-Ca^{2+} exchange may be expressed to different degrees in different species. (Supported by HL16152)

Th-Pos223

INHIBITION OF FORWARD I_{NaCa} IN GUINEA PIG VENTRICULAR CELLS USING EXCHANGER INHIBITORY PEPTIDE (XIP).

((T.K. Chin, K.D. Philipson and J.H.B. Bridge)) Nora Eccles Harrison CVRTI, University of Utah, Salt Lake City, UT 94112 and UCLA School of Medicine, Los Angeles, CA 90024.

XIP has recently been shown to inhibit reverse Na-Ca exchange in voltage clamped guinea pig ventricular cells (Chin, Spitzer, Philipson & Bridge (1992), Circ. Res., in press). We studied the effect of XIP on forward I_{NaCa} activated by rapid solution changes in voltage clamped ventricular myocytes. Under conditions favoring forward Na-Ca exchange (pipette Na^+=0 mM, EGTA=20 uM), forward I_{NaCa} was activated by abruptly increasing Na^+_o from 0 to 145 mM during a twitch (when intracellular Ca^{2+} was elevated). I_{NaCa} was unchanged in control cells, but decreased within 15 minutes in cells with 10 uM XIP in the pipette. I_{Ca} was unchanged in both control and XIP-treated cells suggesting that inhibition of I_{NaCa} by XIP is not secondary to its effect on I_{Ca}.

The contribution of Na-Ca exchange to post-rest decay was measured with and without XIP in the pipette. Shortening was measured at 0.5 Hz, and after rests of 0.25, 0.5, 1, 2, 4, 6 and 10 minutes. Rest decay was slowed in XIP-treated cells.

We conclude that XIP inhibits forward I_{NaCa} without inhibiting I_{Ca}. We also conclude that forward Na-Ca exchange contributes to rest decay, presumably by pumping Ca^{2+} that leaks from the sarcoplasmic reticulum to the cell exterior.

Th-Pos224

Sodium acts as a cofactor in proton inhibition of the cardiac sodium-calcium exchanger.

A. E. Doering and W. J. Lederer, Dept. of Physiology, U. of Md. School of Medicine, Baltimore, MD 21212

The Na/Ca exchanger is highly sensitive to block by cytoplasmic protons around physiological pH. Factors which affect the degree of proton block may therefore be important in the physiological function of the Na/Ca exchanger. We measured Na/Ca exchange current in giant excised patches from adult guinea-pig ventricular myocytes using the method developed by Hilgemann (Hilgemann, Nature 344:242 (1990)), and investigated its sensitivity to cytoplasmic pH (pH_i) and sodium concentration ($[Na]_i$). We have previously reported that proton block of the Na/Ca exchanger is enhanced when $[Na]_i$ is raised from 0 to 60 mM (Doering & Lederer, Biophys. J. 61:A390 (1992)). Further investigation has shown that proton block of the Na/Ca exchanger is partially relieved when $[Na]_i$ is lowered from 60 mM to 0. Thus sodium ions seem to act as a cofactor in proton interaction with the Na/Ca exchange molecule. The experimental results could be reproduced by a simple model of Na/Ca exchange function, in which the affinity of the Na/Ca exchange molecule for protons is increased when one or more sodium ions is bound to the molecule. The enhancement of proton block by increased $[Na]_i$ could explain, at least in part, the transient component of outward sodium-activated Na/Ca exchange current often observed in the giant excised patch preparation. This transient component can be removed by pre-exposure to acidic pH. The role of sodium ions in proton block of the cardiac Na/Ca exchanger could be important in predicting the effect of changes in $[Na]_i$ on Na/Ca exchange function *in vivo*.

Th-Pos225

Antibody to the cardiac sodium-calcium exchanger inhibits sodium-calcium exchange current measured in giant excised patches.

A. E. Doering, L. Santana, H. H. Valdivia, and W. J. Lederer, Dept. of Physiology, U. of Md. School of Medicine, Baltimore, MD 21212

The sarcolemmal Na/Ca exchanger has been visualized in guinea-pig cardiac cells using flourescein-labeled immunoglobulin G bound to a polyclonal antibody specific for the rat Na/Ca exchange protein (Kieval, *et. al.*, Am. J. Physiol. 263:C545 (1992)). We measured the effect of the antibody on Na/Ca exchange function. Outward Na/Ca exchange current was activated in giant excised membrane patches from guinea pig ventricular myocytes by raising cytoplasmic sodium concentration from 0 to 60 mM accroding to the method developed by Hilgemann (Hilgemann, Nature 344:242 (1990)). Polyclonal rabbit antiserum raised against the canine cardiac Na/Ca exchanger was diluted 1:100 in the 60 mM Na cytoplasmic solution and applied to the membrane patch. This reduced current amplitude by about 80%. Pre-immune serum was applied to the membrane patch as a control, and had no effect on Na/Ca exchange current. We partially digested the Na/Ca exchange protein by exposing the membrane patch to cytoplasmic chymotrypsin for two minutes. This treatment is known to enhance Na/Ca exchange function by disrupting modulatory sites on the protein. The Na/Ca exchange current amplitude was increased, but it was still sensitive to inhibition by the antibody. This polyclonal antibody to the cardiac Na/Ca exchange protein appears to bind to a site or sites which are important in exchange function. The site of interaction is not closely associated with the calcium-, ATP-, or proton- modulatory sites, all of which are rendered ineffective by chymotrypsin digestion.

Th-Pos226

PHARMACOLOGICAL MODULATION OF CARDIAC Na-Ca EXCHANGE CURRENT IN WHOLE-CELL VOLTAGE CLAMPED GUINEA-PIG AND RABBIT MYOCYTES. ((S.MATSUOKA AND D.W.HILGEMANN)) Dept. of Physiology, UTSW, Dallas, TX, 75235.

The outward Na-Ca exchange current was measured in whole-cell voltage clamped guinea-pig and rabbit ventricular cells, and the effects of several agonists were tested. The current was activated by extracellular application of 2 mM Ca in the presence of 140 mM extracellular Na (40-100 mM cytoplasmic Na and 0.01-0.1 μM cytoplasmic Ca with 50 mM EGTA). Application of α-adrenergic agonists, norepinephrine (10 μM) and phenylephrine (1-8 μM), and purinergic agonists, adenosine (1-100 μM) and ATP (2 mM), had no significant effect on the exchange current. Application of the ß-receptor agonist isoproterenol (1 μM) induced Cl current, and the exchange current was inhibited by about 20%. High concentrations of platelet-activating factor (1-20 μM) and thrombin (1-10 units/ml), 'membrane phospholipid agonists', suppressed the exchange current. Phorbol 12-myristate 13-acetate had no effect. Extracellular application of phospholipase D (1 mg/ml) inhibited the exchange current by >80%, which is opposite to its cytoplasmic effect in excised patches. Thus, the cardiac Na-Ca exchanger is strongly influenced by membrane phospholipid-dependent factors which do not involve the protein kinase C pathway.

Th-Pos227

OXYGEN RADICALS STIMULATE NA^+/CA^{2+} EXCHANGE AND NON-SELECTIVE CATION CURRENTS IN GUINEA-PIG VENTRICULAR MYOCYTES.((R. Jabr and W.C. Cole)) Dept. of Physiology, Univ. of Manitoba, Winnipeg, Manitoba, Canada, R2H 2A6.

Oxygen radicals (O-R) have been implicated as a cause of electrical alterations and elevated intracellular calcium $[Ca^{2+}]_i$ in heart, however, the ionic basis for such alterations are still unclear. In this study we have investigated the effects of O-R using guinea-pig ventricular myocytes via whole-cell patch clamp technique. O-R were generated from 3mM DHF and $FeCl_3$:ADP (0.05:0.5mM). In current clamp, cells dialysed with O-R and 0.1mM EGTA show transient marked depolarization to ~-30mV associated with membrane oscillations, delayed after-depolarizations (DADs) and triggered activity. In voltage clamp, O-R cause; 1) large negative increase in holding current associated with positive shift in quasi-steady state I-V relation resulting from activation of steady-state current with reversal potential of -22.1±1.8mV (n=21) and 2) activation of transient inward current ($I_{t,i}$) upon repolarization back to -85mV. All these changes were blocked when cells were dialysed with 5mM EGTA or pretreated with 10µM ryanodine. Complete replacement of extracellular Na^+ (Na^+_o) with lithium blocked only $I_{t,i}$ with no effect on the steady-state current. In the presence of 10µM TTX, 100µM nicardipine and complete replacement of K^+_i and K^+_o with Cs^+, O-R transiently activated steady-state current which shifted reversal potential from -40.1±4.1 to -18.6±0.9mV (n=6). Moreover, the reversal potential of steady state current was shifted 1) negatively by 8.4±1.9mV (n=4) upon 50% $[Na^+]_o$ replacement with N-methyl glucamine and 2) positively by 8.8±1.6 mV (n=6) upon doubling $[K^+]_o$ (9.6mM). These data provide evidence that intracellular O-R 1) induce Ca^{2+} overload via increasing Ca^{2+} release from sarcoplasmic reticulum leading to abnormal electrical activity; 2) stimulate $Ca^{2+}{}_i$- activated non-selective cation current which is responsible for the transient marked depolarization to ~-30mV and; 3) enhance the activity of Na^+/Ca^{2+} exchange leading to $I_{t,i}$, DADs and triggered activity. Supported by MRC. WCC is a MRC scholar and RJ is a CHF trainee.

Th-Pos228

THE ROLE OF THE Na-Ca EXCHANGER IN THE STRENGTH-INTERVAL RELATIONSHIP IN CARDIAC MUSCLE.
((Simon M. Harrison & Mark R. Boyett)) Department of Physiology, University of Leeds, Leeds LS2 9JT, U.K.

It has been suggested that the increase in contractility at high stimulus frequencies is due to enhanced Ca^{2+} entry via the Na-Ca exchanger in response to the rise of intracellular sodium activity (a^i_{Na}) induced by an increase in stimulus frequency. To test this hypothesis, guinea-pig ventricular myocytes were voltage clamped (switch clamp) from -80 to 0 mV (for 100 ms) in a normal Tyrode solution containing 1.8 mM Ca^{2+} at pH 7.4, 36°C. Increasing the frequency of voltage clamp pulses from 0.5 to 3 Hz led to an increase in contractility (to 130 ± 6 % of that at 0.5 Hz; mean ± SEM, n=18). During 3 Hz stimulation membrane current became progressively more outward at the end of the pulse and more inward (tail current) upon repolarisation with little change in the holding current. The I-V relationship for current at the end of the pulse showed that the shift was greater at more positive potentials (-20 to +60 mV). The magnitude of the shift in current was unaffected if K^+ currents were blocked with caesium. 25 μM TTX was used to reduce the rise of a^i_{Na} during increases in stimulation rate. This reduced both the increase in contractility at 3 Hz and reduced the outward shift of current at the end of the pulse from 0.21 ± 0.02 nA (control, n=7) to 0.13 ± 0.02 nA (TTX, n=7) at 0 mV. The TTX sensitive current (measured at the end of the pulse) became progressively more outward at more positive potentials and reversed between -30 and -40 mV. This outward shift of current during the pulse is likely to reflect increased Ca^{2+} influx via Na-Ca exchange caused by the rise in a^i_{Na}.

Th-Pos229

INTRACELLULAR ACIDOSIS DECREASES Na^+ BACKGROUND CURRENT AND Na^+-Ca^{2+} EXCHANGE DURING CARDIOPLEGIA.
So Ra PARK, Ek Ho LEE, Cheol Joo LEE*, Chang Kook SUH, Department of Physiology, and Department of Thoracic and Cardiovascular Surgery*, Inha University College of Medicine, Inchon, Korea.

Cellular edema and cardiac arrhythmia are problems of clinical cardioplegia and ischemia of the heart, and those are closely related to intracellular ionic alterations, such as intracellular pH and Na^+ activity. In our previous study, intracellular pH during ischemic cardioplegia in guinea pig papillary muscle was decreased, in a Na^+-dependent way, and the degree of acidosis was larger in 0 Ca^{2+} cardioplegic solution than 1.8 mM Ca^{2+} cardioplegic solution (Park et al., 1992). The intracellular acidosis can affect the other transmembrane Na^+ movements such as Na^+ background current and Na^+-Ca^{2+} exchange current. Therefore we studied the effects of intracellular pH on Na^+ background current with whole cell clamp method in guinea pig ventricular myocytes, and the effects of intracellular pH on Na^+-Ca^{2+} exchange current with giant excised patch clamp method. The results are as follows. 1) The Na^+ background current at -40 mV membrane potential was larger at 0 Ca^{2+} cardioplegic solution than at 1.8 mM Ca^{2+} cardioplegic solution. 2) Intracellular acidosis decreased the Na^+ background current. 3) Intracellular acidosis also decreased the Na^+-Ca^{2+} exchange current. These results suggest that Na^+ background current and Na^+-Ca^{2+} exchange may play a major role in regulating intracellular Na^+ activity during cardioplegia.

Th-Pos230

DEVELOPMENTAL CHANGES IN MODULATION OF CALCIUM CURRENTS OF RABBIT VENTRICULAR CELLS BY ISOBUTYLMETHYLXANTHINE (IBMX)

((Toshiaki Akita, Rajiv Kumar, Chengbiao Lu and Ronald W. Joyner)) Department of Pediatrics, Emory University, Atlanta, GA, 30322

We measured whole-cell L-type calcium current (I_{Ca}, pA/pF) in adult (AD) and newborn (NB) cells at room temperature. The isoproterenol (ISO) E_{max} (percentage increase) and the EC_{50} were, respectively, 203 ± 5 % and 51 ± 8 nM for AD, and 111 ± 2 % and 81 ± 5 nM for NB. E_{max} and EC_{50} for IBMX used without ISO were 147 ± 61 % and 61 ± 38 uM for AD, and 267 ± 42 % and 56 ± 24 uM for NB. With a constant concentration of 0.1 uM ISO, the dose-response relationship for IBMX showed a much more potent effect, with an E_{max} and EC_{50} of 108 ± 8 % and 1.17 ± 0.5 uM for AD and 195 ± 29 % and 0.11 ± 0.08 uM for NB. When we applied a constant concentration of 0.1 uM IBMX (lower than that required to show any effect of IBMX applied alone) and then tested the dose-response relationship for ISO, we found that the E_{max} and EC_{50} for ISO were not substantially changed for AD cells from the values for ISO alone (177 ± 18 % and 28 ± 18 nM) while for NB cells the potency and efficacy of ISO were substantially increased by the presence of this low dose of IBMX, with E_{max} = 238 ± 18 and EC_{50} = 10 ± 3 nM. Thus, the effect of IBMX on basal I_{Ca} only occurred at large concentrations of IBMX, while, with a constant stimulation of even a small dose of ISO, the effect of IBMX on L-type I_{Ca} occurred at much smaller doses and decreased after birth. These results suggest that postnatal changes occur in the distribution of types of phosphodiesterases present in rabbit heart cells

Th-Pos231

5-HT-induced modulation of L-type calcium channel activity in human atrial myocytes

Ulrich Jahnel[1], Hermann Nawrath[1], Johanna Rupp[1], and Rikuo Ochi[2]
From the [1]Department of Pharmacology, University of Mainz, Federal Republic of Germany and the [2]Department of Physiology, Juntendo University School of Medicine, Tokyo, Japan

5-Hydroxytryptamine 10 µmol/l (5-HT) exerted a positive inotropic effect associated with an increase in the Ca^{2+} current (I_{Ca}) in the human right atrium. For detailed analysis, L-type Ca^{2+} channel currents were recorded from cell-attached patches using 100 mmol/l Ba^{2+} as charge carrier. Ca^{2+} channel activity was identified, first, by burstlike inwardly directed currents and, second, by the appearance of long channel openings, promoted by Bay K 8644 1 µmol/l, upon repetitive depolarizations from -80 to 0 mV. The unitary conductance of the Ca^{2+} channels amounted to 25.8 pS. During superfusion with 5-HT, ensemble averaged (mean) current was enhanced by about 60%. The increase in mean current was brought about by an increase in the channel availability, defined as the ratio of current containing sweeps to the total number of depolarizations. Unitary conductance, open probability within a current-containing sweep, mean open and mean shut times and the first latency, however, remained unaffected by 5-HT (n=10). These results indicate that the increase in macroscopic I_{Ca} in the human atrium reflects an enhanced activity of Ca^{2+} channels which may involve a cAMP-dependent phosphorylation of the channel protein thereby causing a shift from an unavailable to an available state of the Ca^{2+} channel.

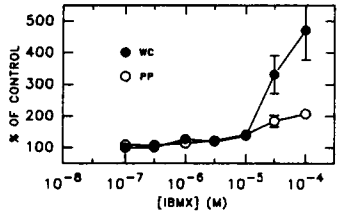
Supported by the Deutsche Forschungsgemeinschaft

Th-Pos232

CARDIAC I_{Ca} IS NO MORE SENSITIVE TO IBMX WHEN USING PERFORATED-PATCH THAN WHEN USING TRADITIONAL WHOLE-CELL RECORDING.

((A. Kawamura and G.M. Wahler)). Dept. of Physiology & Biophysics, Univ. of Illinois, Chicago, IL 60680.

Whole-cell (WC) recording causes cell dialysis, often resulting in current run-down and blunted responses to neurotransmitters. Perforated-patch (PP) recording maintains a more physiological intracellular milieu. Kurachi *et al.* (Naunyn-Schmied. Arch. Pharm. 340:219, 1989) suggested that I_{Ca} is more sensitive to isoproterenol stimulation using PP recording. We hypothesized that the I_{Ca} response to phosphodiesterase (PDE) inhibitors should be much greater using PP versus WC recording, since the response requires a gradual build-up of cAMP. Thus, we determined dose-response curves for IBMX in guinea pig ventricular myocytes using PP and WC recording methods. At low concentrations there was no difference between the two methods. At high concentrations (30-100 *u*M) the IBMX response actually tended to be larger using traditional WC recording. Thus, we conclude that dialysis of cardiac myocytes during traditional WC recording does not limit the response to PDE inhibitors.



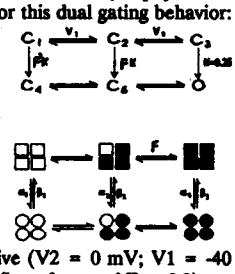
(Supported by HL 42955).

Th-Pos233

A MODEL OF THE DHP RECEPTOR OF SKELETAL MUSCLE AS SLOW Ca CHANNEL AND FAST CONTROLLER OF SR Ca RELEASE.

((Jianjie Ma and Eduardo Rios*)) Dept. of Physiol. and Biophys, Case Western Reserve University and Rush Medical College*.

We have shown that the same DHP-sensitive Ca channel of skeletal muscle is capable of both slow and fast modes of activation (Biophys. J. 61: A130). The proposed model (Fig.) accounts for this dual gating behavior: The model assumes that the last step into the open state of the channel is voltage independent and the route of channel closing is different from that of channel opening. The open symbols represent the resting states of each internal repeat of the DHPR, and the filled symbols their activated states. The channel as a whole can exist in two different configurations (squares or circles). Repeat I differs from the other three in two aspects: its equilibrium transition potential is more positive (V2 = 0 mV; V1 = -40 mV) and its forward transition rate is slower (by a factor of F = 0.2).



Assuming that partially activated DHPR (C2 and C5) could signal opening of the SR Ca release channel (suggested by K. Beam), this model puts together the two functions of the DHPR: explaining slow activation of the Ca current and fast control of SR Ca release.

Th-Pos234

L-TYPE CALCIUM CURRENTS IN VENTRICULAR MYOCYTES FROM ATHEROSCLEROTIC RABBITS

((K.E.Walker, S.R.Houser* and T.N.Tulenko)) *Temple U. School of Med., Medical College of PA, Phila.,PA (Spon. by T.N. Tulenko)

Calcium influx is increased in vascular smooth muscle cells (VSMC's) from diet-induced atherosclerotic rabbits and in cholesterol-enriched VSMC's from control rabbits. It is thought that the L-type calcium channels are a primary route for the larger Ca^{2+} influx. This investigation was designed to determine if similar membrane-associated alterations occur in cardiac cells from diet-induced atherosclerotic rabbits. Ventricular myocytes were isolated from control (C) and diet (D) animals. Both action potentials (AP's) and L-type calcium currents (I_{Ca}) were measured at 35° using a whole-cell voltage clamp technique. There was no difference between the APD_{25}, APD_{50} or APD_{75} in AP's from C (n=7) and D (n=18) cells. There was, however, a significant difference in the resting membrane potential (C vs. D in mV: -76.3±0.9 vs -72.6±0.9; p<0.05). Cell capacitative surface area (CSA) was similar in 13 C and 12 D cells. Peak I_{Ca} normalized for CSA was smaller in the D group but these differences were not significant except at -10 mV (p<0.05). This difference suggests that the voltage dependence of activation may be slightly shifted toward more positive potentials in the D group. It appears that L-type calcium channels are not altered in cardiac cells from atherosclerotic rabbits. It is, however, possible that changes in the inwardly rectifying K^+ current is contributing to the more depolarized rested state in these D cells.

Th-Pos235

REDUCED CALCIUM CURRENTS IN SUBENDOCARDIAL PURKINJE MYOCYTES DISPERSED FROM THE INFARCTED HEART 24 AND 48 HRS AFTER TOTAL CORONARY ARTERY OCCLUSION

((P.A. BOYDEN, J.M.PINTO)) DEPT. PHARMACOLOGY, COLUMBIA UNIVERSITY, NEW YORK, NEW YORK.

Altered function and/or density of T and L type Ca^{++} currents may underlie changes in the action potentials of subendocardial Purkinje myocytes from 24 and 48 hr infarcted canine hearts. Using whole cell voltage clamp techniques we compared Ca^{++} currents in subendocardial Purkinje cells dispersed from non-infarcted (NPs) and infarcted hearts (24 and 48hr IPs). Under 5mM Ca_o, $EGTA_i$ 10mM, 36°C, rates of rundown during the first 25min after membrane rupture were similar. Within 15-25min, peak i_{CaL} density (V_h=-40mV) in 24hr IPs(6.4±4.1 pA/pF, n=12) was similar to NPs(6.3±2.7pA/pF,n=24) but was significantly reduced in 48hr IPs (2.9±1.5pA/pF, n=11)(p<0.05). Decay of peak i_{CaL} was no different in 24hr (τ_1=7±4;τ_2=56±29ms) vs. NPs (τ_1=10± 5; τ_2=80±92ms) and 48hr IPs (τ_1=9±4;τ_2=54±17ms). Steady-state availability and restitution relationships of i_{CaL} were no different among cell types. Peak i_{CaT} density (V_h=-70mV) was reduced in 48hr IPs(0.97± 0.75pA/pF)(p<0.05)and in 24hr IPs(2.3± 1.5pA/pF) compared to NPs(3.4±1.7pA/pF). Therefore, after total coronary artery occlusion, density of i_{CaT} and i_{CaL} decrease without significant changes in voltage dependent activation and inactivation processes. Presumably reduced inward Ca^{++} in these surviving subendocardial Purkinje myocytes reflects persistent adaptive changes occurring in the normal Ca^{++} channels as a result of disease.

Th-Pos236

EFFECT OF ISOPROTERENOL AND BAYK 8644 ON THE REDUCED CALCIUM CURRENTS OF CANINE MYOCYTES FROM EPICARDIAL BORDER ZONE OF INFARCTED HEARTS.((R. Aggarwal, P. Boyden)) Dept of Pharmacology, Columbia Univ., NY, NY

We have previously shown that peak L-type calcium current ($I_{Ca,L}$) density is reduced in myocytes (IZs) obtained from epicardial border zone of 5 day infarcted hearts. To further characterize $I_{Ca,L}$ in IZs, we tested the ability of Isoproterenol (ISO,1μM), and BayK 8644 (BayK,1μM) to modulate $I_{Ca,L}$ and compared it to responses of epicardial myocytes from noninfarcted hearts (NZs). Whole cell voltage clamp technique was used to record $I_{Ca,L}$ (36°C, 5mM Ca_o, 10mM $EGTA_i$). ISO-induced increase in peak $I_{Ca,L}$ density was reduced in IZs (3.6±0.9 pA/pF (control,C) to 7.6±3.0(drug,D), n=9) as compared to NZs (5.9±1.1(C) to 19.5±4.4(D), n=11), representing a 2.2±0.8and 3.3±1.0($P<0.05$)fold increase, respectively. ISO shifted the steady state availability curve towards more negative potentials in both IZs and NZs. Whereas, the ability of BayK to increase peak $I_{Ca,L}$ density was similar in IZs (4.0±1.0 pA/pF (C) to 13.9±3.1(D), n=5) and NZs (5.8±1.5(C) to 20.5± 5.6(D), n=5), representing a 3.5±0.6and 3.5±0.2fold increase, respectively. BayK accelerated the decay of peak $I_{Ca,L}$ and shifted steady state availability to more negative potentials in IZs and NZs. These results suggest that modulation of $I_{Ca,L}$ by BayK is unaltered in IZs. However, the diminished ISO-induced increase in $I_{Ca,L}$ suggests a dysfunction in ß-receptor-effector coupling in IZs.

MEMBRANE TRANSPORT

Th-Pos237

ATP-DEPENDENT REGULATION OF THE CARDIAC NA/CA EXCHANGER EXPRESSED IN CHO CELLS. ((M. Condrescu, J. Aceto, C. Kroupis, and J. P. Reeves)) Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Na/Ca exchange activity is stimulated by ATP in many different cell types, including cardiac myocytes. Phosphorylation and acidic phospholipid transport have both been suggested as possible mechanisms. Exchange activity in CK1.4 cells, a transfected CHO cell line permanently expressing the bovine cardiac Na/Ca exchanger, was reduced to 30% by treatment of the cells for 15 min with 2 mM 2-deoxyglucose + 2 µM rotenone (DGR). DGR treatment reduced cellular ATP to <20% of control. The decline in exchange activity was not due to interference with intracellular Ca sequestration since treatment with the mitochondrial uncoupler Cl-CCP (10 µM) plus the SERCA ATPase inhibitor thapsigargin (2 µM) produced <15% inhibition of exchange activity. The decline in exchange activity during DGR was not retarded by the protein phosphatase inhibitors okadaic acid or calyculin. Moreover, the non-specific protein kinase inhibitor staurosporin (1 µM) inhibited exchange activity <15% after 30 min of incubation. Immunoprecipitation of the exchange protein from ^{32}P-labelled cells did not reveal a phosphorylated form of the exchanger, although phosphorylation of minor contaminating proteins could readily be detected. The results indicate that ATP-dependent regulation of the cardiac exchanger is maintained in the CHO cell environment, but do not support phosporylation as the mechanism of this effect.

Th-Pos238

TRANSMEMBRANE ASYMMETRY OF NA^+-CA^{2+} EXCHANGE INHIBITORS. ((G. Chernaya and J. P. Reeves)) Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Dichlorobenzamil (DCB) and quinacrine (Q) inhibit Na^+-Ca^{2+} exchange activity with K_i's of 10-25 µM in sarcolemmal vesicles. In CK1.4 cells, a transfected CHO cell line permanently expressing the bovine cardiac Na^+-Ca^{2+} exchanger, exchange activity (30 sec $^{45}Ca^{2+}$ uptake) was unaffected by the presence of 100 µM DCB in the assay medium. However, cells pre-incubated with 100 µM DCB gradually developed ($t_{1/2}$ ~ 5 min) complete inhibition of exchange activity. The inhibition by DCB was only partially reversed when the cells were subsequently incubated in DCB-free medium. Q inhibited exchange activity ($K_{1/2}$ ~ 200 µM) when present in the assay medium, but was more effective ($K_{1/2}$ ~ 50 µM) when cells were preincubated with Q (10 min) and then assayed in the absence of external Q. The inhibition by external Q was fully developed within 10 sec, while cells preincubated with 200 µM Q developed inhibition over a time course of several minutes. The results suggest that Q inhibits from both the cytoplasmic and extracellular membrane surfaces, whereas DCB, at concentrations up to 100 µM, inhibits only from the cytoplasmic membrane surface.

Th-Pos239

IN DIALYZED SQUID AXONS THE EFFECTS OF VANADATE ON THE Mg-ATP STIMULATED Na/Ca EXCHANGE SUPPORT A PHOSPHORYLATION - DEPHOSPHORYLATION PROCESS ASSOCIATED TO A KINASE-PHOSPHATASE SYSTEM ((R. Dipolo and L. Beauge)) CBB, IVIC, Apdo. 21827 Caracas, Venezuela and Inst. M. y M. Ferreyra, PO box 389 Cordoba, Argentina.

Vanadate (V_0) and P_0 are powerful inhibitors of ATPases and phosphatases. We reported earlier that, in squid axons dialyzed with Mg-ATP, both V_0 and P_0 stimulate Na/Ca exchange. In this work we have extend these studies by following the V_0 effects on the steady-state $Na_{(o)}$-dependent Ca efflux in dialyzed and voltage clamped squid axons. At concentrations up to 100 uM, V_0 stimulated $Na_{(o)}$-dependent Ca efflux only in the presence of Mg-ATP and provided there was a nucleotide stimulation of the Na/Ca exchanger. Thus, when the axons were dialyzed with the non-hydrolyzable ATP analog AMP-PCP, or with Cr-ATP that completely abolished Mg-ATP stimulation, no V_0 effects were detected. Furthermore, in axons fully activated by Mg-ATP-γ-S, V_0 has no effect on the $Na_{(o)}$-dependent Ca efflux. In the presence of 2 mM Mg-ATP, the dose response curve for V_0 stimulation followed Michaelian kinetics with a Km of 5.6 ± 0.4 uM and a V_{max} of 216 ± 10 fmoles.cm-2.s-1 ($[Ca2^+]_i$ =0.85 uM). On the other hand, 100 uM V_0 increased five fold the apparent affinity for Mg-ATP reducing the Km from the reported 220 ± 14 uM to 40 ± 4 uM. This behavior is to be expected in a system where the rate of exchange is proportional to the level of phosphorylation, which results from the balance between a kinase and a phosphatase activity. Finally, higher concentrations of V_0 inhibited solely the Mg-ATP stimulated $Na_{(o)}$-dependent Ca efflux (K_0 = 5.7 ± 0.3 mM; $I_{(max)}$ = 99 ± 4 %), perhaps acting on the kinase reaction. (Supported by grants from NSF (BNS-9120177), CONICIT (S1-2231), Venezuela and CONICET (3000800/88), Argentina.

Th-Pos240

MOLECULAR CLONING OF A RAT RENAL Na, Ca-EXCHANGER. ((S.L. Lee, A. Yu and J. Lytton)) Renal Div., Dept. of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

The kidney is the key organ responsible for whole body calcium homeostasis. The tubular reabsorption mechanism is thought to be an active transcellular process in the distal portion of the nephron, where a sodium gradient-dependent calcium exchange transporter (NCE) has been suggested to play an essential role. The NCE cDNA has been cloned from heart. The operation and regulation of a renal NCE, however, might be expected to differ from the cardiac enzyme since the molecules play quite different roles in the two tissues. Therefore, the goal of this study is to clone the rat renal NCE and to compare its molecular identity and regulatory mechanism with the cardiac NCE. Previously our laboratory had used the polymerase chain reaction (PCR) to clone a fragment of the rat renal NCE, which was called NCX1. Based on nucleotide sequence and Northern blot analysis, it was concluded that NCX1 and the cardiac NCE were products of the same gene (Yu *et al.* Am. J. Physiol., 1992, in press). A rat kidney cortex cDNA library was screened using PCR and with NCX1 as a probe. A 3.4 kb clone (denoted F1) was obtained. Comparison of this sequence with other reported NCE sequences revealed a high degree of identity within the coding region, but very little similarity within the 5'-UTR. Fragments from the coding region (NCX1) or from the unique 5'-UTR were used to probe Northern blots of RNA from various tissues. The coding region probe identified a prominent band of ~7 kb and a minor band of ~13 kb in all tissues examined. The 5'-UTR probe also identified both of these species in kidney cortex, with a similar intensity to those seen with NCX1. No signal was observed, however, in any other tissue even after prolonged exposure. Therefore, our renal NCE clone F1 possesses a 5'-end which is unique to the kidney. We are currently investigating the relative abundance and localization within kidney of NCE molecules with renal versus cardiac 5'-ends.

Th-Pos241

THE INHIBITORY EFFECT OF AMYLOID PEPTIDE ON HUMAN BRAIN Na^+/Ca^{2+} EXCHANGE. ((R.A. Colvin and A. Wu)) Department of Biological Sciences, Ohio University, Athens, OH 45701.

These studies were performed to determine the effects of addition of Alzheimer's disease beta- amyloid peptide (ß-AP) on Na^+/Ca^{2+} exchange activity. Alzheimer's disease (AD) is characterized by certain neuropathological lesions, including deposition of ß-AP, however, its pathogenic role is unknown. Na^+/Ca^{2+} exchange activity was measured in cerebral plasma membrane vesicles (PMV) purified from either rat brain or human postmortem frontal cortex tissues. The effects of synthetic full length peptide (ß-AP $_{1-40}$), ß-AP_{25-35}, and scrambled (ß-$AP_{25-35sc}$) were examined. PMV were preincubated with each peptide for 15 minutes (37°C) in buffer containing 137 mM NaCl, 10 mM HEPES (pH 7.4), and protease inhibitors. Ca^{2+} uptake was initiated by diluting PMV 20 fold with buffer containing either 137 mM NaCl or 137 mM choline Cl and $^{45}CaCl_2$ then terminated by addition of 5 mM $LaCl_3$ and rapid filtration. An inhibitory effect of ß-AP_{25-35} or ß-AP_{1-40} was seen at concentrations as low as 10 μM. No effect was seen when ß-$AP_{25-35sc}$ was used. The inhibitory effect was evidenced as a decrease in the initial velocity of Ca^{2+} uptake (measured 30 seconds after start of the reaction) and the peak uptake (5 minutes). The inhibitory effect of ß-AP $_{25-35}$ could not be overcome by increasing Ca^{2+}. When ß-AP_{25-35} was added in the dilution buffer instead of during the preincubation step to load the vesicles with Na^+, no inhibition was seen. These data suggest that preincubation with ß-AP_{25-35} was neccesary to observe an inhibitory effect. ß-AP may inhibit Na^+/Ca^{2+} exchange activity by making PMV "leaky" to Na^+.

Th-Pos242

SUBSTRATE DEPENDENCE OF NA-CA EXCHANGE 'GATING' CURRENTS ACTIVATED BY PHOTOLYSIS OF CAGED CA^{2+}

R. Niggli & P. Lipp, Department of Physiology, University of Bern, Bern, Switzerland.

Ca^{2+} concentration jumps performed in cardiac myocytes by flash photolysis of 'caged' Ca^{2+} activate an inward Na-Ca exchange current and a 'gating' current lasting ~ 2 ms. The gating current (I_{gate}) has been associated with a conformational rearrangement of the Na-Ca exchanger molecules after Ca^{2+} binding involving an outward movement of negative charge with the Ca^{2+} translocating step. Assuming a consecutive Na-Ca exchange model the amount of charge moved during I_{gate} would reflect the number of exchanger molecules with the Ca^{2+} binding sites facing the inside of the cell. We have now combined a rapid superfusion system ($t_{1/2} < 1$ s) with photorelease of Ca^{2+} to investigate the effects of extracellular substrates (Na^+_o and Ca^{2+}_o) on I_{gate} and thus on the steady-state distribution of exchanger states. Rapid superfusion should minimize uncontrolled intracellular substrate changes (i.e. Na^+_i and Ca^{2+}_i) during exposure to varying extracellular ionic conditions. Using solutions based either on Li^+, NMG or Ni^{2+} the following effects of extracellular substrates were observed: Compared to substrate-free conditions, a brief (~2 s) exposure to 10 mM Na^+ reduced I_{gate} to 92.2% ± 2.4% (mean ± S.E, n = 11). Application of 1 mM Ca^{2+} had no significant influence on I_{gate} (99% ± 3.9%, n = 9). These observations do not reflect the predicted inward shift of the steady state distribution of Na-Ca exchanger molecules. From these results we conclude that I_{gate} may arise from a conformational change of the Na-Ca exchange molecules after Ca^{2+} binding other than a membrane crossing transition. Changes of I_{gate} reported after prolonged exposure to substrate free solutions may therefore be the result of uncontrolled variations of $[Ca^{2+}]_i$. Supported by Swiss National Science Foundation.

Th-Pos243

INTERACTION OF CARDIAC NA-CA EXCHANGE AND THE EXCHANGE INHIBITORY PEPTIDE (XIP) WITH PHOSPHOLIPIDS AND PROTEASE TREATMENT. ((T.R. Shannon, M.A. Milanick and C.C. Hale)) University of Missouri, Columbia, MO 65211.

The Na-Ca exchanger catalyzes the exchange of Na for Ca across the plasmalemma. While endogenous regulation of Na-Ca exchange is not well understood, in vitro exchange activity is stimulated by mild proteolysis and the addition of negative phospholipids. Within loop f of the exchange protein there is a 20 amino acid primary sequence that contains 8 positive amino acids that resembles a calmodulin binding region. A synthetic peptide (XIP) corresponding to this region is a potent inhibitor of Na-Ca exchange. We have initiated a series of experiments to test the hypothesis that the endogenous XIP region is an autoregulatory site on Na-Ca exchange possibly, under the control of the membrane lipid environment. XIP peptide binds to liposomes composed of phosphatidyl choline (PC) and phosphatidyl serine (PS) with maximal binding observed at 1:1 PC:PS. No binding was observed in liposomes composed only of PC. Reconstituted Na-Ca exchange activity was observed in proteoliposomes composed of 1:1 PC:PS but not PC proteoliposomes. Na-Ca activity in 1:1 PC:PS proteoliposomes was inhibited by XIP peptide. When detergent solubilized Na-Ca exchange protein was proteolyzed prior to reconstitution into 1:1 PC:PS proteoliposomes, the resulting activity was also inhibited by XIP which suggested that the XIP binding site on the exchanger remained intact. These data are consistent with a model of Na-Ca exchange regulation in which the endogenous XIP region interacts with another area of the protein to inhibit activity or is lipid-bound prohibiting inhibition of the exchange reaction. Supported by NIH DK 37512, NIH DK 02006, and the AHA-Missouri Affiliate.

Th-Pos244

THE CARDIAC Na^+-Ca^{2+} EXCHANGER BINDS TO THE CYTOSKELETAL PROTEIN ANKYRIN ((Z. Li, E.P. Burke, J.S. Frank, V. Bennett and K.D. Philipson)) UCLA, Los Angeles CA 90024; Duke University, Durham NC 27710

Na^+-Ca^{2+} exchange is the major pathway of Ca^{2+} efflux during excitation-contraction coupling in cardiac muscle. It was reported recently that the Na^+-Ca^{2+} exchanger is present in cardiac transverse tubules with an apparent high density (Frank et al., J. Cell Biol. 117:337, 1992). The mechanism for this localization is unknown but may involve interactions with the cytoskeleton. In the present study, we examined the interaction of the Na^+-Ca^{2+} exchanger with the cytoskeletal protein ankyrin. On immunoblots of isolated canine cardiac sarcolemma, an antibody raised against purified rabbit red blood cell ankyrin (RBC-ankyrin) recognized a 220 kD protein, which is the same size as RBC-ankyrin. Alkaline extraction of sarcolemma removed this protein. The Na^+-Ca^{2+} exchange protein, purified from recombinant baculovirus-infected insect cells, bound ^{125}I-labeled-RBC-ankyrin with a K_D of 30 nM. ^{125}I-RBC-ankyrin was precipitated by either polyclonal or monoclonal antibodies raised against the canine sarcolemmal Na^+-Ca^{2+} exchanger after preincubation with solubilized cardiac sarcolemma. The cardiac ankyrin co-localized with the Na^+-Ca^{2+} exchanger in guinea pig ventricular myocytes as shown by immunofluoresence techniques. These results demonstrate that the cardiac Na^+-Ca^{2+} exchanger binds ankyrin with high affinity. This interaction may be important for localizing the Na^+-Ca^{2+} exchanger to specific domains of the sarcolemma.

Th-Pos245

IDENTIFICATION OF REGULATORY REGIONS OF THE CARDIAC SARCOLEMMAL Na^+-Ca^{2+} EXCHANGER. ((S. Matsuoka, D.A. Nicoll, R.F. Reilly, D.W. Hilgemann, and K.D. Philipson)). UCLA, Los Angeles, CA 90024; Univ. Texas, Southwestern, Dallas, TX 75235; Yale, New Haven CT 06510

We have analyzed the regulatory properties of the wild-type cardiac exchanger expressed in *Xenopus* oocytes using the giant excised patch technique. The expressed exchanger displays the same properties observed in the native sarcolemmal exchanger: Activation by chymotrypsin, Na^+-dependent inactivation, secondary regulation by intracellular Ca^{2+}, and inhibition by a specific exchanger inhibitory peptide (XIP). These properties were localized to the large hydrophilic domain of the exchanger by deletion mutagenesis and construction of a chimeric exchanger. Analysis of the mutants demonstrated that residues 240-679 are not essential for ion transport; that residues 562-679 are involved in regulation by Ca^{2+}_i and inhibition by XIP; that Na^+-dependent inactivation is separable from secondary Ca^{2+} regulation and localized to residues 240-561; and that the two hydrophobic domains of the exchanger are involved in ion binding and transport. A chimera made from renal and cardiac exchangers helps to further define the residues involved in Ca^{2+}-regulation and XIP interaction.

Th-Pos246

BIOSYNTHESIS AND INITIAL PROCESSING OF THE CARDIAC Na^+-Ca^{2+} EXCHANGER. ((L.V. Hryshko, D.A. Nicoll, J.N.Weiss, and K.D. Philipson)) UCLA, Los Angeles CA 90024.

Based on the primary sequence of the cardiac Na^+-Ca^{2+} exchanger, there are 6 potential N-linked glycosylation sites and a potential cleaved signal sequence at the NH_2-terminus. To study the post-translational modifications of the exchanger, *in vitro* translation was examined in the presence and absence of canine pancreatic microsomes. Glycosylation, detected as endoglycosidase-H induced shifts in molecular size, was examined for proteins obtained using full and partial length RNA transcripts containing different numbers of the potential N-linked glycosylation sites. In the presence of microsomes, the M_r of the full length clone increased 3 kDa to 113 kDa. Endoglycosidase H treatment led to a reduction of M_r to 108 kDa indicating that glycosylation increases M_r by ~5 kDa and a signal sequence of ~2 kDa is cleaved during processing. Analysis of M_r shifts obtained with partial transcripts suggested that glycosylation occurs only at position N9. This was confirmed by site directed mutagenesis studies. Western blots of the wild-type and N9Y mutant revealed that eliminating this potential glycosylation site reduced M_r by ~10 kDa. The giant excised patch technique was used to determine the functional consequences of glycosylation. Na^+-Ca^{2+} exchange current was examined in patches from oocytes expressing either the wild-type or N9Y mutant. The non-glycosylated mutant exhibited the same properties as the native exchanger with respect to voltage and sodium dependence as well as to the effects of chymotrypsin. These results suggest that glycosylation does not affect exchanger function and help to define the exchanger topology.

Th-Pos247

K+-DEPENDENT NA+/CA2+ EXCHANGE IN HUMAN PLATELETS: EVIDENCE FOR A RETINAL ROD-TYPE OF EXCHANGER ((M. Kimura, A. Aviv and *John P. Reeves)) Hypertension Research Center, University of Medicine and Dentistry of NJ, Newark, NJ, 07103 and *Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ, 07110.

Ca^{2+} influx via Na^+-Ca^{2+} exchange was examined using fura-2 fluorescence techniques in human platelets loaded internally with Na^+ by pretreatment with ouabain. In 140 mM LiCl, Ca^{2+} influx via the exchanger was completely dependent upon the presence of extracellular K^+ (K_m ≈ 1 mM). In 140 mM N-methyl-D-glucamine (NMDG), Ca^{2+} influx was stimulated by K^+ but was not competely dependent upon it. The K^+-dependence of Ca^{2+} influx was confirmed by $^{45}Ca^{2+}$ flux studies. The presence of extracellular Ca^{2+} stimulated $^{86}Rb^+$ influx in ouabain-treated platelets in 160 mM LiCl or NMDG. The Rb^+/Ca^{2+} influx ratio was 0.42 ± 0.04 (N=3) at $[Rb^+]$ = 0.2 mM and 0.89 at $[Rb^+]$ = 1.3 mM. Neither K^+-dependent Ca^{2+} influx nor Ca^{2+}-dependent Rb^+ influx was observed in 160 mM NaCl or in platelets that had not been treated with ouabain, indicating that these fluxes resulted from Na^+-Ca^{2+} exchange activity. These properties are essentially identical to those of the Na^+/Ca^{2+}-K^+ exchanger found in retinal rods, as distinct from the more widely distributed cardiac-type of exchanger, which is not K^+-dependent. The K^+-dependence of the exchanger may be important in promoting platelet activation at sites of tissue damage, where extracellular $[K^+]$ is likely to be elevated.

Th-Pos248

SARCOLEMMAL CALCIUM PUMP IN MYOCARDIAL ISCHEMIA. ((Malcolm M. Bersohn)) V.A. Medical Center and University of California, Los Angeles, CA 90073

To investigate the effect of 1 and 2 hrs of global ischemia in the rabbit heart on the sarcolemmal Ca^{2+} pump, sarcolemmal vesicles were purified (measured by the ratio of sarcolemmal to homogenate K^+ *p*nitrophenylphosphatase activity), and the initial velocity of ATP-dependent $^{45}Ca^{2+}$ uptake into inside-out vesicles was measured at pH 7.4 at 37°. One and 2 hrs of ischemia were separate experiments, hence the different control values below. T = duration of ischemia. Means ± S.E. are shown.

T(hr)	n	Km *	Vmax **	Purification
0	6	0.43±.06	1.3±0.1	27±2 fold
1	6	0.48±.08	1.3±0.1	20±1 fold
0	5	0.13±.02	2.0±0.2	40±6 fold
2	5	0.18±.03	2.0±0.2	43±9 fold
		*μM Ca^{2+}	**nmol Ca^{2+}·mg^{-1}·min^{-1}	

Sarcoplasmic reticulum contamination was negligible, and the percentage of intact inside-out vesicles was unaffected by ischemia. Thus the activity of the sarcolemmal ATP-dependent Ca^{2+} pump, a high affinity but low capacity mechanism for cellular Ca^{2+} efflux, is preserved for at least 2 hrs of global ischemia.

Th-Pos249

THE CYTOSOLIC pH in SYNAPTOSOMES IS INCREASED BY Cl^- REMOVAL OR HIGH K^+-SOLUTION. Sergio Sanchez-Armass., Raúl Martínez-Zaguilan* and Robert Gillies*. Dept. Fisiologia, Univ. San Luis Potosi., Mexico and Dept. Biochemistry, Arizona University*, AZ 85724.

In rat brain synaptosomes the Na^+/H^+ exchanger has been the only carrier mediated mechanism involved in the regulation of pH_i. The H^+_i was assessed with the fluorescent indicators pyranine or BCECF. Cl^-_o removal, in the absence of HCO_3^- and Ca^{2+}, rapidly induces a net raise in the pH_i by ca. 0.20 pH units. This effect is abolished by 100 μM DIDS. Cytosolic Cl^- measured with the fluorescent probe N-(6-methoxy-quinolyl) acetoxy ester (MQAE) was estimated as 50 and 10 mM in Normal (145 mM Cl^-) and Cl-free solution, respectively. The levels in Normal solution indicates that Cl^-_i is distributed out of equilibrium. Experiments designed to simultaneously monitor external $[Cl^-]$ (with MQAE) and pH (with SNARF-1) in Cl^--free conditions suggest a Cl^--H^+ symporter that normally operates as an acid loader. The acidification seen in low Na^+ medium is abolished 80 % by 24 mM K^+ and a net alkalinization is observed in 75 mM K^+ solution. These K^+ effects are Ca^{2+} independent. The data suggest a K^+/H^+ antiporter. Supported by CONACYT F140-I9112 to S.S-A and NIH R01 GM43046-01 to RJG and R.M-Z.

Th-Pos250

Na^+-H^+ EXCHANGE ACROSS HUMAN ERYTHROCYTE MEMBRANES AS PROBED BY MULTINUCLEAR NMR SPECTROSCOPY. ((D.M. de Freitas, S. Mo and Y. Chi)) Department of Chemistry, Loyola University of Chicago, 6525 North Sheridan, Chicago, IL 60626.

Whether Na^+-H^+ and Na^+-Li^+ exchange proteins are structurally unrelated or are the same protein but regulated differently in human red blood cells (RBCs) is not known. We studied Na^+-H^+ exchange in Na^+-loaded RBC suspensions by using a combination of ^{23}Na and ^{31}P NMR methods. The cells were suspended at 20% hematocrit in two shift reagent-containing media at two pH values, 6.0 and 8.0. The changes in Na^+ concentrations were monitored by ^{23}Na NMR, and ^{31}P NMR was used to measure changes in intracellular pH by loading the indicator methylphosphonate in intact RBCs. The rates of RBC Na^+-H^+ exchange measured by NMR were within the range of those previously measured by atomic absorption. In intact RBC and RBC membrane suspensions, a decrease in pH caused an increase in both ^{23}Na and ^7Li NMR relaxation time values indicating that protons compete with Na^+ and Li^+ for the same membrane binding sites. Addition of the transport inhibitor amiloride caused an increase in both ^{23}Na and ^7Li NMR relaxation values measured in Na^+- or Li^+-treated RBC membrane suspensions suggesting that Na^+-H^+ and Na^+-Li^+ exchange are mediated by the same RBC membrane transport protein. NMR spectroscopy is therefore a powerful tool for obtaining a molecular understanding of the mechanism responsible for elevated rates of Na^+-H^+ and Na^+-Li^+ exchange in RBCs of hypertensive patients.

Th-Pos251

CLONING AND CHARACTERIZATION OF A PUTATIVE CA^{2+}/H^+ ANTIPORTER GENE FROM *ESCHERICHIA COLI*. ((J. Zemsky, D.M. Ivey, A.A. Guffanti, S. Schuldiner, E. Padan, and T.A. Krulwich)) Mount Sinai School of Medicine of CUNY, NY, NY and Institute of Life Sciences, Hebrew University, Jerusalem, Israel.

DNA libraries from alkaliphilic *Bacillus firmus* OF4 had been screened, in earlier studies (Ivey *et al.*, 1991, J. Biol. Chem. 266, 23483-23489) for clones that would functionally complement a strain of *Escherichia coli* (NM81) with a deletion in one of its Na^+/H^+ antiporter genes. During those studies, in which one putative alkaliphile antiporter gene was cloned, a second alkaliphile antiporter gene was hypothesized to have been incorporated into the chromosome of strain NM81. During attempts to reisolate this gene, a new *E. coli* gene was identified on the basis of its ability to restore Na^+-resistance and membrane Na^+/H^+ antiporter activity (but not Li^+/H^+ activity) to antiporter-deficient mutant strains. The active orf in the clone maps at 27 min on the *E. coli* chromosome and would be predicted to encode an extremely hydrophobic protein with multiple membrane-spanning regions and a molecular weight of 39,200 daltons. A region in one of the predicted hydrophilic loops in the gene product structure possesses striking sequence similarity to calsequestrin. The Ca^{2+}/H^+ antiporter activity of membranes from an *E. coli* transformant with a clone possessing only this orf was therefore assayed. A significantly higher, Ca^{2+}/H^+ antiporter activity was found in this transformant relative to a control. We propose that the new gene, designated *cha*A, is the structural gene for a Ca^{2+}/H^+ antiporter whose overexpression led to sufficient activity with Na^+ to lead to its identification in the functional complementation assay.

Th-Pos252

RELATIONSHIP BETWEEN CYTOSOLIC [ATP], [ATP]/[ADP] AND Na^+,K^+-ATPase ACTIVITY IN PERFUSED RAT HEART. ((V. Kupriyanov, L. Stewart, R. Deslauriers)) Institute for Biodiagnostics, NRC, Canada (Spon. by I.C.P.Smith)

The objective of this study was to assess whether cardiomyocyte Na^+ pump activity is inhibited by decreased [ATP] and [ATP]/[ADP], when other variables ([Pi], $[H^+]$) are kept constant. With this aim, $[Na^+]_i$, Rb^+ influx rate (RIR) and phosphates were measured in Langendorff-perfused rat hearts by ^{23}Na (with the shift reagent DyTTHA^{3-}), ^{87}Rb and ^{31}P NMR. First, we verified the relation between RIR, reflecting Na^+ pump activity, and $[Na^+]_i$, reflecting the balance between Na^+ influx and efflux. Ouabain, which inhibits the Na^+ pump, reduced RIR by 70% and increased $[Na^+]_i$. Monensin, which increases Na^+ influx, increased $[Na^+]_i$ and RIR, whereas procaine, which blocks Na^+ entry, decreased $[Na^+]_i$ and RIR. Thus, changes in both RIR and $[Na^+]_i$ reflect Na^+ pump activity. We then used ^{23}Na NMR to determine the effect of changes in adenine nucleotides (AdN) on Na^+ pump activity. Pyruvate-perfused hearts were treated (30 min) with 2-deoxyglucose (DG) + insulin, resulting in a depletion of the AdN due to phosphate trapping into DG-6-phosphate and decreases in phosphocreatine, cytosolic [ATP] (to 0.8 mM) and [ATP]/[ADP] (to 6%) without affecting Pi, pH_i or $[Na^+]_i$. We suggest that the Na^+ pump is not sensitive to such variations in AdN, and that the increase in $[Na^+]_i$ that is characteristic of hypoxia is caused by inhibition of the pump by increased Pi and/or fatty acid derivatives.

Th-Pos253

PROBABLE IDENTIFICATION OF AMINOPHOSPHOLIPID TRANSLOCASE ACTIVITY IN CARDIAC MYOCYTES. ((D.W. Hilgemann & A.J. Schroit[2])), *Dept. of Physiol., UTSW Med. Ctr., Dallas, TX., 75235 & [2]Dept. of Cell Biology, UT M.D. Anderson Cancer Ctr., Houston, TX. 77030.*

In red blood cells the preferential distribution of phosphatidylserine (PS) to the cytoplasmic bilayer leaflet is maintained by an ATP-dependent aminophospholipid transporter (Signeuret, M. & Devaux, P.F., Proc. Natl. Acad. Sci. U.S.A. 81, 3751, 1984). To test whether this transport system is active in cardiac sarcolemma (Hilgemann D.W. & Collins A., J.Physiol., 454, 59, 1992), ventricular myocytes were incubated with 6-carbon-chain (C6), NBD-labelled phospholipid analogues (Connor, J. & Schroit, A.J., Biochemistry 26, 5099, 1987), and fluorescence from a 6 μm^2 area of single myocytes was monitored. Cells were eliminated from the assay if they were hypercontracted and/or took up rhodamine-labelled pentalysine, which was used to quench extracellular phospholipid fluorescence (1-10 μM). After 30 min incubation with C6-PS, a 3.4-fold greater uptake of label was found at 37°C than at 4°C (guinea-pig myocytes, $p<0.01$, $n=10$). N-ethyl-maleimide (0.5 mM) reduced the temperature-sensitive uptake fraction by >80% (guinea-pig and rat myocytes, $p<0.01$; $n=10$). Preincubation of cells with 10 mM deoxyglucose and 0.15 mM dinitrophenol for 15 min to deplete ATP reduced the temperature-sensitive fraction by 68% (guinea pig myocytes; $p<0.05$; $n=16$). Uptake of the equivalent fluorescent phospatidylcholine analogue was low and temperature-insensitive (guinea pig myocytes, $n=9$). These results suggest that cardiac cells express an aminophospholipid transporter, similar to that found in red blood cells.

Th-Pos254

INSULIN-DEPENDENT AND INSULIN-INDEPENDENT GLUCOSE TRANSPORT IN BARNACLE MUSCLE CELLS. ((R. Quinones, D. Erlij*, C. Peña-Rasgado & H. Rasgado-Flores)) UHS/The Chicago Medical School. N. Chicago, IL 60064. *Downstate Medical Center, SUNY. Brooklyn, NY 11203.

Glucose transport in skeletal muscle is regulated by exercise, anoxia, exposure to metabolic poisons and to insulin. However, little is known about the underlying mechanisms responsible for such regulation. This lack of information is due in part to the small size of mammalian muscle cells which precludes experimental control of the myoplasmic composition. Barnacle muscle cells (bmc) are well suited for the study of glucose transport and regulation because: i) insulin stimulates glucose transport in these cells; and ii) this preparation allows control of the composition of intra and extracellular media and membrane potential. Glucose transport was studied in intact and internally perfused bmc. The transport of ^{3}H-3-O-Methylglucose (^{3}H-3OMG) was chosen as a substrate since it is not metabolized by the cells. The intact cell preparation consisted of bundles (30-40) of cells attached to the carapace but detached from each other allowing measurement of ^{3}H-3OMG uptake. The perfused cell preparation consisted of cells cannulated and mounted in an experimental chamber allowing measurement of both influx or efflux of ^{3}H-3OMG. The results show that: i) insulin (18 μM) stimulates ^{3}H-3OMG influx in both intact (~2.5 μmoles/gr dry weight) and perfused cells (~4.5 pmoles·cm^{-2}·s^{-1}); ii) insulin (up to 18 μM) does not stimulate ^{3}H-3OMG efflux from perfused cells; and iii) increases in temperature (from 12 to 20 °C) produce a 15 fold stimulation of an insulin-insensitive but cytochalasin-B (25μM) -sensitive ^{3}H-3OMG efflux from perfused cells. Our results show that two ^{3}H-3OMG transport mechanisms can be measured in bmc: one system is insulin-sensitive while the other is insulin-insensitive but temperature and cytochalasin-B-sensitive.

Th-Pos255

OPPOSITE ROLES OF cAMP AND cGMP ON REGULATORY VOLUME DECREASE IN BARNACLE MUSCLE CELLS. ((C. Peña-Rasgado and H.Rasgado-Flores)) Dept.Physiol. UHS/The Chicago Medical School. N. Chicago, Il 60064.

Cell volume regulatory mechanisms are studied in bundles of isolated barnacle muscle cells (dissected from each other but attached to the carapace). In response to hyposmotic conditions the cells present an extracellular Ca^{2+} (Ca_o)-dependent regulatory volume decrease (RVD) with the following characteristics: i) it is accompanied by the net loss of KCl, glycine and taurine; ii) the ratio of KCl loss is about 2K:1Cl; and iii) it is blocked by external Li^+. The aims of this work are the following: i) to discern if Ca^{2+} influx is necessary for RVD; ii) to analyze if the ratio of 2K:1Cl loss during RVD is due to loss of K^+ accompanied not only by Cl^- but by another anion (e.g., OH^-) as well; and iii) to study if the block of RVD by Li^+ is due to alterations in the levels of cAMP and/or cGMP since it is known that Li^+ modifies the levels of these messengers in cells including barnacle muscle. The strategies followed to answer these questions consisted in evaluating: i) if the Ca^{2+} channel blocker verapamil blocks the Ca_o-dependent RVD; ii) if there is an external alkalinization during RVD; and iii) if increases in the levels of cAMP [by incubating the cells in the presence of dibutyryl cAMP (dcAMP) or forskolin] or cGMP [by incubating the cells in the presence of dibutyryl cGMP (dcGMP)] affect the RVD. The results showed that: i) presence of 0.1 mM verapamil completely inhibited the RVD indicating that Ca^{2+} influx is indeed necessary for RVD; ii) RVD is accompanied by alkalinization of the external medium indicating that K^+ is loss not only as KCl but also as KOH (or being exchanged with H^+); iii) 10 mM dcAMP or 20μM Forskolin promoted RVD in cells exposed to hypotonic conditions containing 0Ca_o suggesting that increases in cAMP promote RVD; and iv) 2 mM dcGMP supresses RVD (in the presence of Ca_o) suggesting that cGMP inhibits RVD. Supported by NIH R29-AR39522.

Th-Pos256

CHARACTERIZATION OF GENES INVOLVED IN NA^+-EXTRUSION BY *BACILLUS SUBTILIS*. ((J. Cheng, A.A. Guffanti, and T.A. Krulwich)) Department of Biochemistry, Mount Sinai School of Medicine of CUNY, NY, NY (sponsor: M. Sassaroli)

Transposon-mediated insertional libraries, prepared from *Bacillus subtilis* using the Tn917-containing plasmid pLTV1, were screened for mutant strains that were more sensitive than the wild type to Na^+ concentrations in the range of 0.2-0.5 M, at either pH 7 or pH 8.5. At the elevated pH, several distinct Na^+-sensitive strains, with different levels of sensitivity, were isolated. Two highly sensitive strains have been characterized initially. These strains exhibit reduced extrusion of $^{22}Na^+$ in a protocol in which energy-starved, $^{22}Na^+$-loaded whole cells were re-energized by the addition of 10 mM K^+-malate. Assays of membrane antiport activity also indicated a reduction in the respiratory-coupled Na^+/H^+ antiporter activity of the two strains relative to the wild type. The chromosomal DNA region that flanked the Tn917 insertion site of one of the two mutants has been cloned and sequenced. The data on this region are consistent with the insertion having disrupted an open reading frame that could encode a very hydrophobic protein whose partial deduced sequence displays marginal, if any homology with the sequence of previously reported Na^+/H^+ antiporters.

Th-Pos257

AMPLIFICATION OF A DNA FRAGMENT WHICH ENCODES A PORTION OF THE MITOCHONDRIAL CITRATE TRANSPORT PROTEIN. ((R.S. Kaplan[1], J.A. Mayor[1] and D.O. Wood[2])) Depts. of [1]Pharmacology and [2]Microbiology and Immunology, College of Medicine, University of South Alabama, Mobile, AL 36688.

The polymerase chain reaction (PCR) has been used to amplify a DNA fragment which encodes approximately two-thirds of the rat liver mitochondrial citrate transport protein (CTP). For these studies degenerate oligonucleotide primers were designed based on partial amino acid sequence data that we obtained. Rat liver cDNA was employed as the template. Amplification conditions were developed which yielded one major product (584 bp) which was cloned and sequenced. Translation of the resulting nucleotide sequence yielded a deduced amino acid sequence, portions of which displayed complete identity with the chemically determined partial amino acid sequence data. We have determined and/or deduced 203 residues of the CTP (i.e., the amino terminal two-thirds of the entire CTP sequence). Hydrophobicity analysis predicts four membrane-spanning segments, a finding that is consistent with the secondary structures predicted for other mitochondrial transporters. Inspection of the partial CTP sequence indicates regions that display strong similarity with sequences that are highly conserved in other mitochondrial carriers. In summary, to our knowledge this report provides the first sequence information on the mitochondrial CTP. (Supported by NIH Grants GM38785 to R.S.K. and AI20384 to D.O.W.).

Th-Pos258

CHARGE TRANSPORT BY ION TRANSPORTING MEMBRANE PROTEINS MEASURED ON SOLID SUPPORTED MEMBRANES.

((K. Seifert, K. Fendler and E. Bamberg)) Max-Planck-Institut für Biophysik, Kennedeyallee 70, 6 Frankfurt/M. 70, Germany. (Spon. by W. Stoeckenius)

A new method for the investigation of ion transporting membrane proteins is presented. Protein containing membrane fragments or vesicles are adsorbed to a solid supported membrane (SSM). The SSM consists of a lipid monolayer on a gold substrate which is coated with a mercaptan monolayer ($CH_3(CH_2)_{15,17}SH$). Both monolayers are prepared by spontaneous self-assembly from solutions. The specific conductance and specific capacitance of the SSM are comparable to those of black lipid membranes (BLM). Therefore, the SSM is used as a capacitive electrode in the same way as a BLM to measure charge translocation generated by ion transporting membrane proteins. An important advantage of the SSM over the BLM is its mechanical stability which opens the opportunity to rapid stirring or fast exchange of the electrolyte. The electrical activity of bacteriorhodopsin, bacterial reaction center, Na,K-ATPase, H,K-ATPase, and Ca-ATPase on the SSM is measured and compared to signals obtained on a BLM. The behavior of these ion pumps is similar to that in the BLM system. The SSM therefore represents an alternative method for the investigation of electrical properties of ion transporting transmembrane proteins.

Th-Poa259

EFFECTS OF OUABAIN ON ION AND AMINO ACID CONTENT IN HYPOTONICALLY SWOLLEN CULTURED CHICK HEART CELLS.

((R.L. Rasmusson, T.W. Smith, D.G. Davis and M. Lieberman)) Dept. of Cell Biology, Div. of Physiology, Duke U. Med. Ctr., Durham, N.C. 27710

Osmotically induced volume changes in spherical aggregates of cultured chick heart cells were measured optically as changes in diameter. Na, K and amino acid contents of confluent cultures were measured by atomic absorption spectroscopy or HPLC. Solution osmolality (Iso: 293 mOsm) was reduced by removing NaCl (Hypo: 164 mOsm); 0-Ca solutions contained 1 mM Na_2EGTA. Control volume(CV) was measured after 20 min. in Iso. Hypo solutions produced a rapid (3 min.) initial swelling to 1.42x CV, followed by a time-dependent regulatory volume decrease (RVD) to approximately 1.07x CV during continuous(20 min.) exposure to Hypo. This RVD was mediated by the loss of taurine, aspartate, glutamate and glycine as well as loss of inorganic ions. 0-Ca-Hypo produced only a very minor effect on RVD; loss of amino acids, Na and K were unperturbed from those measured in Hypo. However, RVD increased abruptly when Hypo treated cells were exposed to ouabain (0.1 mM). This ouabain induced effect was inhibited by 0-Ca but was insensitive to Cl^- replacement with methanesulfonate. Hypo+ouabain resulted in a 35% increase in amino acid loss compared to Hypo. Iso+ouabain did not cause significant loss of amino acids. These results suggest that Ca^{++} loading alone is insufficient to activate volume regulatory amino acid loss, but that Ca^{++} can stimulate amino acid loss once activated by swelling. Supported in part by NIH grants: HL-07063 and HL-27105. TWS is a Medical Student Research Fellow of the AHA.

Th-Poa260

CYCLIC AMP MEDIATES INACTIVATION OF THE SWELLING-INDUCED CHLORIDE CURRENT IN ISOLATED HEART CELLS.

((S.K. Hall, J. Zhang & M. Lieberman)) Dept. of Cell Biology, Division of Physiology, Duke University Medical Center, Durham NC 27710.

Hypoosmotically-induced swelling of cultured embryonic chick heart cells is associated with sustained activation of an outwardly rectifying chloride current (I_{Cl}), measured using whole cell patch clamp. I_{Cl} is attenuated by agents which elevate cAMP, *e.g.* 10μM forskolin or 20μM isoproterenol (Zhang *et al*; Physiologist 35(5):A18; 1992). We used the nystatin-perforated patch clamp technique to investigate this current without disturbing the cell interior. I_{Cl} activated rapidly following hypoosmotic challenge (from control 310mOsm to 280mOsm), reaching a peak of 575±175% control (SD, n=8) at ~1min; the current magnitude then declined, in most cases returning to control levels (5.2±4.0pA/pF; SD, n=8) within 3mins while the cell remained in hypoosmotic solution. Rp-cAMPS (11μM), an antagonist of cAMP at protein kinase A (PKA), inhibited inactivation of I_{Cl}, so that the current remained elevated (~400% control) in hypoosmotic solution. This agent also reversed the inhibition of I_{Cl} by forskolin in the whole cell configuration. When cAMP (10μM) and IBMX (1mM) were included in the patch pipette solution, the time course of I_{Cl} in the whole cell configuration showed the same inactivation profile seen using the perforated patch. These data demonstrate that cAMP levels are important in regulating the swelling-induced I_{Cl}, possibly by a PKA-mediated phosphorylation mechanism which inactivates the current.

Supported by NIH HL27105, AHA NC91F3 and The Walter P. Inman Fund.

Th-Poa261

PROTEIN SYNTHESIS INHIBITORS ACTIVATE GLUCOSE TRANSPORT INTO L6 MYOTUBES. ((N. Hayes, C. Biswas, H.V. Strout and J. Berger)) Merck Research Laboratories, Rahway, NJ 07065. (Spon. by G.J. Kaczorowski)

Skeletal muscle is the major insulin-sensitive tissue involved in the postprandial uptake of serum glucose with adipose playing a secondary role. Recently, it has been demonstrated that short-term protein synthesis inhibition leads to large increases in hexose transport by 3T3-L1 adipocytes (Clancy, et. al., JBC, 266,10122-10130 (1991)). In this study, we tested the effect of protein synthesis inhibitors on glucose transport by L6 myotubes, a skeletal muscle cell line which demonstrates a 2-fold increase in hexose transport rates within 30 min of addition of 100 nM insulin. Exposure of these cells to 300 μM anisomycin or 500 μM cycloheximide caused a maximal 2-fold increase in the 2-deoxyglucose transport rate within 6 h. When the cells were treated with both 300 μM anisomycin (6 h) and 100 nM insulin (30 min) the observed increase in hexose transport was greater than that elicited by either stimulant alone but was not completely additive. The effects due to either insulin (30 min) or anisomycin (6 h) on hexose transport kinetics were similar, resulting in a 2-fold increase in V_{max} but no significant alteration in the apparent K_m. Both protein synthesis inhibitors caused only small increases in GLUT4 expression and negligible effects on GLUT1 expression. Anisomycin, as well as insulin, induced the translocation of both transporters from intracellular vesicles to the plasma membrane though not in quantities sufficient to entirely account for the activation of transport. The results indicate that protein synthesis inhibitors stimulate hexose transport into L6 myotubes by increasing the number of transporters in the plasma membrane and augmenting the intrinsic catalytic activity of the transporters.

Th-Poa262

Mg^{2+} TRANSPORT ACROSS THE PLASMA MEMBRANE OF CARDIAC AND LIVER CELLS DEPEND ON THE PRESENCE OF PHYSIOLOGICAL CONCENTRATIONS OF EXTRACELLULAR Na^+ AND Ca^{2+}. (A. Romani*, C. Marfella and A. Scarpa) *Ist. Patologia Generale, Universita di Siena, Siena, 53100, Italy and Dept. Physiology & Biophysics, Case Western Reserve Univ., Cleveland, OH 44106, USA.

We have previously shown in cardiac and liver cells that the increase in cytosolic cAMP level induces the release of approx. 10% of total cell Mg^{2+} (1 mM) in the extracellular compartment, whereas the activation of the protein kinase C induces an appreciable influx (approx. 1 mM) in both cell types. The sequential application of these stimuli rapidly reverts the efflux into an uptake or vice versa. The Mg^{2+} transports across the plasma membrane do not depend on the extracellular Mg^{2+} concentration, over the range of 0 to 1.2 mM, but strictly depend on physiological concentrations of external Na^+ and Ca^{2+}. By decreasing the extracellular concentration of NaCl (from 120 to 0 mM), a concomitant decrease to a complete block in Mg^{2+} efflux and influx is observed both in cardiac and liver cells. These movements are restored by reintroducing 120 mM NaCl in the incubation system. A qualitatively similar decrease in Mg^{2+} movements is also observed by reducing the extracellular $CaCl_2$ (from 1.2 to 0 mM). When the external Ca^{2+} concentration is replaced by an equimolar concentration of other cations (i.e. Ba^{2+}, Mn^{2+}, Sr^{2+}) or when 100 uM Cd^{2+} is used as a Ca^{2+}-blocker, consistent modifications in Mg^{2+} efflux and influx are observed. Ba^{2+} can replace Ca^{2+} only in Mg^{2+} efflux but not Mg^{2+} influx. Mn^{2+} and Sr^{2+} abolish both Mg^{2+} efflux and influx. 100 uM Cd^{2+} reduces or abolishes the Mg^{2+} movements, in the presence or in the absence of 1.2 mM $CaCl_2$ in the medium, respectively. Finally, the inhibition of the ER or SR Ca^{2+} pumps by thapsigargin completely blocks the Mg^{2+} influx, while is ineffective on the Mg^{2+} efflux. These data indicate: I) the presence of physiological concentrations of extracellular NaCl and $CaCl_2$ are necessary for favouring both Mg^{2+} efflux and influx, II) the presence of a hormone-mobilizable Ca^{2+} store(s) is necessary for Mg^{2+} influx.

Th-Poa263

CHARACTERIZATION OF cAMP BINDING TO ADENINE NUCLEOTIDE TRANSLOCASE (AdNT). ((C. Marfella, A. Romani, J. Hwa, and A. Scarpa)) Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106. (Spon. by A. Scarpa)

We have previously observed in perfused hearts or livers, in isolated myocytes or hepatocytes, and in isolated mitochondria that increased [cAMP] results in a sizeable efflux of Mg^{++} from mitochondria (A. Romani, et al., 1991, J.Biol. Chem., a266, 24376, 1991). In permeabilized hepatocytes and isolated rat liver mitochondria, cAMP induced a Mg^{++} efflux which is fully inhibited by two inhibitors of the AdNT. Autoradiography (of non denaturating PAGE or denaturating SDS-PAGE) of the total mitochondrial protein or purified 32 KD protein (identified as AdNT) indicated that 32P-cAMP, 32P-α ATP or 32P-γ ATP bind to the AdNT. The protein labelled with 32P-γ ATP was electroeluted and digested by HCl. An aminoacid TLC was performed on the acid extract, but no incorporation of 32P-γ ATP to single aminoacids was observed suggesting the presence of binding rather than phosphorylation. Similar binding of both ATP and cAMP was observed in purified AdNT reconstituted in phosphatidylcholine liposomes. Both in isolated AdNT and in AdNT incorporated into liposomes, dot blot analysis indicates that cAMP binds to the AdNT with a Km of 22.5 nM. Maximal binding to AdNT was observed with 50 nM cAMP, a concentration similar to that which induces maximal Mg^{++} efflux from mitochondria. These data suggest that cAMP in vitro and in vivo can bind to the AdNT of mitochondria and that this binding is accompanied by mitochondrial Mg^{++} efflux. Supported by N.I.H. HL 18708.

Th-Poa264

TRANSSARCOLEMMAL LACTATE TRANSPORT IN GUINEA-PIG VENTRICULAR MYOCYTES ((R-C Shieh, J. S. Stuart, J. L Goldhaber, and J.N. Weiss))UCLA School of Medicine, Los Angeles, CA90024.

Previously, we reported that cardiac transmembrane L-lactate transport was predominately carrier-mediated and electrically-silent. We have further characterized properties of transmembrane L-lactate movement in isolated patch-clamped guinea-pig ventricular myocytes loaded with the pH-indicator carboxy SNARF-1 (0.1 mM) via the patch electrode. Under conditions where the ionic currents and other transport processes were blocked, a rapid increase in extracellular L-lactate ($[L^-]_o$) from 0 to 30 mM at a constant pH_o caused an intracellular acidification averaging 0.18 ± 0.02 units. Acidification in response to increased $[L^-]_o$ was saturable with a K_m~4 mM. The lactate transport inhibitor α-cyano-4-hydroxycinnamate at 5 and 1 mM inhibited the intracellular acidification by 81 ± 4 and 46 ± 14%, respectively. The lactate carrier showed stereospecificity, as the acidification produced by 30 mM D-lactate was only 64 ± 9% of that observed with 30 mM $[L^-]_o$. Insulin (0.1u/ml), isoproterenol (1μM), and phenylephrine (20 μM) did not significantly affect the acidification induced by elevated $[L^-]_o$. Replacing extracellular N-methyl-D-glucamine with 120 mM K^+ reduced the acidification in response to 30 mM $[L^-]_o$ by 52 ± 15% suggesting possible competition between H^+ and K^+ for cotransport with L-lactate. Further studies to investigate this possible interaction are underway.

Th-Pos265

INHIBITION OF ENDOSOMAL IRON TRANSPORT BY CHELATORS: LOWER LIMITS ON INTERMOLECULAR FE TRANSFER RATES.

((Y.C. Ho, J.D. Altazan, J.A. Watkins, C-Y. Li, and J. Glass)) Hematology-Oncology Section, Center for Excellence in Cancer Research, Treatment, and Education, LSUMC-S, Shreveport, LA, 71130.

The effects of chelators on iron (Fe) transport by isolated reticulocyte endosomes provides a method for probing Fe transfer by perturbing the kinetics and equilibria of mobilization. Ferrozine (FZ), Ferrene S (FS), α,α'-dipyridyl (DP), tripyridyl triazine (TPTZ), and EDTA added to the intra or extravesicular solution inhibit Fe mobilization with a pattern of inhibition that is significantly different between NADH and ascorbate mediated mobilization. In the absence of reductants, extravesicular chelators were unable to mobilize more than 3% of the total initial Tf derived Fe suggesting that most Fe was bound to intravesicular sites. Extravesicular chelators (200 μM) inhibit iron transport with a pattern that is consistent with measured membrane permeability. The pathway for ascorbate is more susceptable to inhibition by membrane permeable chelators compared to NADH confirming multiple Fe binding sites and alternate pathways of Fe transfer and reduction. In the absence of reductants, fluorescence spectra show minimal Fe(III) binding to the intravesicular chelators supporting models where Fe transfer occurs after reduction and a $K_a > 10^{15}$ M^{-1} for Fe(III) binding sites on the intravesicular surface. On the basis of considerations of expected competition kinetics, measured chelator Fe(II) and Fe(III) complex formation rates, using an ascorbate-citrate system, suggests a lower limit for intermolecular Fe transfer rates of 10^{-2} s^{-1}. Overall, insights into sequence of reduction and iron transfer as well as some properties of the Fe(II) binding sites of the pathways leading to iron mobilization are revealed.