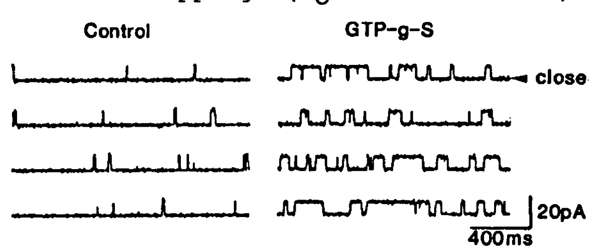
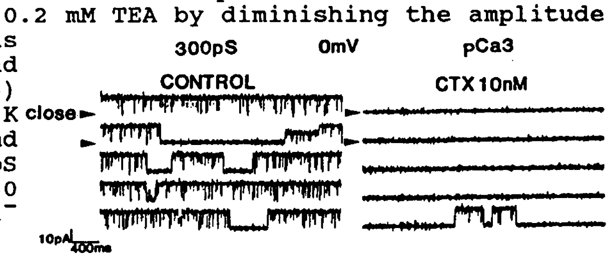
W-Pos209 ARACHIDONIC ACID AND OTHER FATTY ACIDS ACTIVATE K+ CHANNELS IN ISOLATED GASTRIC SMOOTH MUSCLE CELLS. Richard W. Ordway, John V. Walsh Jr., and Joshua J. Singer, Department of Physiology, Univ. of Massachusetts Medical School, Worcester, MA 01655
The effects of fatty acids (FAs) on membrane currents in smooth muscle cells isolated from the stomach of the toad, Bufo Marinus, were examined using patch clamp techniques. A variety of structurally diverse FAs were applied to the external surface of cells by pressure ejection from a micropipette. Whole-cell recording revealed that arachidonic acid (AA) and numerous other FAs which, unlike AA, are thought not to be oxygenated through cyclo-oxygenase and lipoxygenase metabolic pathways, elicited a large, outwardly rectifying K^+ current. This current was activated by micromolar concentrations of FAs and was shown to be a K^+ current by ion substitution. Effective FAs included long chain unsaturated FAs such as arachidonic (20:4 cis-5,8,11,14), linolenic (18:3 cis-9,12,15), linoelaidic (18:2 trans-9,12), and oleic (18:1 cis-9) acids, as well as a medium chain length saturated FA, myristic acid (14:0). FAs which were ineffective included the long chain saturated FAs stearic (18:0) and palmitic (16:0) acids, and the short chain saturate caprylic (8:0) acid. Single channel recording was employed to examine the underlying channel events. Arachidonic, linolenic, linoelaidic, and myristic acids were each applied to excised patches and found to activate a 22-25 pS K^+ channel (20 mM $[K^+]_o$/130 mM $[K^+]_i$). FA-activated channels were found in virtually all patches and were shown to be K^+ channels by ion substitution. The activation of K^+ channels by FAs suggests that these molecules may function as biological signals. The release of AA and other fatty acids from cellular phospholipids by phospholipase activation may lead to a direct action of these FAs on K^+ channels or their associated structures. Supported by NSF DCB-8511674 and NIH DK-31620.

W-Pos210 GTP-g-S ENHANCES THE OPEN PROBABILITY OF K_{Ca} CHANNELS FROM MYOMETRIUM INCORPORATED INTO BILAYERS. J. Ramos-Franco, L. Toro and E. Stefani. Dept. Physiol. Mol. Biophys. Baylor College of Medicine. Houston, TX 77030.
Membrane vesicles were isolated from non-pregnant rat uteri and collected from a 25:30% sucrose gradient interface. K_{Ca} channels were incorporated into lipid bilayers formed from equimolar mixtures of PS/PE (25 mg/ml). The channel conductance was 264 ± 7 pS (± S.E.; n=16) in a 261/61 K^+ gradient. The open probability, P_o was 0.97 ± 0.02 (n=14) at 0 mV and pCa=4. This value was practically unchanged during the burst activity of the channel, up to V_H of 80 mV (applied to the intracellular site of the channel). At V_H > +20 mV, the channel had burst activity with long closed states or "Ca^{2+}-trapping" (P_o=0.12 at 100 mV). The addition of 10 μM GTP-g-S and 1 mM $MgCl_2$ to the intracellular site of the channel increased the P_o by a factor >2, at 0 mV. The addition of norepinephrine (NE) (5-10 μM) potentiated this effect. On the contrary, NE (1-2 μM) by itself diminished the P_o by 10%. These data suggest that NE has opposite effects on K_{Ca} channels depending upon the activation of G proteins. Supported by NIH.



W-Pos211 IONIC CHANNELS FROM CORONARY SMOOTH MUSCLE INCORPORATED INTO BILAYERS. L. Vaca, L. Toro and E. Stefani. Dept. Physiology and Molecular Biophysics. Baylor College of Medicine. Houston, TX 77030.
Membrane vesicles were isolated from 20-30 porcine coronary arteries after scraping off the endothelium. K^+ channels were detected at the 20:25% and 25:30% interfaces of a sucrose gradient. Cl^- channels were preferentially encountered at the 20:25% interface. Channels were incorporated into lipid bilayers formed from 3:1 mixtures of POPE/POPC (40 mg/ml). Several K_{Ca} conductances were found, that differed from each other in their Ca sensitivity. K_{Ca-1} (298 ± 5 pS; n=4) had a P_o<0.5 at 0 mV and pCa=4. In contrast, K_{Ca-2} (313 ± 10 pS; n=7) had a P_o>0.9 under the same conditions. Both channels were closed at a pCa near 7 and were CTX sensitive (5-10 nM). K_{Ca-2} was blocked by 0.2 mM TEA by diminishing the amplitude of the channel. K_{Ca-3} (250 ± 1 pS, n=5) was open at a pCa near 7 (P_o, 0.2-0.3) and had a P_o=0 at pCa 4. K_{Ca-4} (183 ± 3 pS, n=4) had a P_o=0 at pCa 7. Also small K conductances were detected (94 ± 5, n=3 and 51 ± 6 pS, n=5). Cl^- channels had a 10 pS conductance and a reversal potential of +40 mV when recorded using a 250/50 Cl^- gradient. Supported by AHA, Texas Affil.



W-Pos212 SELECTIVITY OF POTASSIUM CHANNELS OF LOCUST MUSCLE IS MODIFIED BY THE PLANT TOXIN, RYANODINE. Ely Gorczynska[1], Patrick L. Huddie[2], & Peter N.R. Usherwood[3]; [1]Department of Biophysics, School of Physics, University of New South Wales, PO Box 1, Kensington, Sydney, NSW, Australia; [2]NINCDS Laboratory of Molecular and Cellular Neurobiology, National Institutes of Health, Building 9, Room 1w125, Bethesda, MD 20892; [3]Department of Zoology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK.

Ryanodine, a lipid soluble alkaloid, is a powerful inhibitor of contraction of vertebrate cardiac muscle and insect muscle, and is believed to affect the Ca sequestration mechanisms of the sarcoplasmic reticulum. However, ryanodine also converts the graded electrogenic potentials of locust muscle to all-or-none action potentials (Usherwood, 1962, Comp. Biochem. Physiol 6, 181-189), and our experiments suggest that the actions of ryanodine are not limited to the SR.

Two types of potassium channels were studied in isolated membrane patches of locust muscle, a 'large' conductance channel of ≈ 100 pS, and a 'medium' conductance channel of ≈ 20 pS. A 'small' K^+ channel was also observed, but not studied in detail. At concentrations of 10^{-9}M - 10^{-5}M ryanodine alters the cation selectivity of the 'large' and 'medium' channels. The P_{Na}/P_K ratio before ryanodine treatment was ≈ 0.07, but after exposure to the drug the P_{Na}/P_K ratio approached unity, indicating a profound decrease in K^+ selectivity.

W-Pos213 NOISE ANALYSIS OF QUINIDINE-INDUCED BLOCKADE OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN DISSOCIATED GASTRIC SMOOTH MUSCLE CELLS. Brendan S. Wong, Department of Physiology, Baylor College of Dentistry, Dallas, TX 75246

Single-channel studies on enzymatically dissociated single smooth muscle cells from Bufo marinus have demonstrated that quinidine induced rapid flickerings between the open and blocked states of Ca^{2+}-activated K^+ channels. Noise analysis was used to analyze data obtained from multiple-channel patches. In the absence of quinidine, the power spectrum was well fitted by a single Lorentzian with a corner frequency of approximately 20 Hz (τ = 7.96 ms). The spectra, in the presence of quinidine, had to be fitted by double Lorentzians, with one corner frequency very similar to that observed under control conditions (< 30 Hz). The other Lorentzian component varied with membrane potential and showed a corner frequency ranging from 350 to 3700 Hz (τ = 0.46 ms and 0.04 ms, respectively). These values are considerably larger than that due to the intrinsic gating kinetics of the channel. The corner frequency was also found to increase with membrane depolarization and quinidine concentration. Using a sequential channel blockade model, the blocking (k_+) and unblocking (k_-) rate constants were found to be voltage-dependent and increase with membrane depolarization. The apparent dissociation constant was found to be 253 μM and 209 μM for +20 mV and -60 mV, respectively. The results are consistent with quinidine being a moderately fast channel blocker, with the observed flickering and increased open-channel noise caused by rapid transitions between the open and blocked states.

W-Pos214 APPLICATION OF THE POLYMERASE CHAIN REACTION TO K^+ CHANNEL MOLECULAR BIOLOGY. S. Roberds* K. Knoth*, C. Poteet+, M. Tamkun+* (Intr. by D.B. Zimmer), Departments of Pharmacology* and Molecular Physiology and Biophysics+, Vanderbilt Medical School, Nashville, TN 37232.

The polymerase chain reaction (PCR) uses thermal cycling to sequentially denature the DNA, allow the annealing of oligonucleotide primers, and synthesize new DNA via primer extension. Repeated cycling can amplify sequences between two primer binding sites by 10^{10}. Our goal is to isolate cDNA encoding new voltage-sensitive K^+ channels by targeting conserved protein domains essential to ion transport with PCR primers. Variable codon usage requires degenerate oligonucleotides as primers. Therefore we first confirmed that mixed, inosine-containing primers amplify voltage-sensitive Na^+ channel cDNA from brain. Degenerate primers (18-20 bp) based solely on Na^+ channel amino acid sequence specifically amplified 700 bp fragments of rat brain Na^+ channels. No other bands were amplified even though each primer was capable of hybridizing to as many as 1000 different nucleotide sequences. We then applied this approach to brain and cardiac K^+ channels by designing degenerate, inosine-containing primers based on amino acid sequences that are highly conserved between the *Drosophila* and mouse brain K^+ channels (Tempel, L., et al, 1988, Nature 332, 837-839). PCR amplified 300 or 800 bp fragments from both rat brain and rat heart, depending on the pairs of primers used. Nucleotide sequencing of subcloned fragments revealed high amino acid sequence homology between brain and heart K^+ channels, especially in the membrane spanning domains and the putative voltage-sensing region. However, the codon usage was much less conserved. Studies using cDNA and oligonucleotide hybridization to various brain K^+ channel PCR fragments indicated that multiple isoforms exist in this tissue. We are currently examining the number of K^+ isoforms in heart. The use of degenerate primers makes PCR an attractive substitute for low stringency library screening to identify new channel isoforms.

W-Pos215 RESTING POTASSIUM CURRENT IN ISOLATED BULLFROG SYMPATHETIC NEURONS.
Stephen W. Jones, Dept. Physiol. & Biophys., Case Western Reserve University, Cleveland, OH 44106.

The origin and magnitude of the resting potential in frog sympathetic neurons has been controversial. Microelectrode recordings from cells in the excised ganglion generally give values positive to -60 mV, but this has been attributed by some to impalement damage.

Under whole-cell recording, at -60 mV or more negative, a steady-state current remained that showed little voltage dependence. The steady-state current varied between cells, with average conductance 5.3 ± 0.6 nS ($\pm$ s.e.m., n = 28) and average reversal potential of -56 ± 4 mV. The resting potential, estimated as the zero-current point under voltage clamp, was -67 ± 2 mV, approximately as expected for a mixture of voltage-independent steady-state current and M-current (24 ± 3 nS at -30 mV, reversing at -88 ± 1 mV).

In several cells (12 of 28) the reversal potential of the resting current was quite negative (between -65 and -95 mV), and was essentially equal to the resting potential of the cell, indicating that the M-current contributed little to setting the resting potential.

Preliminary evidence indicates that the resting current is (1) potassium selective, (2) blocked by mM Ba^{2+}, (3) not blocked by agonists that inhibit M-current, (4) not calcium-dependent.

These results suggest that a resting potassium current distinct from known voltage-dependent currents plays a major role in setting the resting potential of isolated bullfrog sympathetic neurons. This supports the idea that the normal resting potential of these cells is quite negative, so that little M-current is active at rest. However, it will be necessary to verify that dialysis of isolated cells under whole-cell conditions does not affect these resting currents.

W-Pos216 CHARACTERIZATION OF MACROSCOPIC OUTWARD CURRENTS OF CANINE COLONIC SMOOTH MUSCLE CELLS. Cole, W.C. and K.M. Sanders. (Intr. by J. Sutko), Dept. of Physiology, University Nevada School of Medicine, Reno, NV 89557 and Dept. of Physiology, University of Manitoba, Winnipeg, MB, R2H 2A6.

Macroscopic outward currents of isolated smooth muscle cells from the circular muscle of the canine proximal colon were examined by whole-cell voltage clamp method. Outward currents were carried by K^+ ions based on sensitivity to TEA and shift in reversal potential of tail currents as a function of transmembrane potassium gradient. Four components of outward current were observed; a time-independent current and three time-dependent currents. The time-independent component showed strong outward rectification positive to -25 mV and was blocked by external TEA. The time-dependent components include: 1) a transient Ca^{2+}-activated K^+ current sensitive to manipulations of external Ca^{2+} and Ca^{2+} influx through dihydropyridine-sensitive channels (blocked by EGTA (0.1 mM) and nifedipine (1 uM) and increased by elevated Ca2+ (8 mM) and BAY K 8644 (1 uM)). It demonstrated apparent voltage-dependent inactivation, with peak suppression at -20 mV and slight recovery positive to -10 mV, likely as a result of the inactivation properties of inward Ca^{2+} current. 2) A delayed rectifier current was observed that decayed very slowly with time and showed no dependence on Ca^{2+}. 3) Spontaneous transient outward currents (STOCs) that were blocked by ryanodine (2 uM) were also recorded. Ca^{2+}-activated K^+ current may contribute a significant conductance to the repolarizing phase of the electrical slow wave. Supported by NIADDKD grant DK38717 and Career Development Award to KMS DK01209.

W-Pos217 INHIBITION OF Ca^{2+} -ACTIVATED K^+ CURRENT IN COLONIC SMOOTH MUSCLE CELLS BY ACETYLCHOLINE INVOLVES PERTUSSIS TOXIN SENSITIVE G-PROTEINS. Cole, W.C., A. Carl, K.M. Sanders. (Intr. by W. Gerthoffer) Dept of Physiology, University Nevada School of Medicine, Reno, Nevada, 89557 and Dept. of Physiology, University of Manitoba, Winnipeg, MB, R2H 2A6.

The role of G proteins in the transduction mechanism involved in ACh inhibition of Ca^{2+}-activated K^+ current in isolated myocytes from the circular smooth muscle of the canine proximal colon was assessed. ACh induced depression of whole-cell Ca^{2+}-activated K^+ current was reversed within 2-4 min upon washout when GTP (0.1 mM) was included in the pipette solution. However, the effect of ACh could not be reversed when the non-hydrolyzable GTP analogs, GTP S (0.1mM) or GppNHp (0.1 mM) were employed. We also noted that the time dependent relaxation of the Ca^{2+} activated K^+ current during long depolarizing pulses (10 sec) was more rapid in the presence of added GTP (or its analogs) (time constant of 1500 ms versus control 3500 ms). Dialysis of the cell interior with pipette solution containing pertussis toxin (10 ng/ml) for 30 min had no effect on the whole cell currents evoked upon depolarization. However, inhibition of Ca^{2+}-activated K^+ current by ACh (10uM) was completely abolished. We conclude that pertussis toxin-sensitive G-proteins are involved in muscarinic suppression of Ca^{2+}-activated K^+ currents in colonic smooth muscle cells. Supported by research grant from NIADDKD (DK38717) and a Career Development Award (DK01209) to KMS.

W-Pos218 ACh SUPPRESSES A Ca^{2+}-DEPENDENT K^+ CURRENT IN COLONIC MYOCYTES OF THE CANINE PROXIMAL COLON. Carl A., W.C Cole and K.M Sanders. (Intr. by J. Peacock) University Nevada, School of Medicine, Reno, NV 89557. and Dept. of Physiology, University of Manitoba, Winnipeg
Previous intracellular microelectrode studies on intact colonic muscles have shown that ACh increases the amplitude and duration of the plateau phase of electrical slow waves. These findings suggest, that ACh either increases an inward current and/or suppresses an outward current. Whole-cell voltage clamp experiments from freshly dispersed smooth muscle cells from canine proximal colon were performed to test this hypothesis. ACh (10^{-5} M) in the bath solution reduced the time-dependent outward currents evoked by depolarizing test steps to all potentials in the range of -45 to +30 mV. Tail currents were also reduced by ACh suggesting that the effect of the drug was suppression of an outward current. When cells were pretreated with 1 uM nifedipine to abolish the Ca^{2+} dependent component of the outward current, no further reduction of the outward current with ACH was seen. Single channel experiments were performed to determine whether ACh has a direct effect on Ca^{2+} activated K channels in this tissue. The voltage dependent activation of these channels was determined in on-cell configuration using voltage ramp protocols. The patch potential was changed over 4 sec from +150 mV to 0 mV and current traces of several ramps were averaged and leak corrected. However, when 10^{-5} M ACh were included in both the bath and the pipette solution, a shift in voltage dependent activation by 21 ± 15 mV (n=5) occurred. This effect of ACh on the voltage dependent activation of Ca^{2+}-activated K^+ channels can explain most of the electrophysiological effects of muscarinic, excitatory stimulation in the circular layer of the proximal colon. Supported by research grant from NIADDKD (DK38717) and a Career Development Award (DK01209) to KMS.

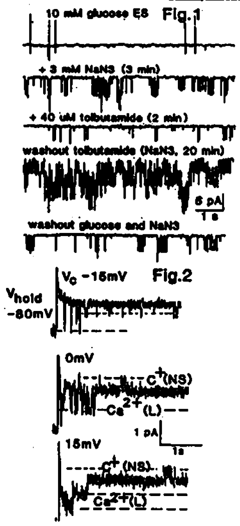
W-Pos219 OUTWARDLY RECTIFYING K^+ CURRENT AS A MARKER OF CELLULAR ACTIVATION IN HUMAN MACROPHAGES. B. JOW AND D.J. NELSON Committee on Cell Physiology, The University of Chicago, Chicago, Il. 60637.

Although an outwardly rectifying K^+ conductance has been described in murine peritoneal macrophages and a murine macrophage-like cell line (Ypey and Clapham, Proc. Natl. Acad. Sci., 1984, 81:3083; Gallin and Sheehy, J. Physiol., 1985, 369:475) the expression of this conductance in human monocyte-derived macrophages (HMDM) is rare. Whole-cell current recordings were obtained using HMDM differentiated in adherent culture for varying periods of time following isolation using a Ficoll-Hypaque density gradient centrifugation step. Of the 596 cells in the study obtained from a total of 22 donors, we were unable to observe the presence of this inactivating, holding potential dependent outward current. In contrast, whole-cell current recordings from human alveolar macrophages obtained from bronchoalveolar lavage and maintained in short term culture, revealed a much higher frequency of current expression (58%; n = 128). Based on the dramatic increase in outwardly rectifying K^+ current frequency in the human alveolar macrophage over that observed in the HMDM, we investigated the possibility that current expression might be linked to state of cellular activation. HMDMs were activated and activation electrophysiologically assayed using a two signal protocol. Cultures were stimulated with combinations of recombinant interferon-gamma (gamma-IFN, 10 U/ml) or phorbol myristate acetate (PMA, 0.01 or 1 μg/ml) as the "priming" signal and/or lipopolysaccharide (LPS, 2 μg/ml) as the "activation" signal for periods of up to 24 hours. The combination of LPS and gamma-IFN or PMA greatly enhanced the frequency of current expression, 49 of the 183 cells investigated (27%) expressed the transient outward current. The time course of the observed increase in current expression is consistent with a mechanism which would involve gene transcription and/or expression. The increase in K^+ current appears to provide an electrophysiological marker of cellular activation and might alter the potential of the mononuclear phagocyte to respond to extracellular signals mediating chemotaxis, phagocytosis, tumoricidal functions. Supported by NIH GM36823.

W-Pos220 Chimeric Shaker cDNA transcripts induce the expression of kinetically distinct K^+ currents in Xenopus oocytes. Richard Swanson, Joanne Antanavage, Robert B. Stein, Mark Tanouye*, and Jeffrey S. Smith, Merck, Sharp & Dohme Research Laboratories, West Point, PA 19486 and *Division of Biology, California Institute of Technology, Pasadena, CA 91125

Chimeric Shaker locus clones were constructed by cleaving H4 and H37 cDNAs at their observed XbaI site and ligating the 5' and 3' ends. The resulting chimeras encode proteins containing the N-terminus of H4 linked to the C-terminus of H37 (H4/H37) or the N-terminus of H37 followed by the C-terminus of H4 (H37/H4). In vitro synthesized transcripts of the parental and chimeric cDNAs were polyadenylated and injected into Xenopus oocytes at at 10 ng poly A^+ RNA/cell. After 3-5 days incubation at 25°C, oocytes were screened for the presence of K^+ currents using the two microelectrode voltage clamp technique. Cells injected with H4 or H37 poly A^+ RNA expressed rapidly activating A-type currents identical to those reported by Iverson, et al., (1988) PNAS 85: 5723-5727. H4/H37 injected oocytes expressed a rapidly activating A current (time to peak at 40 mV = 5 ms) which inactivated completely during a 25 ms pulse. This current appeared kinetically similar to H4 induced currents except that recovery from inactivation was slowed compared to H4. H37/H4 injected oocytes expressed a rapidly activating A current (time to peak at 40 mV = 11 ms) similar to H37 except that currents showed only partial inactivation during 12s depolarizing pulses. These experiments lend further support to the hypothesis that K^+ channel diversity may arise from the alternative splicing of RNA transcribed from the Shaker locus of Drosophila.

W-Pos221 <u>ION CHANNEL CURRENTS IN CANINE PANCREATIC ISLET B CELLS</u>. David Pressel and Stanley Misler. Washington University Medical Center, St. Louis, MO.



Ion channel currents were recorded from surface cells of isolated, functional canine pancreatic islets (gift of David Scharp). Cell attached patches formed with 138mM KCl pipettes (Ringers bath) displayed three currents with E_{rev} of $V_c=(-V_{pipette})=70$mV: 1)A 60 pS conductance, voltage insensitive channel reversibly closed by bath applied glucose or α-ketoisovalerate (3-10mM) or tolbutamide (20-40uM), but reopened by sodium azide (NaN_3); action currents appear with channel closure (Fig. 1). These features are characteristic of an ATP sensitive K^+ channel, [K^+(ATP)]. 2) A 20pS channel with longer open times; these appear to be nonselective cation channels, [C^+(NS)]. 3) A 200pS voltage dependent channel carrying outward currents at V_c>100mV, identified as "maxi $K^+(Ca^{++})$" channels. Cell attached patches formed with "Ba^{++}" pipettes (bath: 138mM KCl + 5uM BAY K 8466) display voltage dependent inward currents at V_c>-30mV. These have an extrapolated $E_{rev}\approx50$mV, an estimated conductance of 25pS, and resemble an "L-type" Ca^{++} channel. Also seen is a 10pS channel with an E_{rev} of $V_c\approx$-5mV and presumed to be C^+(NS) (Fig 2). The finding of these channels in a classic preparation used to study in vivo insulin secretion suggests the canine islet is a good model for the study of neurohumoral and metabolic control of ion channels in the B cell.

W-Pos222 **EXTERNALLY APPLIED TETRAALKYLAMMONIUM IONS BLOCK OPEN DELAYED RECTIFIER K CHANNELS AT BOTH INTERNAL AND EXTERNAL SITES.** **by T.E. DeCoursey, W.B. Im* & F.N. Quandt, Dept. of Physiology, Rush Medical Center, Chicago, IL & *Upjohn Co., Kalamazoo, MI.**

Internally applied quaternary ammonium ions (QA^+) block K channels in nerve only when the channel is open (*e.g.* Armstrong & Hille, 1972, *J.Gen.Physiol.* *59*:388). However, little information is available concerning the nature of interactions between QA^+ and the external blocking site of the K channel. Long chain tetraalkylammonium ions (TAA^+) including tetrabutylammonium ($TBuA^+$), tetrapentylammonium ($TPeA^+$), and tetrahexlyammonium ($THexA^+$), potently block whole–cell delayed rectifier K currents in rat type II alveolar epithelial cells when applied externally at 0.1–100 μM. This block resembles that described in nerve for internal application: (*1*) channel opening kinetics are not much affected, (*2*) K currents "inactivate" (are blocked) rapidly after channel opening, (*3*) the block rate increases with [TAA^+], (*4*) recovery from block is much faster when $[K^+]_o$ is increased. The difference between internal and external TAA^+ blocking sites was studied by examining single delayed rectifier channel currents in mouse neuroblastoma (N1E–115) cell membrane patches. Long chain internal TAA^+ reduce open time and exhibit discrete blocked states. For example, internal 30 μM $TBuA^+$ reduced mean open time from 164 ms to 26 ms (at 11° C) with a block time of 50 ms. The block time for external $TBuA^+$ was only 3 ms, indicating that $TBuA^+$ binds to a distinct external receptor site with a much faster dissociation rate than that associated with the internal site. In contrast with $TBuA^+$, block times for external or internal $TPeA^+$ were similar. Although the K channel has distinct internal and external receptors for TAA^+, longer chain length TAA^+ may reach the internal site through the membrane phase. *Supported by NIH grants SK04–HL01928 & R01–HL37500 (TD).*

W-Pos223 HISTAMINE AND METHACHOLINE INHIBIT VOLTAGE-DEPENDENT POTASSIUM CURRENTS IN AIRWAY SMOOTH MUSCLE CELLS. M.I. KOTLIKOFF, Dept. of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104-6046.

Potassium currents were studied in isolated canine airway smooth muscle cells using the whole-cell method. Voltage clamped cells depolarized to potentials positive to -30 mV revealed inactivating and non-activating outward currents that were identified as potassium currents on the basis of blockage by Cs^+ and TEA^+, and by current reversal potential. The currents displayed different voltage activation and inactivation relationships, and different sensitivity to TEA^+. Neither current was altered under conditions in which the inward current was completely blocked by Mn^{++} or nifedipine, when the inward current was carried by Ba^{++}, or when the charybdotoxin (50 nM) was added to the bath, suggesting that the currents can best be classified as delayed rectifier currents. The delayed rectifier currents were reversibly inhibited by histamine and methacholine. Evidence for the inhibition of these currents includes: 1) a decrease in the amplitude of the apparent outward current evoked by voltage clamp steps; 2) a decreased ohmic jump at the end of the clamp steps, indicating a conductance decrease; 3) a depressed conductance observed during ramp depolarizations; 4) a decreased conductance during current clamp; and 5) no effect of histamine or methacholine on the inward currents under conditions in which the delayed rectifier currents were blocked. This finding represents a previously unreported effect of agonists on delayed rectifier potassium currents in smooth muscle.

W-Pos224 **BARIUM INHIBITS Ca-ACTIVATED K CHANNELS IN PANCREATIC B-CELLS.** A.A. Gonçalves, M.X. Li, I. Atwater and E. Rojas. (Intr. by C. Colton) LCBG, NIDDK, NIH, Bethesda, MD and Depto. Fisiol. e Biofísica, IB-UNICAMP, Campinas, S.P. Brasil.

In mouse pancreatic B-cells, glucose reduces K permeability, depolarizes the membrane, induces Ca action potentials and increases insulin release. Ba mimics these effects. In order to determine which K channels are involved in glucose sensing, we have studied the effects of Ba and its competition with Ca on the K permeability in B-cells from mouse and rat using standard intracellular potential recording and patch clamp techniques. Ba (0.5 - 5 mM) depolarized mouse B-cells, induced spikes and increased the input membrane resistance either in the absence or presence of 2.5 mM Ca. Spikes augmented in duration with increasing [Ba]. In inside-out patches from cultured rat B-cells (high Na in the pipette), 0.1 to 10 mM Ba decreased the open time probability of the Ca-activated K-channel, K(Ca), in the presence of 0.1 mM Ca. Increasing [Ca] from 0.1 to 0.5 mM partially reversed this inhibition. Also, the K(Ca) channel exhibited a sub-state conductance when Ba and Ca were present. In whole cell configuration, Ba inward currents were larger than Ca currents and did not inactivate. In conclusion, Ba replaces Ca in the inward current, but competes with it for the membrane depolarization and increased imput resistance, indicating that the K(Ca) channel plays an important role in the control of B-cell membrane potential. (A.A.G. was supported in part by FAPESP and CNPq).

W-Pos225 PARTIAL PURIFICATION OF THE HIGH CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNEL FROM BOVINE AORTIC AND TRACHEAL SMOOTH MUSCLE. M.L. Garcia, J. Vazquez, A. Galvez, F. King, P. Feigenbaum and G.J. Kaczorowski, Merck Institute, Rahway, N.J. 07065.

We have characterized a single class of high affinity binding sites for charybdotoxin (ChTX), a specific blocker of the ca. 200 pS Ca^{2+}-activated K^+ channel ($P_{K,Ca}$) in purified sarcolemmal membrane vesicles derived from either bovine aortic or tracheal smooth muscle. The properties of these sites suggest that they are associated with $P_{K,Ca}$. To verify this assumption, the ChTX receptor has been subjected to purification from these membrane sources. Using [^{125}I]ChTX binding as an assay, receptor activity has been solubilized from aortic or tracheal sarcolemma with 1% digitonin in 60% yield after double extraction of vesicles. Solubilized material maintains identical binding properties as native receptor, and can be reconstituted in asolectin liposomes. The solubilized receptor has been partially purified in the presence of 0.1% digitonin by employing two sequential steps: a Wheat Germ Lectin Sepharose 6MB column which retains ChTX binding activity, followed by elution in the presence of N-acetyl-D-glucosamine and NaCl; a mono Q ion exchange HPLC column eluted with a discontinuous NaCl gradient at pH 9.0 in which binding activity appears at 0.3-0.5 M NaCl. Together, these steps purify the ChTX receptor ca. 100-fold with 20% yield of initial activity. Crosslinking studies with [^{125}I]ChTX and either membrane preparation demonstrate specific covalent incorporation of ChTX into a 105 kDa protein after SDS-PAGE. Enrichment of a 105 kDa protein is also observed in the partially purified preparation. Final purification steps are under development using separation techniques based on molecular size. The homogeneous ChTX receptor will be used to prove that this entity is functionally associated with $P_{K,Ca}$ in smooth muscle.

W-Pos226 DIFFERENCES IN K CHANNEL BEHAVIOR IN GLUCOSE-RESPONSIVE AND GLUCOSE-UNRESPONSIVE INSULIN SECRETING TUMOR CELL LINES. G.T. Eddlestone, B. Ribalet and S. Ciani. Dept. of Physiology; BRI; JLNRC. Univ. of Calif., Los Angeles, CA 90024.

Of two insulin secreting cell lines, HIT and RINm5F, the former secretes significant amounts of insulin in response to a glucose challenge, the latter does not. Since insulin secretion is causally associated with membrane electrical activity in the pancreatic islet B-cell, we hypothesized that this difference in secretory behavior is paralleled by differences in the behavior of two glucose-modulated K channels in the cell membrane. To test this hypothesis we performed experiments using the patch clamp technique. In cell-attached patch experiments, glucose dose-dependently inhibited the ATP-sensitive K channel over the range 0 to 10 mM in both the HIT and the RINm5F cell, in each case half maximal inhibition was attained with about 0.5 mM glucose. Similarly, glucose inhibited a larger K channel, subsequently identifed as a voltage- and calcium-dependent (K(Ca,V)) channel, in the range 0 to 25 mM in both cell types. In this case half maximal channel inhibition was attained with 7 mM glucose in the HIT cell and 4.5 mM in the RINm5F cell. In inside-out excised patches the concentration dependent blocking effect of ATP on the K(ATP) channel in the two cell types was similar, in each case half maximal channel inhibition required about 60 uM ATP. Investigation of the calcium dependence of the K(Ca,V) channel in the HIT cell inside-out patch indicated that 50% of maximal channel activity occurred at pCa5, 6 and 7 at patch potentials of -45, +5 and +100 mV respectively; a similar response to that in the adult rat B-cell (Findlay et al. J. Membrane Biol. 83:169-175 (1985)). In contrast, experiments on the RINm5F cell inside-out patch indicated a much reduced calcium sensitivity of the channel with 50% of maximal activity at pCa5 observed with a patch potential of +40 mV and the activity at pCa6 reaching only about 5% at +120 mV. These data suggest that the aberrant behavior of the K(Ca,V) channel of the RINm5F cell may be a factor underlying the lack of glucose responsiveness of this cell type. Supported by a NSF grant DCB-85 17413, an award from the ADA (W-P860812) and a grant from the MDA.

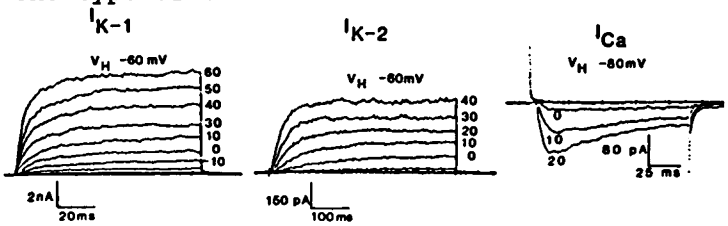
W-Pos227 ATP-DEPENDENT K CHANNEL WITH MULTIPLE CONDUCTANCE SUBSTATES IN CRUSTACEAN GLIAL CELLS. Christian Erxleben, (Intr. by A. Hermann). Fakultät für Biologie, Universität Konstanz, W. Germany.

Single potassium channels were characterized in cell-attached and excised patches of glial cells which ensheath the abdominal stretch receptor neuron of the crayfish (*Orconectes limosus*). In cell-attached patches the predominant channel is a non-voltage-dependent K channel. It shows multiple subconductance states of smaller as well as larger conductance than the main state. The current-voltage relationship in cell-attached patches (160mM KCl in the pipette) and outside-out patches excised in 160mM K saline was linear, with a slope of the main conductance state of 120pS. The channel is insensitive to 5mM TEA and 1mM 4-aminopyridine applied from the outside. In outside-out patches with minimal salt solution in the pipette (that is, for the cytoplasmic site: 160mM KCl, 10mM HEPES, 1mM $MgCl_2$ and 0.2µM free Ca^{++}) channel activity decreased within minutes after excision of the patch. In contrast, activity could be maintained if the pipette solution was supplemented with 2mM ATP. The gradual rundown of channel activity in minimal saline and the ability of intracellular Mg-ATP to conserve channel activity suggests that the subconductance K channel may be regulated via phosphorylation, by mechanisms that have been established for other K and Ca channels. This would provide a means of modulation of glial K channels, channels that are thought to be involved in the process of spatial buffering.

Supported by the DFG (SFB 156).

W-Pos228 K^+ AND Ca^{2+} CURRENTS FROM CORONARY SINGLE SMOOTH MUSCLE CELLS. L. Toro, S. D. Silberberg, A.M. Brown and E. Stefani. Dept. Physiology and Molecular Biophysics. Baylor College of Medicine. Houston, TX 77030.

Freshly dissociated smooth muscle cells from the coronary artery of the pig were studied using the whole-cell patch clamp technique. Cells were elongated in shape and measured 100- 200 μm length x 5-10 μm width. The input capacity of the cells was 20 ± 2 pF (± S.E.; n=21). Two types of K^+ currents (I_{K1} and I_{K2}) were found. I_{K1} had an activation time constant, τ_{on}, of 15 ± 1 ms (n=9) at 0 mV and a maximum slope conductance, G of 3.8 ± 0.5 mS/cm^2 (n=7). I_{K2} had a slower τ_{on} (146 ± 13 ms) at 0 mV (n=10) and a smaller G (0.48 ± 0.04 mS/cm^2; n=10). I_{K1} was detected at -30 mV and I_{K2}, at -20 mV. Both currents did not inactivate during the test pulses (100 and 500 ms). Only one type of Ca^{2+} current was recorded when the holding potential was changed from -80 mV to -40 mV. I_{Ca} had a time to peak of 14 ms at 20 mV from a V_H of -80 mV. With 10 mM Ca in the external solution and 10^{-7} Ca internal, I_{Ca} was detected at 0 mV and had a peak amplitude at 40-50 mV. Supported by AHA, Texas Affil. and NIH.



W-Pos229 AGE DEPENDENT EFFECTS OF cAMP AND cGMP ON THE DELAYED OUTWARD K^+ CURRENT IN THE AORTA. Peyrow, M., Chahine, M. and Bkaily, G. Department of Physiology and Biophysics, University of Sherbrooke, Sherbrooke, Quebec, Canada. J1H 5N4.

The increase of $[cAMP]_i$ and $[cGMP]_i$ were reported to regulate Ca^{2+} channels in heart and vascular smooth muscle (VSM). Agents that increase $[cAMP]_i$ or $[cGMP]_i$, such as Isoproterenol(Iso) or Nitroprusside relax vascular smooth muscle. Recently, we reported that increasing $[cAMP]_i$ and $[cGMP]_i$ decreased the slow inward current (I_{si}) in the rabbit's aorta; however, the regulation of K^+ current in VSM by cyclic nucleotides is not known. Thus, we studied the effects of Iso, Nitroprusside, 8-Bromo cAMP and 8-Bromo cGMP on single (VSM) cells of rabbit aorta at different ages, using the whole-cell voltage clamp technique. The increase of $[cAMP]_i$ by addition of 8-Bromo cAMP or Iso, decreased the delayed outward K^+ current in young and adult rabbit VSM. However, increasing [cGMP]i by the addition of 8-Bromo cGMP or Nitroprusside increased the delayed outward K^+ current in adult rabbit VSM and decreased I_K in young rabbit. Thus, the modulation of the delayed outward K^+ current by cAMP is not age-dependent, however the modulation of the same current by cGMP is age dependent. Furthermore, the increase of the delayed outward K^+ current by cGMP in adult rabbit VSM may explain in part the vasodilating effect of Nitroprusside on VSM. The blockade of I_{si} and I_K by increasing $[cAMP]_i$ may explain the vasorelaxation on VSM by Iso. This work was supported by MRCC (MA 8920) and Dr. Bkaily is a scholar of CHF.

W-Pos230 **LOW Ca^{2+}-AFFINITY MAXI-K^+ CHANNELS IN MITOTIC AND INTERPHASIC HUMAN FIBROBLASTS.**
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A previous patch-clamp study revealed that human fibroblasts possess large conductance K^+ channels which are fully activated only at high, non physiological, internal Ca^{2+} concentrations (Galietta, L.J.V. et al. Pfluegers Archiv: 1988, in press). Since this low Ca^{2+} affinity could be related to the mitotic phase at which the cells were studied, two types of experiments have been performed in order to test the channel Ca^{2+}-sensitivity in fibroblasts during the interphase. The first type consisted in direct patch-clamping of flat cells whereas, in the second one, the cells were used after rounding up by trypsine treatment. In both cases, inside-out patches showed maxi-K^+ channels with a Ca^{2+}-sensitivity not significatively different from that of mitotic cells. The channel activation, at physiological membrane potentials, was only evoked after application of internal Ca^{2+} concentrations as high as 0.2 mM. However, there was a great variability in different experiments. Cell-attached experiments on trypsinized cells revealed a peculiary behavior of channels activity. In fact, the cell resting potential of -40 mV, estimated from the reversal of the maxi-K^+ channels (pipette solution: 130 mM KCl), shifted to -75 mV after the trypsinization. Furthermore, in trypsinized cells it was present a novel maxi-channel which, at resting, showed bursts of strong activity separated by long quiescent intervals.

W-Pos231 TEMPERATURE DEPENDENCE OF K^+ CHANNEL PROPERTIES IN LYMPHOCYTES.
S. Lee and C. Deutsch. Department of Physiology. University of Pennsylvania, Phila., PA 19104
Many functional responses of the lymphocyte are sensitive to temperature, including antibody and lymphokine secretion, growth and proliferation, and killing of foreign and virus-infected cells. In order to understand the potential role of ion channels in human peripheral blood T cell function, we have used the whole-cell patch clamp to determine the temperature dependence of the biophysical properties of the voltage-gated potassium channel. Threshold for activation and steady-state inactivation are the same at 22°C and 37°C. The reversal potential is likewise unchanged. However, the time constants for activation, inactivation, and deactivation are decreased. The temperature coefficient (Q_{10}) for each of these processes is approximately 5. Peak outward current is approximately twofold greater at 37°C than at 22°C. This increase is also manifest at the single channel level. Agents that block this channel at 22°C, including quinine, tetraethylammonium, and charybdotoxin, also block this conductance at 37°C with similar potencies. Supported by NIH grant GM 41467.

W-Pos232 YEAST ION CHANNELS. SELECTIVITY AND INHIBITION OF NUTRIENT UPTAKE BY K^+ CHANNEL BLOCKERS.
F. Gómez-Lagunas*, A. Peña* and A. Darszon+. *Dept. of Bioenergetics, IFC-UNAM, Apdo.Postal 04510 and +Dept. of Biochemistry, CINVESTAV-IPN, Apdo.Postal 14-740, México City.
Yeast cells have a H^+-ATPase which generates the membrane potential, and is regulated by the fluxes of K^+ ions. These ions regulate many of the physiological processes dependent on the electro chemical potentials across the plasma membrane. With the purpose of understanding the relationships among the different ionic fluxes occurring in yeast, we have studied the *Saccharomyces cerevisiae* ion channels, both by the fusion of purified plasma membranes into black-lipid bilayers, and by doing transport experiments *in vivo*. In this work we report that regardless of the amount of protein added, the fusion of the yeast membrane always incorporated several conductances. Independently of the bilayer lipidic composition, the two most frequent conductances were around 64 and 108 pS in 300 mM KCl. These conductances seem to correspond to channels that under biionic conditions select K^+ over Na^+. In these latter conditions, a 51 pS conductance with a maximum $(P_{Na^+})/(P_{K^+}) = 0.5$ was observed. At present, we do not know if this conductance belongs to the 64 or the 108 pS channels. On the other hand, K^+ channel blockers inhibit two kinetically different amino acid transport systems, although at this moment we can not make any physiological correlation. Also, rapid K^+ effluxes from the cells can be observed upon placing them in K^+-free media, or when CO_2 is washed from the incubation mixture. These effluxes might be related to the presence of K^+ channels in the plasma membrane. This work was partially supported by CONACYT (Fellowship to FGL) and by OEA.

W-Pos233 HORMONE-INDUCED CHANGES IN VOLTAGE-DEPENDENT CURRENTS DURING MATURATION OF STARFISH OOCYTES.
L. Simoncini and W.J. Moody. Department of Zoology, University of Washington, Seattle WA 98195.
Changes in electrical properties of starfish oocytes during hormone-induced maturation were studied under voltage-clamp. *Asterina miniata* oocytes have three major voltage dependent ionic currents: 1) An inward K current recorded during hyperpolarizing steps; 2) a transient inward Ca current blocked by Cd; and 3) an A-current similar to the one found in molluscan neurons that is blocked by 4-aminopyridine. During the first 30 min of 1-methyladenine induced maturation, the inward K current decreases by 50-75%, the A-current by more than 50%, while the Ca current remains unchanged. Capacitance measurements indicate that the total membrane area remains constant. These changes in electrical properties precede the other major biological effect of 1-MA, breakdown of the nuclear membrane. Preliminary evidence indicates that nuclear membrane breakdown can be prevented under conditions that do not alter the electrophysiological actions of 1-MA. In other starfish oocytes (*Leptasterias, Henricia*) the loss of currents during maturation happens concurrently with net loss of membrane. Three hypotheses could account for this difference: 1) The electrical actions of 1-MA are not secondary to membrane loss in any starfish; 2) they are explained by membrane loss in all species, but in *Asterina* net area is preserved by recycling, or 3) the basic mechanisms of 1-MA action are different in different species.

W-Pos234 RECONSTITUTED AORTIC Ca^{2+}-ACTIVATED K^+ CHANNELS: SELECTIVITY AND PHARMACOLOGICAL MODULATION BY CROMAKALIM (BRL 34915) AND PINACIDIL. **Craig H. Gelband and Cornelis van Breemen, Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.**

Large conductance Ca^{2+}-activated K^+ channels from rabbit aorta were incorporated into planar lipid bilayers (Latorre *et al.*, P.N.A.S. 77:805, 1982). This channel was characterized by its high conductance, 232 ± 7 pS in 250 mM KCl, as well as by its $[Ca^{2+}]_i$ and voltage sensitivity. Shifts in the reversal potentials of single channel currents, and the Goldman-Hodgkin-Katz equation were used to calculate relative permeabilities when $[K^+]_i$ was substituted with various cations. The selectivity series obtained was $K^+ > Rb^+ > NH_4^+ >> Na^+, Cs^+$ with permeability ratios of 1.0, 0.68, 0.14, for K^+, Rb^+, and NH_4^+ respectively. Na^+ and Cs^+ were not measurably permeant.

Cromakalim (BRL 34915) and pinacidil have been hypothesized to be K^+ channel agonists in vascular smooth muscle (Hamilton *et al.*, Br. J. Pharmacol. 88:103, 1986). Cromakalim (50 nM), in the presence of 1 μM $[Ca^{2+}]_i$, increased the probability of channel opening (P_o) by 56% from a control value of 0.09 ± 0.01 to 0.14 ± 0.01 at -40 mV ($p<0.01$, n=4 experiments). This occurred primarily by a 45% decrease in the time constant of the long closed state from 266 ± 32 ms to 147 ± 17 ms ($p<0.01$, n=4) with no significant effect on the open time constant (19 ms). The relationship between P_o and bilayer potential (-70 to +30 mV) was shifted 10-15 mV in the hyperpolarizing direction by 50 nM cromakalim.

Pinacidil (1 μM) in the presence of 1 μM $[Ca^{2+}]_i$, increased P_o by 82% from a control value of 0.17 ± 0.04 to 0.31 ± 0.07 at -40 mV ($p<0.05$, n=3). Pinacidil significantly decreased the time constant of the long closed state of the channel by 50% from 173 ± 50 to 86 ± 19 ms ($p<0.05$, n=3). Like cromakalim, no significant change in the open time constant was observed (20 ms). In conclusion, both cromakalim and pinacidil increased the probability of Ca^{2+}-activated K^+ channel opening, mediated by a decrease in the time constant of the long closed state of the channel. This may account in part for their anti-hypertensive properties in vascular smooth muscle. Supported by NIH HL-40184 and HL-07188.

W-Pos235 WHOLE-CELL RECORDING OF SPONTANEOUS TRANSIENT OUTWARD CURRENTS IN ISOLATED SINGLE SMOOTH MUSCLE CELLS FROM PIG TRACHEA. **Hsiu-Ming Saunders and Jerry M. Farley, Dept. Pharmacol. and Tox., Univ. Miss. Med. Ctr., Jackson, Ms. 39216-4505.**

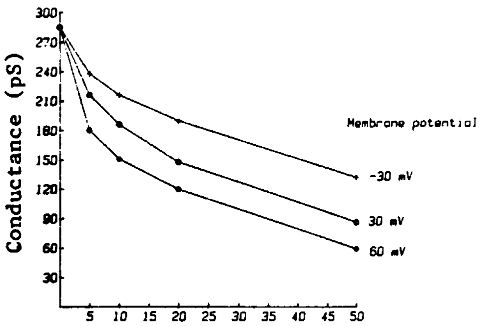
We have studied spontaneous transient outward currents (s.t.o.c.) in isolated single smooth muscle cells from pig trachea using the patch-clamp whole-cell recording technique. The s.t.o.c. could be observed at membrane potentials near the resting level of -60 mV. They increased in frequency and amplitude as the membrane potential became more positive. The removal of extracellular Ca^{2+} ions and addition of manganese (Mn^{2+}) ions had little influence on the frequency or amplitude of s.t.o.c. The direction of the s.t.o.c. could be predicted by the Nernst potential for K^+ ions when extracellular K^+ ion concentration was changed. The s.t.o.c. were reversibly blocked by potassium channel blockers such as TEA and Ba^{2+} (10 mM) applied from the extracellular side. When a high concentration (10 mM) of a Ca^{2+} ion chelator (EGTA or BAPTA) was present in the patch pipette, the s.t.o.c. amplitudes recorded were much smaller than that recorded when lower concentrations of EGTA (1 or 4 mM) were present. Caffeine (1 mM) stimulated s.t.o.c.. Caffeine (5 mM) caused an initial stimulation and then inhibition of s.t.o.c. Acetylcholine (1 uM) also stimulated then inhibited these currents. Extracellular application of 2.5 mM Mn^{2+} ions did not prevent the action of acetylcholine. We conclude that the s.t.o.c. in pig tracheal smooth muscle cells result from the activation of Ca^{2+}-sensitive potassium channels by transient release of intracellular Ca^{2+} ions. The initial stimulation of s.t.o.c. by acetylcholine may be due to an increased release of intracellular Ca^{2+}. The mechanism of ACh-induced inhibition has not been defined. (Supported by The Mississippi Lung Association and NIDA Grant 05094).

W-Pos236 LARGE CONDUCTANCE CA-ACTIVATED K CHANNELS IN ADULT RAT DIAPHRAGM: SINGLE CHANNEL PROPERTIES AND BLOCK BY PROTONS. Andrew L. Blatz, Dept. of Physiology, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235.

Single channel currents from large-conductance Ca-activated K channels (BK-channels) were recorded from acutely dissociated adult rat diaphragm muscle fibers using the patch clamp technique. Diaphragms were removed and soaked for 60-90 minutes in a Ca-free MEM containing 1-2 mg/ml collagenase (Gibco). Portions of single muscle fibers were dissected out and placed into the recording chamber for study. Cell-attached and inside-out configurations of the patch-clamp technique were used. Pipette and bath solutions normally contained (mM) 140KCl, 5 TES, and 0-40 uM added Ca. BK channels were studied at negative membrane potentials where the channels are less Ca-sensitive, and hence the highly pH-sensitive Ca-EGTA buffer system was not required. Properties of BK channels from adult muscle were very similar to those of channels from tissue-cultured myotubes. Single channel conductances averaged 240 pS in 140 mM KCl and increased to over 350 pS in 1 M KCl. Analysis of the distributions of open and shut dwell times revealed that, as in tissue-cultured channels, the underlying kinetic schemes must contain 3-4 open states and at least 5 shut states. The characteristic moding behavior of the channel was also readily apparent. Lowering intracellular pH had two major effects on currents through single BK channels. The probability of channels being in the open state decreased and the single channel current was reduced. At -40 mV and 40 uM intracellular [Ca], lowering intracellular pH from 7.0 to 5.0 reduced the open probability from 0.34 to 0.07 and the single channel conductance fell from 240 to 210 pS. This suggests that there must be at least two mechanisms underlying the proton block of BK channels, one perhaps involving the Ca-binding site(s) and another involving the permeation pathway. Supported by NIH grant GM39731.

W-Pos237 MAGNESIUM IONS REDUCE THE CONDUCTANCE OF THE LARGE CONDUCTANCE CALCIUM ACTIVATED POTASSIUM CHANNEL (BK CHANNEL) IN CULTURED RAT SKELETAL MUSCLE. W. B. Ferguson and K. L. Magleby, Dept. of Physiology and Biophysics, University of Miami School of Medicine, Miami FL 33101.

Currents through single BK channels were recorded using the patch clamp technique. The inside of the membrane was then exposed to solutions containing various concentrations of Mg. Conductance was calculated from the amplitude of the single channel current divided by Em - Er. Mg decreased single channel conductance in a concentration and voltage dependent manner as



illustrated. The channel blocking model of Woodhull was not sufficient to account for all the effects of Mg, suggesting additional or separate phenomenon may be involved. For example, fixed negative charges located inside the membrane field and near the channel pore would increase the local concentration of Mg in a voltage dependent manner. This increased local [Mg] could reduce the local K concentration by repulsion. Decrease in the [K] at the mouth of the channel would reduce the conductance. Supported by the NIH and MDA.

Figure. Experiments were performed in symmetric 150 mM KCl and 10 mM MOPS (pH 7.2). 5µM Ca++ was added to the inner solution to activate the channel. The lines

W-Pos238 CHARACTERIZATION OF OUTWARD IONIC CURRENTS IN TOADFISH SACCULE HAIR CELL: DIFFERENTIAL ACh RESPONSES. A. Steinacker and A. Romero, Dept. Otolaryngology, Washington University Sch. Med., St. Louis, MO. 63110

To study regional differences in morphology and ionic currents in the saccule of the toadfish, Opsanus tau, we used whole cell patch clamp methods to record ionic currents from hair cells of different regions of the saccule. Two populations of hair cells were found based on types of outward currents. A TEA (25 mM) sensitive outward current was present in all cells. The kinetics of activation and tail current decay varied widely between cells. In a minority of cells, all outward current was rapidly blocked by 25 mM TEA, exposing the inward calcium current. More commonly, a TEA insensitive current was also seen which was resistant to 5 mM Ba^{++}, apamin and 0 Ca^{++}. This current was slowly activating and showed no inactivation. The tail current decay was not related to the rate of current activation but matched tail current decay of the TEA sensitive current for that cell. A third transient outward current activated from hyperpolarized potentials was also seen. One population of hair cells shows an increase and another a decrease in outward current in response to ACh. These differences in ionic currents are not associated with a major division or cell type of the saccule but were distributed uniformly, with the exception of cells from the rim of the macula, whose kinetics were always slow.

W-Pos239 CHARACTERIZATION OF THE INWARDLY-RECTIFYING CONDUCTANCES IN AtT-20 CELLS. A.G. Dousmanis and P.S. Pennefather, Dept. of Physiology and Faculty of Pharmacy, U. of Toronto, M5S 2S2

We have measured the effects of somatostatin (S-14) and carbachol on membrane currents in AtT-20 cells using patch electrodes (7-10 MΩ) in the whole-cell configuration in the discontinuous voltage-clamp mode. Pipette filling solution contained in mM: 140 KCl, 5 HEPES,1 $MgCl_2$, 0.9 $CaCl_2$, and 1 EGTA, pH=7.2. External solution contained in mM: 140 NaCl, 1.3 $CaCl_2$, 25 HEPES, 35 Glucose, and either 5,10, or 20 KCl, pH=7.4. Extracellular application of 10^{-7} M S-14 induced an inwardly-rectifying conductance which was sensitive to extracellular K^+ concentration. The induced currents reversed at potentials close to E_K, and exhibited maximal slope conductances of 1.29 ± 0.24 (SEM, n=5), 1.36 ± 0.30 (n=7), and 3.03 ± 0.41 (n=16) nS in 5, 10, and 20 mM K^+ respectively. In 20 mM K^+ 10^{-5} M carbachol induced an inwardly-rectifying conductance very similar to that induced by S-14. The reversal potential of the carbachol-induced conductance was the same as that of the S-14-induced conductance, and exhibited a maximal slope conductance of 2.88 ± 0.38 nS (n=12). Normalized S-14 and carbachol I-V relations superimposed, suggesting a similar voltage dependence. In five of eight cells, addition of 10^{-5} M carbachol to 10^{-7} S-14 already present in the perfusate did not evoke any additional current. Thus, both S-14 and carbachol receptors appear to be coupled to the same pool of channels. In 20 mM K^+, in the absence of agonist, voltage steps from -30 mV to -80 mV elicit a K^+ conductance that also exhibits inward rectification. Like the agonist-dependent current, the backround current was completely blocked by 1 mM barium (n=9). In contrast to the agonist-induced current, the backround current inactivates to a steady-state level with a time constant of approximately 100 ms. In five of six cells, lowering external sodium (by substituting choline for Na^+) prevented the decay of the current, suggesting that Na^+ is involved in inactivation of the backround current. In summary, three inwardly-rectifying K^+ currents have been detected in AtT-20 cells: an S-14-induced current, a carbachol-induced current, and a backround current. Experiments are in progress to test the hypothesis that all three currents are generated by the same type of channel. (AGD is a recipient of an OGS; PSP is an Ontario Ministry of Health Career Scientist. Supported by the MRC Canada).

W-Pos240 BLOCK OF K(Ca) CHANNELS BY MONO-IODINATED CHARYBDOTOXIN DERIVATIVES AND A NEWLY IDENTIFIED HOMOLOG. Kathryn J. Lucchesi and Edward Moczydlowski, Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Two charybdotoxin peptides (Lq1 and Lq2) were purified from Leiurus q. venom by ion exchange and reverse phase chromatography. Both peptides were sequenced after deblocking the N-terminus with pyroglutamate aminopeptidase. The sequence of Lq1 was identical to that reported by Gimenez-Gallego et al. 1988 (Proc. Nat. Acad. Sci. USA 85: 3329). Lq2 differs by 8 (of 37) residues:

Lq1 Z F T N V S C T T S K E C W S V C Q R L H N T S R G K C M N K K C R C Y S
Lq2 Z F T Q E S C T A S N Q C W S I C K R L H N T N R G K C M N K K C R C Y S

Single channel blocking kinetics of Lq1, Lq2 and iodinated derivatives were analyzed for the 200 pS K(Ca) channel from rat skeletal muscle in planar bilayers (22°). At 100 mM KCl in and 50 mM KCl out, Kd values for the two isotoxins are 1.3 nM (Lq1) and 43 nM (Lq2). This affinity difference results from an 8-fold faster dissociation rate and a 4-fold slower association rate for Lq2 vs Lq1. Both peptides inhibited K^+ efflux elicited by A23187 in human red cells with similar affinity (IC_{50} = 43 nM), suggesting that the Gardos channel is less discriminating than the rat muscle channel for these two scorpion toxins. Two mono-^{125}I derivatives of Lq1 were prepared by a lactoperoxidase/glucose oxidase method. Iodination impaired the biological activity of Lq1 by increasing k_{off} and decreasing k_{on}. The blocking affinities were decreased to Kd = 0.86uM for ^{125}I-Lq1a and Kd = 15uM for ^{125}I-Lq1b. Specific binding (4°) of ^{125}I-Lq1a to rat muscle microsomes was of low affinity (Kd = 30 to 50nM) and was 50% displaced by 250nM native Lq1. We conclude that monoiodination of Lq1 greatly reduces its affinity for the large conductance K(Ca) channel and ^{125}I-Lq1a binds with low affinity to sites on skeletal muscle plasma membranes other than this channel.

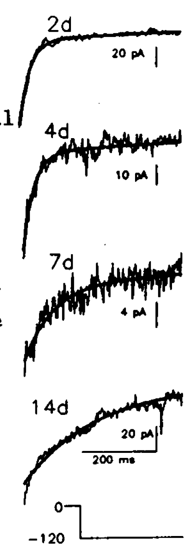
W-Pos241 INWARDLY RECTIFYING K^+ CURRENT GIVES RISE TO TWO STABLE MEMBRANE POTENTIALS IN RAT OSTEOCLASTS. Stephen M. Sims and S. Jeffrey Dixon. Dept. of Physiology and Div. of Oral Biology. University of Western Ontario, London, Ontario, Canada N6A 5C1.

Osteoclasts are large multinucleated cells with the unique ability to mediate extracellular resorption of bone. The activated osteoclast secretes protons and lysosomal enzymes into a confined compartment adjacent to the bone surface, the resorption lacuna. Despite the importance of osteoclasts in bone turnover, little is known about the role of membrane events and ionic currents in controlling their function.

We have used tight-seal whole-cell recording to study freshly isolated rat osteoclasts. The capacitance of cells ranged from 26 to 140 pF, with a mean value of 68 $\pm$ 31 pF ($\pm$ SD, n = 53). Under voltage-clamp, capacitive current transients decayed as single exponentials. In current-clamp, membrane potential changes due to current injection were also single exponentials. Thus, the osteoclast membrane can be considered an isopotential surface. Membrane potential switched between two stable levels, $\simeq$-70 and -15 mV. Under voltage-clamp, only inwardly rectifying K^+ current was evident. The current-voltage relationship of osteoclasts was "N-shaped" and intersected the zero-current level at three potentials, accounting for the existence of two stable membrane potentials. This is the first description of ionic currents and "excitable" behaviour in osteoclasts. Rapid, sustained changes in membrane potential could be responsible for regulating a number of processes, including the transport of ions into and out of the resorption lacuna, thereby modulating bone resorption. (Supported by MRC Canada).

W-Pos242 **THE TIME-DEPENDENCE OF THE INWARD RECTIFIER IN EMBRYONIC CHICK VENTRICLE CELLS CHANGES WITH DEVELOPMENT.** **Jonathan Satin and Robert L. DeHaan, Anatomy and Cell Biology, Emory University Health Sciences Center, Atlanta, GA 30322**

During development of the cardiac action potential, the maximum diastolic potential (MDP) shifts from about -50 mV to -90 mV. We measured inward rectifier current (I_{K1}) in isolated 2d, 4d, 7d and 14d embryonic ventricle cells using the whole-cell recording mode of the patch-clamp technique. Current elicited with hyperpolarizing steps from a holding potential (V_H) of -80 mV was time-independent, while steps from V_H = 0 mV evoked a component that displayed activation and a bi-exponential decay at all stages. The τ_{fast} of the inactivating phase slowed with development from 17 ms at 2d to 184 ms in 14d cells (Figure). In 7d cells, τ_{fast} of the ensemble average of single-channel currents measured in cell-attached patches was the same as that of the whole-cell I_{K1}. Positive levels of V_H removed inactivation. The half-inactivation potential shifted from +11 mV at 4d and 7d to -7 mV in 14d cells. I_{K1} reversal potential (E_{rev}) shifted by 55 mV/decade of $[K]_{out}$. The shift of E_{rev} was not significantly different in 4-, 7-, or 14-day cells, indicating that K selectivity of the channel remains constant throughout development. We conclude that changes in voltage-dependent inactivation of I_{K1} contribute to the negative shift of MDP during development of the ventricular action potential. (Supported by NIH HL-27385 to RLD).



W-Pos243 INACTIVATION OF THE DELAYED RECTIFIER POTASSIUM CHANNEL IN SQUID AXONS. A TAIL CURRENT ANALYSIS. John R. Clay. NINCDS, NIH, Bethesda, MD 20892 and Marine Biological Laboratory, Woods Hole, MA 02543.

The delayed rectifier, I_K, in squid axons inactivates with membrane depolarization with a time course of several seconds (Ehrenstein, G. and Gilbert, D.L., 1966, Biophys. J. 6: 553.; Chabala, L.D., 1984, J. Physiol. 356: 193). Information is lacking, however, concerning the onset of inactivation and reactivation during the initial 1-2 sec of each process. These results are provided in this study using a technique similar to that of Ehrenstein and Gilbert. The external potassium concentration, K_o, was increased so that E_K was within the range of activation of I_K. The I_K kinetics at E_K were measured from tail current amplitudes following prepulses of various durations to E_K. These results demonstrate that I_K inactivates following a delay of ~ 100 msec. Inactivation occurs in two phases, and it is incomplete. The tail current time constant is independent of prepulse duration. Reactivation was measured from tail currents using a three step protocol. This process also occurs following a delay of ~ 100 msec. These results can be described by a model consisting of a homogeneous population of channels with a single open state and three inactivated states arranged in a linear array for each channel. The rate constants of the model are more complicated than single exponential functions of membrane potential, V. Inactivation is independent of V for $-40 < V < 0$ mV and it is also independent of K_o in the 10 to 300 mM range.

W-Pos244 A BIOCHEMICAL "AND" GATE MAY ACTIVATE A LARGE CONDUCTANCE CALCIUM SENSITIVE POTASSIUM CHANNEL IN RAT PINEALOCYTES. John Ives Halperin, Valentin Cena, Stephen Yeandle, David C. Klein, Naval Medical Research Institute, Bethesda MD 20814; Dept. of Neurochemistry, Univ. of Alicante, Alicante, Spain; Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, Md 20892.

Rat pinealocytes possess a large Ca^{+2} sensitive K^+ channel (K(Ca) channel). Using on-cell patches, it was found that the frequency of opening of these channels was increased when 1μM norepinephrine was in the patch pipette or when 10μM norepinephrine was spritzed on to the cell from another pipette. This observation indicates that one or more second messengers are involved in norepinephrine stimulation of these channels.

Tracer flux experiments indicate that the mixed alpha and beta adrenergic agonist norepinephrine (10μM) stimulates ^{86}Rb efflux. In this tissue norepinephrine controls cyclic nucleotides via a biochemical "AND" gate by simultaneously activating $alpha_1$- and beta-adrenergic receptors. Analysis of the mechanism by which norepinephrine stimulates ^{86}Rb efflux indicates that a biochemical "AND" gate is involved in which $alpha_1$ and beta adrenergic receptors regulate the K(Ca) channel. For example, whereas neither the selective beta adrenergic agonist isoproterenol (0.1μM), nor the selective $alpha_1$ adrenergic agonist phenylephrine (0.1μM) stimulates ^{86}Rb efflux when admisistered alone, a near maximal stimulation of ^{86}Rb efflux is produced when they are administered simultaneously. Other studies indicate elevation of cyclic AMP and $[Ca^{+2}]i$ are both required for ^{86}Rb efflux. Cyclic AMP and Ca^{+2} may be the second messengers involved.

W-Pos245 REGULATION OF HIGH CONDUCTANCE Ca-ACTIVATED K CHANNELS (P_{KCa}) BY GMP. L. Roy-Contancin, G. Velasco, G. Katz, R. Winquist, M. Garcia, G. Kaczorowski, and J.P. Reuben, Merck Institute, West Point, PA and Rahway, NJ 07065

Our previous research (Biophys. Abstr. 1988, PNAS in press) has shown that P_{KCa} channels within on-cell patches of bovine aortic smooth muscle (BASM) cells are markedly potentiated by vasodilatory agents that increase cytosolic cGMP levels. Experiments using excised inside out patches showed that GMP was 5-10X more effective in potentiating P_{KCa} channel activity than cGMP. These data suggest that GMP is the second messenger following hydrolysis of cGMP for mediating the action of the vasodialators. Current research using both BASM and GH_3 cells has revealed block of the potentiation of P_{KCa} channel activity elicited by ANF (10^{-7} M) and nitroprusside (10 uM) when a permeant phosphodiesterase inhibitor isobutylmethylxanthine (IBMX; 1 mM) is present in the bathing medium (in mM; 5 KCl, 2 Mg Cl_2, 135 NaCl, 5 Hepes, pH 7.3). After washing out the IBMX the full potentiating action of ANF and nitroprusside was observed. By itself, IBMX has no or only a minimal affect on channel activity. Consistent with our postulate that relaxation induced by the vasodilatory agents may occur, under some conditions, due to the increased activity of P_{KCa} channels is the finding that charybdotoxin (30 nM) reduces the effectiveness of these agents in causing relaxation. The latter has been demonstrated in isolated segments of rabbit aorta contracted by the α_1 agonist, methoxamine, and subsequently relaxed by titration with ANF (10^{-10} to 10^{-8} M).

W-Pos246 β-ADRENERGIC MODULATION OF DELAYED RECTIFIER K^+ CURRENTS IN BULLFROG ATRIAL MYOCYTES. A.A. Lagrutta, I. Gourdon, and H.C. Hartzell. Dept. of Anatomy and Cell Biology. Emory University School of Medicine. Atlanta, GA. 30322.

The whole cell patch-clamp technique was used to study the regulation of the delayed outward K^+ current, I_K, in amphibian atrial myocytes by β-adrenergic mechanisms. Superfusion with isoproterenol (1 μM) typically increased I_K amplitude 2-fold in this preparation. Blocking the calcium current, I_{Ca}, with 100 μM Cd^{+2} did not affect this increase. Similarly, an increase in I_{Ca} produced by the calcium channel agonist Sandoz (+)202-791 did not change I_K amplitude. These observations suggest that I_K is not affected by I_{Ca}. Forskolin (5 μM), an activator of adenylate cyclase, and internal perfusion with cAMP (30 μM) mimicked the effect of isoproterenol, suggesting that cAMP-mediated phosphorylation of I_K is the most likely mechanism of action of β-agonists on this current. Dose-response studies showed that 2.2 μM cAMP produced a half-maximal effect on I_K, the maximal effect being a 1.9-fold increase over control I_K. Similarly, 1.7 μM cAMP produced a half-maximal effect on I_{Ca}, the maximal effect being a 6.3-fold increase over control I_{Ca}. The current-voltage relation for fully activated I_K showed inward rectification and was best fitted by a second order polynomial. The reversal potential was near E_K (E_{rev} = -90 mV with 2.5 mM K^+ outside), and shifted 2-3 mV toward negative potentials upon stimulation by either isoproterenol, forskolin, or cAMP. Kinetics of activation were faster and kinetics of deactivation were slower in isoproterenol than under control conditions. The steady-state activation curve for I_K shifted approximately 5 mV toward negative potentials upon β-adrenergic stimulation. This shift was seen even when I_{Ca} was blocked by 100 μM Cd^{+2}. In the presence of Cd^{+2}, however, considerable variability in the degree of shift in the steady-state activation was noticed. Overall, our observations support the notion that catecholamines increase I_K by a cAMP-mediated change in the gating properties of this channel. (Supported by an N.I.H. grant).

W-Pos247 **IONIC PATHWAYS OF RESTING CURRENT IN SQUID AXON MEMBRANE** J.R. Hunt and D.C. Chang. Baylor College of Medicine, Houston, Texas 77030. (Introduction by R. Jacob).

At present the nature of the membrane pathways that control the resting current are not well understood. These pathways may include: (1) The excitable K channel (delayed rectifier), (2) a leakage conductance, and (3) unknown resting K channels, which obviously must be ion-selective and presumably V-dependent. In order to understand which of these pathways are the major controlling mechanisms for the resting current, we studied the effects of external cations on the resting potential and the membrane conductance in squid giant axons. A special signal-averaging V-clamp protocol was used to determine membrane conductance over a large potential range (both above and below V_{rest}). Preliminary results suggest the existence of a K-selective resting channel which is activated at a relatively negative potential and has an ion-selectivity sequence slightly different than the delayed rectifier. First, the observed V-dependence of the membrane conductance seems to deviate strongly from the Hodgkin-Huxley kinetic for the delayed rectifier at V near or below the resting potential. Second, at $V < V_{rest}$, the conductance is still strongly ion-selective and is voltage-dependent. Third, the K conductance at -80 mV shows a slight inward rectification which was not observed in the delayed rectifier. Fourth, the ion selectivity of the resting pathways, as determined from V_{rest} measurements, is different than the ion-selectivity of the delayed rectifier measured by reversal potential. In particular, the relative permeability of Cs to K, as determined from V_{rest}, is on the order of 0.16, while Cs is known to be virtually impermeable to the delayed rectifier. (Supported in part by NIH grant #NS25803-01).

W-Pos248 CHARYBDOTOXIN BLOCKS VOLTAGE-GATED AND Ca-ACTIVATED K CHANNELS IN MAMMALIAN BRAIN. M.J. Schneider, B.K. Krueger, and M.P. Blaustein. Physiol. Dept., Univ. Maryland School of Medicine, Baltimore, MD 21201.

Charybdotoxin (ChTX), a component of Leiurus quinquestriatus (Lq) venom, has been shown to block a large conductance (maxi) Ca-activated K channel (CAKC) in many preparations. We used a ^{86}Rb efflux assay to study the effect of ChTX on mammalian brain K channels. Depolarization-induced ^{86}Rb efflux can be used to identify at least three different K channels in rat brain synaptosomes. These include inactivating and noninactivating voltage-gated K channels (VGKC) that are blocked by 10 mM 4-aminopyridine (4-AP). In the presence of 1 mM Ca, a CAKC can be identified that is not blocked by 4-AP but is blocked by tetraethylammonium, a characteristic of maxi CAKCs. We isolated ChTX from Lq venom and found that it preferentially blocked brain CAKC with high affinity (IC_{50} about 10 nM). In the absence of 4-AP and Ca, ChTX also blocked both classes of VGKC, but with lower affinities. The IC_{50} for the noninactivating VGKC appeared to be 100 nM, while that for the inactivating VGKC appeared to be about 1000 nM. These observations complement the report that ChTX and noxiustoxin, a component of Centruroides noxius venom that blocks the delayed rectifier K channel in squid axons, both block a CAKC from skeletal muscle, but with different affinities (Valdivia et al, FEBS Let. **226**:280, 1988). These data suggest that different types of K channels may have some structural homology. (Supported by NIH grants NS-16106 and NS-20106.)

W-Pos249 LEIURUS quinquestriatus VENOM PEPTIDES THAT BLOCK BRAIN VOLTAGE-GATED AND Ca-ACTIVATED K CHANNELS ALSO INHIBIT DENDROTOXIN BINDING TO SYNAPTIC MEMBRANES.

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L. quinquestriatus (Lq) venom contains polypeptides that modulate ion channels. We fractionated acetone-extracted Lq venom by cation exchange HPLC. Five peptide fractions were found that block rat brain synaptosome K channels (as measured by ^{86}Rb flux assay). These same fractions also inhibited binding to synaptic membranes of [^{125}I]α-DaTX ("dendrotoxin", from Dendroaspis angusticeps venom), a blocker of inactivating, voltage-gated K channels (VGKC). Two peptides, charybdotoxin (ChTX) and one that we termed Lq toxin-1 (LqTX-1), were studied further. LqTX-1 blocks both inactivating and non-inactivating VGKCs (IC_{50}'s between 100 and 1000 nM), but has no significant effect on the Ca-activated K channels (CAKC). Conversely, ChTX preferentially blocks a large conductance (Maxi) CAKC. Nevertheless, ChTX inhibits [^{125}I]α-DaTX binding to its membrane-bound and detergent solubilized receptors with high affinity (IC_{50} about 10 nM). LqTX-1 also inhibited [^{125}I]α-DaTX binding but with relatively low affinity (IC_{50} about 200 - 400 nM). ChTX and LqTX-1 show mixed-type inhibition of [^{125}I]α-DaTX binding. In summary, we identified two scorpion toxins (ChTX and LqTX-1) that preferentially block different brain K channels and inhibit the binding of a snake toxin, α-DaTX, to synaptic membranes. ChTX and α-DaTX, which have no sequence homologies, apparently share a common receptor site on synaptic membranes. These data suggest that different types of K channels may have some structural homology. (Supported by NIH grant NS-16106)

W-Pos250 EXPRESSION OF THE INWARDLY RECTIFYING K CONDUCTANCE IN RESTING AND ACTIVATED MURINE MACROPHAGE J774.1 CELLS.

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J774.1 cells express an inwardly rectifying K conductance (G_K) that increases in magnitude several hours after cells become adherent (Gallin and Sheehy, J. Physiol. 369:475, 1985). After adherence or other appropriate stimuli, macrophages undergo morphological changes related to their state of activation. The purposes of this study were to determine (a) whether the observed increase in G_K represented a true increase in current density, (b) whether cells with different morphologies differentially express G_K (c) whether cells activated by radiation or lipopolysaccharide (LPS) show increased G_K and (d) whether protein synthesis is necessary for the initial expression of the inward-rectifying current after adherence to occur. Whole-cell currents were recorded using the patch clamp technique as described in McKinney and Gallin, J. Memb. Biol. 103:55, 1988. Conclusions are: (a) G_K density (whole-cell G_K normalized to membrane capacitance, and corrected for leak) increases after adherence. G_K for all cells measured 0-3 h after adherence was 0.14±0.02 nS/pF (N=34); 24 h after adherence G_K= 0.27±0.03 nS/pF (N=23). (b) Cells adherent for 24 hrs, which were small and round, with no processes, (cap=21 ± 3 pF (N=7) had G_K=0.31 ± 0.07 nS/pF. Larger and more polarized or spread cells (cap=39 ± 4 (N=16) had G_K=0.25 ± 0.03 nS/pF, indicating no significant difference between cells of different morphological types. (c) Cells exposed to 1 ug/ml LPS for 24 hrs did not show increased G_K, at short or long times after adherence, nor did cells exposed to a 20 Gray dose of ionizing radiation, indicating that G_K does not change with the state of activation of the cell. (d) Cells exposed to 100 uM cycloheximide (known to block 90% of ^{3}H-leucine uptake) 1 h before, and also during plating, did not show reduced conductance up to 3.5 h after plating. Thus, cycloheximide did not inhibit the initial expression of G_K after adherence.

W-Pos251 **CALCIUM PERMEABILITY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR CHANNEL DETERMINED FROM SINGLE-CHANNEL MEASUREMENTS IN BC3H1 CELLS.** E. Radford Decker and John A. Dani. Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

Nicotinic acetylcholine receptor (nAChR) channels are permeable to monovalent cations and divalent cations (Adams et al., 1980 J. Gen. Physiol. 75:493-510). The purpose of this study is to quantitatively determine the permeability of the nAChR channel to calcium. Single-channel currents were measured in pure calcium and in mixtures of calcium and monovalent cations. In the mixtures, a theoretical description of the data (Dani, 1986 Biophy. J. 49:607-18) enabled us to predict the current carried by calcium or by the monovalent cation. When low millimolar concentrations of calcium are added to pure solutions of NaCl or CsCl, the current carried by the monovalent cations decreases. As higher concentrations of calcium are added, a significant fraction of the current is carried by calcium. With pure 150 mM NaCl bathing both sides of the membrane, the Na current at -100 mV is 5 pA. When 10 mM Ca is added externally the current at -100 mV falls to 2.8 pA: more than 10% of that current is carried by calcium. With pure 110 mM $CaCl_2$ outside and with pure 45 mM CsCl inside, the inward calcium current at -100 mV is 1 pA, giving a cord conductance of 10 pS. AChR channels are packed very densely at the neuromuscular junction. Therefore, the calcium current through nAChR channels in physiological solutions could be important in the development, maintenance and regulation of the nicotinic synapse. Supported by NIH grant NS21229.

W-Pos252 FORSKOLIN-INDUCED DESENSITIZATION AT THE FROG NEUROMUSCULAR JUNCTION IS INDEPENDENT OF ADENYLATE CYCYLASE ACTIVATION. R.A. MASELLI, S.M. PALOMBI, AND D.J. NELSON, (Intr. by L. Ford) Department of Neurology, The University of Chicago, Chicago, Illinois

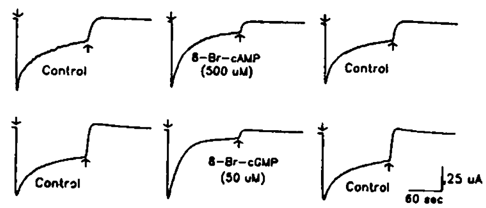
The actions of forskolin an activator of adenylate cyclase were studied at the frog neuromuscular junction. Forskolin (20-100 μM) produced a progressive decrease in the amplitude of end-plate currents (EPCs) evoked by repetitive nerve stimulation and in end-plate potentials obtained by pulses of iontophoretically applied acetylcholine consistent with desensitization. This effect was reversible and dependent on the concentration of forskolin, frequency of stimulation and concentration of Ca^{++} in the extracellular bath solution but was relatively voltage-independent. The forskolin-induced desensitization could not be reproduced by exposing the preparation to agents that increase the intracellular levels of c-AMP (dibutyryl-c-AMP, isoproterenol or theophylline) but was reproduced by the membrane permeant inactive analog 1,9 dideoxy forskolin (20 and 50 μM). Succinyl-forskolin (50 μM) a more hydophilic derivative had no effect on the iontophoretically induced amplitude of endplate potentials. Both forskolin and dibutyryl-c-AMP produced a reversible increase in the frequency of miniature end-plate currents (MEPCs). However, the forskolin-induced increase in MEPC frequency was independent of extracellular Ca^{++} suggesting a separate pathway mediating the response. Forskolin (0.5μM to 100μM) had no effect on MEPC or EPC amplitude or the time constant describing MEPC or EPC decay. Neither the post-synaptic forskolin-induced desensitization effect nor the pre-synaptic forskolin-induced increase in the MEPC frequency appear to be related to the adenylate cyclase activation. Supported by NIH NS24304.

W-Pos253 **ACTIVATION OF NICOTINIC ION CHANNELS AT THE RAT ENDPLATE BY ACETYLCHOLINE.** **N. Mulrine & D.C. Ogden*, Departments of Pharmacology, University College London, Gower Street, & Kings College London*, U.K.**

The activation of ion channels by a wide range of ACh concentrations was studied with single channel recording from endplates of fibers dissociated from M. flexor digitorum brevis of the adult rat. At 20-25°C, single channel conductance was 55-60pS. When the pipette contained more than 5μM ACh, channels opened in clusters of high activity separated by long silent intervals. We estimated the degree of activation of the receptor by measuring the open probability during clusters. Equilibrium open probability, p_o, increased with ACh concentration to a maximum of 0.87 at 200 μM (at -100mV), with 50% activation at 17 μM. At concentrations higher than 200 μM, p_o declined as a result of open channel block by free ACh (K_B = 540 μM). The equilibrium constant for channel opening, β/α, was 42, and the microscopic binding constant, K, was 108 μM. At positive membrane potentials, the open probability was much lower; p_o was only about 20% at 20 μM ACh, and between 40-60% at ACh concentrations between 100 μM and 2 mM. Experiments were also performed at 14°C and at 37°C. The single channel conductance increased from 40 pS at 14°C to 75 pS at 37°C (Q_{10}=1.3). At low ACh concentrations, (100 nM), channels opened in bursts containing brief gaps. The mean burst length decreased from 5.4 ms at 14°C to 2.6 ms at 20°C and 600μs at 37°C. At 37°C, the open probability during clusters was 90% with 200μM ACh (-100mV).

W-Pos254 CYCLIC NUCLEOTIDES MODULATE ACh-INDUCED CURRENTS IN XENOPUS OOCYTES EXPRESSING nAChR CHANNELS. T. Reuhl, J. Pinkham*, J.R. Moorman, J.A. Dani. Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030 and *Department of Molecular Neurobiology, Yale University, New Haven, CT 06510.

In vitro transcribed mRNAs from cDNAs encoding mouse BC3H1 nicotinic acetylcholine receptor (nAChR) channel subunits were injected into Xenopus oocytes. A two electrode voltage clamp was used to measure ACh-induced currents through nAChR channels expressed in defolliculated oocytes. Consistent with other work (see Steinbach & Zempel 1987 TINS 10:61-4), we found that exposing oocytes to 500 µM 8-Br-cAMP for 1 hr or longer enhanced desensitization. This enhancement of desensitization was long lasting. The Figure shows a more transient effect of cyclic nucleotides. The middle current records show that stimultaneous application of 50 µM ACh with 500 µM 8-Br-cAMP or with 50 µM 8-Br-cGMP speeds the falling phase of the currents. At the downward arrow, the drugs were applied; at the upward arrow the drugs were washed off. The wash continued for 7 min before the next measurement. Control currents induced by ACh without nucleotides bracket the tests. The controls show that the effect of the cyclic nucleotides is largely reversed during the 7 min wash. The difference in the recovery from long term and short term exposure to cyclic nucleotides suggests that more than one process may be involved. Supported by NIH NS21229 and KL01858.



W-Pos255 THE APPARENT OPEN TIME OF ACETYLCHOLINE-ACTIVATED ION CHANNELS SLOWLY DECREASES UPON MEMBRANE PATCH EXCISION. M. Covarrubias and J. H. Steinbach, Dept. of Anesthesiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

The role of the intracellular milieu in ion-channel function is a subject of intensive investigation. Previous studies have indicated that membrane patch isolation can affect ion-channel function (Trautman and Siegelbaum, 1983. In: Single Channel Recording, Plenum; Horn and Vandenberg, 1986. In: Ion Channels in Neural Membranes, Alan R. Liss, Inc.). We studied the single channel properties of nicotinic acetylcholine (ACh) receptors in records obtained before (cell-attached) and after patch excision (inside-out). All experiments were done with BC3H-1 cells at 22°C and symmetrical solutions (pipette/bath) containing high K^+ and EGTA-Mg without added Ca^{++}. Our results show that after patch excision the apparent open time slowly decreases, reaching a plateau in approximately 15 min. The apparent mean open time (Vh = -100 mV) in cell-attached patches was 6.2 ± 1.2 ms (n=5) and in inside-out patches was 1.9 ± 0.6 ms (recorded more than 20 min after excision). Similar results were obtained with a low (0.1µM) or an intermediate (10 µM) concentration of ACh. These observations further indicate that ACh receptor function is sensitive to patch configuration. (Supported by NIH NS22356)

W-Pos256 FUNCTIONAL CHARACTERIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS ON PC12 CELLS. C. K. Ifune and J. H. Steinbach, Depts. of Anesthesiology and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Nicotinic acetylcholine receptors expressed by NGF-treated PC12 cells were studied. 100µM ACh perfused onto cells held at -80 mV elicits an inward current that peaks within 200 ms and then rapidly declines. Responses to 100µM ACh measured at various holding potentials between -80 and +80 mV indicate that the whole-cell I-V relationship for the ACh response over this range is non-linear and displays inwards rectification. This inwards rectification is not due to channel block by divalent cations or single channel rectification. Removing Ca^{++} and Mg^{++} from the intracellular solution did not linearize the whole-cell I-V curve. In addition to this, using Na^+, K^+, or Cs^+ as the major intracellular cation or replacing the HEPES buffer with a phosphate buffer did not affect the I-V curve. In outside-out patches exposed to 1-10 µM ACh, two conductance classes of channels are seen. With symmetrical 145 mM Na^+ solutions, the dominant channel has a conductance of 40 pS and a smaller, less frequently seen channel, 25 pS. The I-V relationship for the larger channel is linear between -80 and +80 mV and therefore cannot explain the rectification seen in the whole-cell response to ACh. This suggests that the rectification is due to the voltage dependence of processes involved in channel activation. Supported by NIH 22356 (JHS) and PHS training grant 2 T32 HL07275-11 (CKI).

W-Pos257 ACETYLCHOLINE ACTIVATED SODIUM AND CHLORIDE CHANNELS IN CULTURED APLYSIA NEURONS.

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Despite the large quantity of data on the neurotransmitter activated membrane currents of *Aplysia* neurons, there is comparatively little information on the single channel properties. Acetylcholine activates three distinct currents in *Aplysia* neurons which are carried by sodium, chloride and potassium. This provides a convenient model system for studying several ionic channels coupled to a single type of neurotransmitter receptor. Tissue culture conditions were manipulated to produce neurons which form giga-ohm seals using standard patch-clamp techniques. Recordings were made in the cell-attached and inside-out mode. Acetylcholine (250 to 1000 nM) activated two types of single channel currents. The first type was inward at membrane potentials more negative than -60 mV, decreased in amplitude as the membrane was depolarized and became outward at holding potentials more postive than -60 mV. Under the recording conditions the chloride reversal potential was calculated to be -60 mV, suggesting that these are acetylcholine activated chloride channels. Single channel conductance measured from the slope of the current-voltage curve was 71 pS and channel lifetime was estimated to be 7 ms (23°C, 0 mV). The second type of currents were inward at holding potentials more negative than 0 mV, and outward at potentials more positive. Conductance was 55 pS and lifetime 3.2 ms (23°C, -40 mV). The reversal potential for sodium was calculated to be +40 mV, suggesting that the channel may not be completely selective for sodium. We propose that these currents underlie the macroscopic acetylcholine currents recorded under voltage clamp. (Supported by NIH grant #NS23807 to D.O.C.).

W-Pos258 PROTEOLYTIC FRAGMENTS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IDENTIFIED BY MASS SPECTROMETRY AND MICROSEQUENCING. C.R. Moore, J.R. Yates, J. Shabanowitz, P. Martino, D.F. Hunt and D.S. Cafiso. Department of Chemistry, University of Virginia, Charlottesville, VA 22901.

The Nicotinic Acetylcholine Receptor (AcChR) from *T. californica* was affinity purified and reconstituted into vesicles of PC:PA:Cholesterol (56/19/25), with a right side-out orientation of 75%. Trypsin digestion as a function of time or trypsin concentration was carried out on this sample. The solubilized peptides were separated from vesicles by centrifugation, fractionated by HPLC and analyzed by tandem quadrupole mass spectrometry and fourier transform mass spectrometry. Membrane-bound fragments were analyzed by gel electrophoresis, electroblotted onto PVDF membranes and sequenced using a gas-phase sequenator. The results indicate that cleavage of the reconstituted AcChR occurs preferentially in the putative cytoplasmic region, including the phosphorylated portions of the protein. The following sequences are released with short digestion times (<30 minutes and 1%(w/w trypsin): αLVANLLENYNK (7-17), IFADDIDISIDISGK (341-354),QVTGEVIFQTPLIK (355-373), QVTGEVIFQTPLIKNPDVK (355-373), SAIEGVK (374-380); βANDEYFIR (351-358), KPAGDFVCPVDNAR (359-372), VAVQPER (373-379), LFSEMK (380-385); γSSFGIMIK (353-360), AEEYILK (361-367), SELMFEEQK (371-379), DLANFAPEIK (405-414); δQIFLEK (328-333), ILHMSR (337-342), AQEYFNIK (369-376), SELMFEK (379-385), HGLVPR (390-395), IGFGNNNENIAASDQLHDEIK (400-420), SGIDSTNYIVK (421-431). With additional digestion, peptides are released from the amino terminal region of the AcChR. From microsequencing of the electroblots, the peptides that remain membrane bound are consistent with the cleavage sites observed by mass spectrometry. This data will be discussed in terms of current models for the folding of the AcChR peptide.

[This work was supported by NSF grant BNS 8604101 to DSC and NIH grant GM 37537 to DFH.]

W-Pos259 BINDING OF HALOTHANE TO THE ACETYLCHOLINE RECEPTOR. by Howard H. Wang, Donald D. Koblin, Lily Lin, Francis Namkoong, and Annmarie Kappl. Department of Biology, University of California, Santa Cruz, CA 95064, Department of Biology, Eastern Montana College, Billings, MT 59101 (LL) and Department of Anesthesia, University of California, School of Medicine, San Francisco, CA 94121 (DDK).

Recent advances in understanding the mechanism of anesthesia have shifted attention to membrane proteins as the targets of anesthetic action. The hypothesis that anesthetics act directly on synaptic proteins is attractive, but requires experimental support. If this hypothesis is correct, we expect to observe specific binding of inhalation anesthetics to synaptic receptors. The nicotinic acetylcholine receptor (AChR) is an ideal model for this research. The receptor is a chemically-gated, cation channel which can be blocked by non-competitive inhibitors such as phencyclidine (PCP). PCP has been shown to bind at high affinity (Kd = 0.4 μM) to a single class of sites on AChR. The stoichiometry of high affinity allosteric site has been found to be approximately one per receptor monomer. The inhibition of ^{3}H-PCP binding to AChR by halothane was examined by radioligand assays. In our results, halothane dosage is expressed in volume percent of halothane in air or nitrogen. The binding of ^{3}H-PCP (20 nM) to enriched AChR was clearly inhibited by halothane at 20°; IC_{50} of the fitted inhibition curve was at 1.6% halothane. The ^{3}H-PCP binding at 6.4% halothane was 4% of the control (the control value is the ^{3}H-PCP binding to AChR after equilibration with halothane-free nitrogen gas). The inhibitory effect is reversible; the recovery curve showed an 84% recovery after equilibration with halothane-free nitrogen for one hour. By using the Cheng and Prusoff correction, the IC_{50} of 1.6% halothane gives a corresponding K_i of 1.5%, approximately twice the ED_{50} for anesthetizing tadpoles.

W-Pos260 **Isolation of a cDNA encoding the *epsilon* subunit of the rat muscle AChR.** Patricia Camacho, Richard H. Goodman+, Gail Mandel+, and Paul Brehm. Tufts University School of Medicine, Dept. of Physiology, Boston, MA and +New England Medical Center Hospital, Division of Molecular Medicine, Boston, MA 02111.

The nicotinic AChR on embryonic muscle is an integral membrane glycoprotein with subunit stoichiometry *alpha* 2, *beta, gamma and delta.* A fifth subunit, *epsilon*, has been cloned from bovine tissue (Takai et al. (1985) Nature, **315**, 761-764) and functionally shown to substitute for *gamma* subunit in conferring "adult" type properties to the AChR (Mishina et al. (1986) Nature, **321**, 406-411). Based on a partial genomic sequence of rat *epsilon* (Witzeman et al. (1987) FEBS Lett., **223**, 104-112) we synthesized two 21 base-pair oligonucleotides which were used to screen a denervated rat skeletal muscle cDNA library. One full length cDNA (clone pPC7) was isolated under high stringency hybridization conditions. The cDNA has an open reading frame of 1479 base pairs coding for a protein of 493 amino acids. Sequence analysis revealed 86% overall amino acid homology with bovine *epsilon* with a still greater degree of conservation in the putative membrane spanning regions (96%). Hydrophilicity plots of rat *epsilon* suggest the existence of a mostly hydrophilic amino terminal (except for a short leader peptide) as well as one amphipathic and four hydrophobic regions corresponding to the putative transmembrane domains. Two cysteines at positions 128 and 142 are also conserved in rat *epsilon*. Three potential glycosylation sites were predicted from the amino acid sequence. Furthermore, as in the bovine protein, rat *epsilon* has a potential cAMP dependent protein phosphorylation site at serine 358. Funded by NIH grant NS 18205 to PB.

W-Pos261 B-N-METHYLAMINO-L-ALANINE (BMAA) DIRECTLY ACTIVATES THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR. Charles N. Allen, Peter S. Spencer* and David O. Carpenter, Wadsworth Cntr. for Labs and Research, NYS DOH & School of Public Health, Albany, NY 12201 & Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461. (Intr. by K.W. Kinnally)

BMAA is a neurotoxic amino acid found in the seeds of the false sago palm, Cyas circinalis, and may play a role in the development of Guam amyotrophic lateral sclerosis. BMAA's neurotoxicity is attenuated by APV, ketamine and MK-801 suggesting that BMAA is acting via the NMDA receptor. Since BMAA lacks the dicarboxylic acid structure, characteristic of the excitatory amino acids, it has been proposed to act at the NMDA receptor via a metabolite or an interaction with bicarbonate ions. The whole cell voltage-clamp technique was used to record BMAA- and NMDA-activated currents from cultured hippocampal neurons. In HEPES buffered extracellular solutions containing 0.1 mM Ca and no added Mg, BMAA (0.5 mM) activates an inward current which increases membrane hyperpolarization. BMAA currents had a mean amplitude of -76 pA (n = 24 cells) at -70 mV compared to NMDA (0.5 mM) activated currents which had a mean amplitude of -82 pA (n = 32 cells). In the absence of Mg the BMAA and NMDA current voltage curves were linear over a membrane potential range of -90 to +50 mV. In the presence of Mg the BMAA- and NMDA-induced currents decreased in amplitude with membrane potentials more hyperpolarized than -20 mV. APV (1 mM), an antagonist of the NMDA binding site, reversibly antagonized the BMAA and NMDA currents. Ketamine (0.25 mM), a NMDA channel blocker, reversibly inhibited the BMAA- and NMDA-activated currents in a voltage dependent manner. These data demonstrate that BMAA is interacting with the NMDA type excitatory amino acid receptor to activate an ionic current. (NIH Grant NS23807 to DOC)

W-Pos262 **Diffusion Models of Ion Channel Gating: Effects of Dimensionality and Drift.** G.L. Millhauser, E.E. Salpeter† and R.E. Oswald*,Department of Chemistry, University of California, Santa Cruz, CA 95064, †Newman Laboratory of Nuclear Studies and *Department of Pharmacology, NYSCVM, Cornell University, Ithaca, NY 14853.

In recent work we proposed a diffusion model of ion channel gating in which the closed time distributions are controlled by a one-dimensional diffusion process. With such a model the closed time distribution follows a power law of the form t^{-a} where $a \approx 1.5$ (Millhauser *et al. Proc. Natl. Acad. Sci.*, **85**, 1503-07, 1988). In subsequent work we demonstrated that if a power law is assumed then the exponents for four different channel systems fall in the range $1.39 < a < 1.71$ when the transmitter-gated channels are exposed to saturating quantities of agonist (Millhauser *et al.*, *Biophys. J.*, in press). Recently a three-dimensional defect diffusion model constrained to a limited configuration space was shown to give a good accounting for the closed time kinetics of the Acetylcholine Receptor (AChR) when exposed to 100 nM suberylcholine (P. Lauger, *Biophys. J.*, **53**, 877-84, 1988). In light of these findings we have investigated the effects of dimensionality on diffusion models and we find that the effects can be quite profound. For systems with dimension greater than one the exponent a can be much larger than our predicted value of 1.5. We have also examined the effects on diffusion that arise from an additional drift term which, in turn, might originate from transmembrane potentials. In such cases we find that the closed time distributions will initially follow a power law at short times but at longer times the distribution can have an exponential tail. It is our hope that clarification of such issues will serve as an aid in the development of physical models for gating processes.

W-Pos263 HEME PROTEINS EQUILIBRIUM FLUCTUATIONS AND LOW TEMPERATURE KINETICS MODELS - CONCEPTS - CONTRAVERSIES.

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Association in dark and dissociation in presence of light simulates physiological function of heme proteins with axial ligands like O_2, CO & NO. The illinois group (1) initially studied the recombination kinetics of MbCO — Mb + CO — MbCO, from 4k to room temperature and in time scales of 10^{-6}sec to 10^{2}sec. Frauenfelder etal (1) quickly realized that classical kinetics is severly limited by its inability in estimating the rate constants and interpreted as multiexponential system. The nonexponential behaviour was explained as heme protein having many different conformational substates with different activation enthalpies. They come to this conclusion by fitting the kinetic data to enthalpy spectrum and assumed to be the result of combination of many rate constants with single path. The above assumption is true only when the photoproduct is same as deoxyMb and the intramolecular relaxation is absent in the photoproduct. But EXAFS, Resonance Raman, Optical and other spectroscopic studies (2) indicate the photoproduct contains unrelaxed heme also our optical pumping experiments indicates the presence of relaxation Here we describe various physical models which describe nonexponential nature in disordered systems as well in proteins and outline possible relation between low temperature kinetics and protein fluctuations. Also we report the rsults of our recent experiments with MbCO below 80k using light, temperature jump and optical pumping methods under various experimental conditions and propose a new model based on streched exponential.

W-Pos264 THE STRUCTURAL BASIS FOR CROSS LINKING INDUCED FUNCTIONAL EFFECTS IN HbXL99α: A RESONANCE RAMAN STUDY,

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HbXL99α is a selectively cross linked form of HbA with oxygenation and dissociation properties that make it a strong contender for an acellular blood substitute.[1] In an attempt to understand the interplay between the cross linking of subunits and the resulting functional properties, we have used cw and time resolved Raman to probe the structure, dynamics and reactivity associated with the local environment at the site of ligand binding.

Our findings indicate that the cross linking limits, in a pH dependent fashion, the response of the protein tertiary structure to ligand binding. At high pH (8.5) the protein is seemingly locked into a deoxy T state like conformation even when fully liganded; whereas at lower pH values the fully liganded species has what appears to be a strained R state structure. In all cases the structure of the liganded sample exhibits a more strained proximal environment relative to HbA. Consistent with this finding is the substantial reduction in the geminate yield of the CO photoproduct of HbXL99α. These findings can probably account for the reduction in ligand affinity induced by the cross linking.

[1] S. Snyder et al., PNAS 84, 7280 (1987).

W-Pos265 RABI BROADENING OF SUB-NANOSECOND TRANSIENT RESONANCE RAMAN SPECTRA OF DEOXY-HEMOGLOBIN.

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The sub-nanosecond photodynamic behavior of hemoglobin has been extensively studied using a variety of techniques. In order to investigate the structural changes of the heme activity site of hemoglobin and the immediate protein matrix, we have employed 30 ps pulses to generate transient Resonance Raman spectra of the heme dynamics of the protein. It was found that deoxy-hemoglobin demonstrates inhomogeneous broadening of ν_4, a vibrational mode sensitive to the electron density of the porphyrin macrocycle, using time resolved and transient Raman techniques. The broadening of the mode is only observed upon interrogation of the sample with short laser pulses of high fluence (>2 x 10^9 W/cm^2) which are in near resonance with the intense B(0,0) optical transition at 430 nm. This finding is consistent with the theoretical effects of strong radiation fields at high Rabi frequency. (Supported by the NSF DMB-8604435)

W-Pos266 TIME-RESOLVED RESONANCE RAMAN SCATTERING BY CYTOCHROME OXIDASE: EVIDENCE FOR THE FORMATION AND PHOTOLABILITY OF THE $Fe\text{-}O_2$ SPECIES

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The molecular dynamics of biochemical reactions can be elucidated with respect to structural and conformational changes by TR^3 spectroscopy. Specifically, the TR^3 spectra of heme proteins that bind molecular oxygen can be utilized to obtain quantitative kinetic data on the formation and decay of transient species. Cytochrome oxidase catalyzes a reaction in which 4 electrons, donated by cytochrome c, are added to molecular oxygen, thereby producing water. Time-Resolved Resonance Raman Studies (**JACS 106**, 8305 (1984)) suggest that a 40 μs photolabile intermediate is formed during the cytochrome oxidase/oxygen reaction at room temperature. We used a combination of rapid mixing, laser photolysis and a continuous stream in air to detect a 10 μs photolabile intermediate. The continuous stream in air allows us to obtain TR^3 spectra with good resolution at low laser powers (0.3 mJ). With this approach we are able to avoid photoylsis of the transient intermediates and thus to detect these species. Moreover, because the stream is not contained by a capillary in the scattering region, we are able to observe low frequency as well as high frequency vibrational modes. This approach has allowed us to characterize the transient species in considerably more detail than in the original work. We will present the time resolved spectra of the early intermediates in the cytochrome oxidase/oxygen reaction.

W-Pos267 Resonance Raman Characterization of Room Temperature Dioxygen Intermediates of Cytochrome c Oxidase

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Two physiologically relevant dioxygen intermediates of cytochrome c oxidase have been characterized at room temperature by resonance Raman spectroscopy. Compound C (2 electron reduction of O_2) was prepared by mixing CO-mixed valence enzyme with O_2 saturated buffer followed by a photolyzing laser pulse. The sample was interrogated downstream by a probe laser pulse. Pulsed enzyme (4 electron reduction of O_2) was formed by mixing fully-reduced enzyme with O_2 saturated buffer. The sample was probed downstream several minutes after mixing. High frequency spectral characteristics of compound C include a 2-3 cm^{-1} shift in ν_4 and a decrease in the high spin marker band ν_2 for heme a_3, consistent with low temperature data. The pulsed enzyme displays a high frequency spectrum similar to that of the resting form. These results will be discussed within the context of previous cryogenic studies of oxidase intermediates.
(Supported by the NIH GM33330 and GM22432)

W-Pos268 RESONANCE RAMAN CHARACTERIZATION OF THE HEME ELECTRON TRANSFER SITES IN CYTOCHROME bc_1 COMPLEXES FROM R. Rubrum. J.D. Hobbs,[1] A. Kriauciunas,[2] D.B. Knaff,[2] and M.R. Ondrias.[1] [1]Department of Chemistry, University of New Mexico, Albuquerque, NM 87131 and [2]Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409.

High (1000-1700 cm^{-1}) and low(200-700 cm^{-1}) frequency resonance Raman spectra of the heme electron transfer sites in the cytochrome bc_1 complexes isolated from the photosynthetic purple non-sulfur bacteria, Rhodosprillum Rubrum, have been obtained. The isolated cytochrome bc_1 complex consists of three peptide subunits containing two b-type hemes, a Rieske iron-sulfur center and one c-type heme. The midpoint redox potentials fall into three well separated regions: Rieske Fe-S/heme c (E'm = 350 mV), heme b_{560} (E'm = -30 mV) and cytochrome b_{562} (E'm = -90 mV). Further, absorption differences in both the B and Q bands of the b- and c-types present allow selective chromophore enhancement using a tunable dye-laser light source. Thus, by redox poising of the heme active sites and resonance enhancement techniques, we have been able to discern individual contributions to the Raman vibrational spectrum from the ferrous and ferric states of the two distinct protoporphyrins present. In particular, the core-size marker bands ν_{10}, ν_{11} and ν_{19} have been identified for both the reduced b- and c-type hemes of the isolated bc_1 complex. Interestingly, ν_{19} found at 1589 cm^{-1} for heme c_1 of the isolated complex differs substantially from ν_{19} found at ≈1583 cm^{-1} for an aqueous c-type cytochrome such as that isolated from horse heart. Also, our data indicate that there may be significant perturbations to the b-type hemes present as well. These results will be discussed in the context of cytochrome bc_1's role in physiological electron transfer. (Supported by the NIH GM33330 and GM21277.)

W-Pos269 HYDROGEN EXCHANGE EVIDENCE FOR RESIDUAL STRUCTURE IN DENATURED CYTOCHROME C. M-F. Jeng, G.A. Elöve, H. Roder and S.W. Englander. Dept. of Biochemistry & Biophysics, University of Pennsylvania, Philadelphia, PA 19104.

Cytochrome c is known to undergo several pH-dependent conformational transitions. Besides the well known alkaline transition, the protein is believed to assume a globular high spin form at mildly acidic pH before it becomes extensively unfolded below pH 2. Hydrogen exchange rates for individual amide NH, observed by one and two-dimensional NMR, can provide information on stability and structural mobility of the various conformational states at a site-resolved level. Under conditions that favor the globular high-spin form (pH 2.2, 1.5 M NaCl), slow exchange rates are observed for the amide protons of the N- and C-terminal helices, while all other NH sites are much less protected. This exchange pattern resembles that of an early folding intermediate recently characterized by H-exchange labeling methods (Roder, Elöve & Englander, Nature, in press). This observation suggests that His 18 ligation is not required for the formation of the folding intermediate, since the ligand is believed to be absent at acidic pH. Such a change in iron coordination is consistent with the observation that in 4.2 M guanidine HCl, the His 18 backbone NH is the only proton significantly protected against exchange at neutral pH, while at acidic pH its exchange rate is as expected for a disordered polypeptide. Supported by NIH grants GM 31847 (S.W.E.) and GM 35926 (H.R.).

W-Pos270 HYDROGEN EXCHANGE LABELING AND 2D NMR STUDIES OF ALKALINE CYTOCHROME C.
S.M. Dohne, G.A. Elöve, H. Roder and B.T. Nall*, Dept. of Biochemistry & Biophysics, University of Pennsylvania, Philadelphia, PA 19104; *Dept. of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78284.

At alkaline pH, cytochrome c undergoes a conformational transition to a non-native, but globular low-spin form. Spectroscopic evidence indicates that in the alkaline form the axial methionine ligand is probably replaced by lysine side chain. To obtain more detailed structural information, we are using hydrogen exchange labeling in conjunction with rapid mixing techniques and 2D NMR analysis. These methods, initially developed for protein folding studies (Roder, Elöve & Englander, Nature, in press), can reveal the parts of the protein where changes in H-bonded structure are associated with the conformational transition. The strategy is to allow the alkaline transition to proceed in D_2O for variable time periods before the initially deuterated protein is exposed to a brief H_2O labeling pulse. The pulse conditions are chosen such that exposed NH sites become protonated while protected (H-bonded) sites remain deuterated. The pH is then rapidly lowered to quench H-exchange and to reverse the alkaline transition, and the proton labeling pattern imprinted in the alkaline form is analyzed for individually assigned amide protons, using 2D J-correlated NMR spectroscopy. Samples that are only briefly exposed to alkaline pH remain fully deuterated, but numerous amide sites throughout the protein become available for exchange labeling after extended exposure, following the known kinetics of the alkaline transition. Other amide sites are partially protected (residues 7,10,11,32,68,95,95). The NH sites in the center of the C-terminal helix (res. 96-99) are fully protected, indicating that this part of the native structure is preserved in the alkaline form. (Supported by NIH grant GM 35926).

W-Pos271 RESONANCE RAMAN STUDIES OF SIROHEME MODEL COMPOUNDS. A. D. Procyk, J. M. Peloquin, and D. F. Bocian (Intr. by C. R. Worthington), Carnegie Mellon University, Pittsburgh, PA 15213

Resonance Raman spectra have been recorded for siroheme model compounds, including copper and iron octaethylisobacteriochlorin. Excitation into the B and Q absorption regions result in vibrational patterns that differ markedly from those exhibited by the parent porphyrin. Normal coordinate calculations on the siroheme model indicate that the vibrational eigenvectors for many of the high frequency skeletal modes also differ from the porphyrin. In particular, a number of the vibrations are localized on quadrants of the macrocycle. This effect is typical of low-symmetry hydroporphyrins and occurs as a result of the geometrical changes in the π-bonded system.

W-Pos272 SUB-FREEZING TEMPERATURE JUMP STUDY OF BINDING PROCESS OF FLUORIDE TO HEME PROTEINS.
A.-S. Yang and A.S. Brill, Dept. of Physics, Univ. of Virginia

50 μℓ of a ferric hemeprotein solution, [heme] ∿.2 mM with KF at a concentration which (at room temperature) produces fractional formation of the fluoride complex, is rapidly frozen by immersion in liquid nitrogen. After this process, the concentration of fluoride in the solvation layer around the protein molecule exceeds that in the original solution by a factor of over 50. Because of the rapid rate of cooling, locally concentrated fluoride cannot equilibrate with the heme before the temperature drops into the region (<200 K) where there is insufficient energy for exchange to take place with water bound to the ferric ion. Thus, at 77 K the system is frozen into metastable states that do not permit additional fluoride ligation to occur. When the temperature is then jumped into the range 200 to 230 K, channels for ligand passage to and from the heme are activated and further fluoride complex formation occurs (while the intermolecular ice matrix remains rigid, preventing dissipation of the local high fluoride concentration). The kinetics of fluoride binding at 200 to 230 K is on the time scale of minutes, and the extent of complex formation can be measured with relatively good time resolution (several seconds) at points along the kinetic curve by re-immersing samples into liquid nitrogen and taking EPR spectra. In this manner, kinetic curves of the combination of fluoride with horse aquometmyoglobin and human aquomethemoglobin in the temperature range 200-230 K have been obtained. For myoglobin in aqueous buffer, transition to first-order kinetics occurs above 220 K; 50% sucrose affects the temperature dependence. Throughout the range 200-230 K, the kinetic curves for hemoglobin are more complex than those for myoglobin.

Supported by NSF DMB85-17819.

W-Pos273 DES-IRON MYOGLOBIN: STRUCTURAL STUDY BY PROTON NMR SPECTROSCOPY. M.J. Cocco and J.T.J. Lecomte*, Chemistry Department, Pennsylvania State University, University Park, PA 16802

The structure and stability of myoglobin (Mb) depend on the interactions between the protein matrix and the heme. When the heme (iron-protoporphyrin IX, Fe-PP-IX) is extracted from Mb, the protein is destabilized by 37 kcal/mol (1) and its α-helix content decreases by 20 % (2). PP-IX forms a 1:1 complex with apoMb (des-Fe Mb). The α-helix content of des-Fe Mb is similar to that of Mb, but its stability is reduced (3). Thus, the presence of the iron atom affects the stability of Mb but not its conformation. In order to describe the structural and thermodynamic role of the heme, we are studying apoMb and des-Fe Mb by NMR spectroscopy.

Des-Fe Mb was obtained by reconstituting horse apoMb with PP-IX. Des-Fe Mb is a stable, homogeneous complex, whose proton NMR spectrum can be compared directly with that of carbonmonoxy-Mb. Several protein side chains forming the environment of PP-IX were identified by using correlated spectroscopy (COSY); their orientation was determined through dipolar contact analysis (NOESY). We find that PP-IX binds at the same site as the heme. In the only observable isomer, it is locked in the heme cavity and oriented as in the holoprotein. Overall, the structure of the iron-free protein is remarkably similar to that of the holoprotein.
(Supported by ACS-PRF 20334-G3)
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W-Pos274 AN ANALYSIS OF THE FORMYL VIBRATIONAL MODES IN HEME A Yuan-chin Ching, Sanghwa Han, Sharon L. Hammes, and Denis L. Rousseau, AT&T Bell Laboratories, Murray Hill, NJ 07974

The formyl group on the heme in cytochrome *c* oxidase may play a critical role in the functional properties of the enzyme. Although resonance Raman scattering has been used successfully to probe the properties of the heme *a* macrocycle, the assignment of the modes associated with the formyl substituent has not been resolved. We report isotopic substitution studies of the formyl group in heme *a* ($^{16}O \rightarrow ^{18}O$) which have allowed for identification of the modes present in the Raman spectrum as well as a determination of the coupling properties of the C=O stretching mode of the formyl group. We have recorded the spectra for both oxidation states in both organic and aqueous solvents. The isotopic frequency shifts were analyzed by Raman difference spectroscopy and curve fitting techniques. These procedures were necessary in analyzing the data because we discovered an unexpected degree of complexity in that the carbonyl stretching mode of the formyl group is highly coupled to porphyrin modes. Consequently, the frequency shifts of this formyl stretching mode did not follow simple theory and porphyrin modes also changed intensity and shifted. Conclusions about cytochrome *c* oxidase properties based on analysis of the formyl modes must be viewed cautiously.

W-Pos275 pH-DEPENDENCE OF ELECTRON TRANSFER BETWEEN ASCORBIC ACID AND CYTOCHROME b_{561}.
Patrick M. Kelley, Vishram Jalukar and David Njus, Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202.

Electron transfer across the membranes of secretory vesicles, both in the adrenal medullary chromaffin cells and the vasopressin/oxytocin-secreting cells in the posterior pituitary, is thought to be needed for the maintenance of intravesicular ascorbic acid (vitamin C). Cytochrome b_{561} in the secretory-vesicle membrane imports electrons and regenerates ascorbate from the ascorbate free radical, semidehydroascorbate. To understand better the mechanism of this process, we have analyzed the kinetics of electron transfer from internal ascorbate to external ferricyanide. The rate constant for the reaction between cytochrome b_{561} and external ferricyanide increases markedly with the ionic strength of the medium, consistent with an effect of negative membrane surface potential on the ferricyanide anion. At high ionic strength, that rate constant ($\sim$40 μM^{-1} min^{-1}) and the rate constant for the reaction between cytochrome b_{561} and internal ascorbate ($\sim$3 x 10^{-3} $\mu M^{-1} min^{-1}$) are both insensitive to pH. This implies that the rate constant for the reaction between cytochrome b_{561} and internal semidehydroascorbate should be strongly pH-dependent, because the midpoint reduction potential of ascorbate is pH-dependent while that for cytochrome b_{561} is not. Consequently, regeneration of intravesicular ascorbate should be kinetically favored at the acidic pH prevailing in the interior of secretory vesicles. This work was supported by NIH Grant No. GM-30500 and by the American Heart Association.

W-Pos276 EVIDENCE FOR A HYDROXIDE INTERMEDIATE IN CYTOCHROME *C* OXIDASE Sanghwa Han, Yuan-chin Ching, Denis L. Rousseau, AT&T Bell Laboratories, Murray Hill, NJ 07974

A transient intermediate of cytochrome *c* oxidase has been generated by exposing the resting enzyme to a laser beam in the presence of oxygen. This intermediate appears to be generated by simultaneously reducing the enzyme photoreductively and oxidizing it chemically, thereby forcing the enzyme to turn over. Under these conditions a form of cytochrome *c* oxidase is generated with a line at 476cm^{-1} in the resonance Raman spectrum. From isotopic substitution studies ($H_2O \rightarrow D_2O$; $H_2^{16}O \rightarrow H_2^{18}O$) we find that there is more than one contribution to the intensity of this line. Clear deuterium and oxygen isotope shifts are detected for a component of the line. Based on these isotopic substitution studies, the line at 476cm^{-1} has a contribution from an Fe-OH stretching mode, and thus originates from a hydroxide intermediate. Although postulated in the past this is the first direct evidence for a hydroxide intermediate.

W-Pos277 MODIFICATION AND IDENTIFICATION OF AN FITC LABELED LYSINE ON CYTOCHROME P-450$_{scc}$ WHICH IS INVOLVED IN THE BINDING OF ADRENODOXIN. Lois Geren, Jody Tuls, and F. Millett, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701.

Modification of P-450 with fluorescein isothiocyanate (FITC) resulted in the protein being selectively labeled with a single fluorescent molecule. Using HPLC maps of tryptic and chymotryptic digestions, an FITC labeled peptide was isolated, repurified, and sequenced. Lysine 338 was identified as the singly labeled lysine group. Binding studies in the presence of cholesterol revealed decreased binding of adrenodoxin when labeled P-450 was substituted for the native enzyme. Protection studies with the intermediate in the side chain cleavage reaction, 22(R)-hydroxycholesterol, in which no adrenodoxin was present revealed that the labeling did not occur on a region of cytochrome P-450$_{scc}$ where the substrate binds since there was no decrease in the modification of Lys 338 when compared to a sample modified in the absence of 22(R)-hydroxycholesterol. Furthermore, modification of cytochrome P-450$_{scc}$ in the presence of adrenodoxin indicated that the peak identified as containing FITC labeled Lys 338 decreased in size when compared to an unprotected sample. This confirms that the association of adrenodoxin with cytochrome P-450$_{scc}$ protected its binding domain on the cytochrome from modification by the FITC. Thus the view that adrenodoxin acts as a shuttle is further aided by showing that there is at least one positively charged group on cytochrome P-450$_{scc}$ that could interact with one of the known carboxyl groups on adrenodoxin which have shown to be involved with both adrenodoxin reductase activity and P-450 binding. Supported by NIH GM 20488.

W-Pos278 SUBSTRATE BINDING TO HORSERADISH PEROXIDASE AS REVEALED BY HIGH RESOLUTION FLUORESCENCE SPECTROSCOPY. J.Fidy, J.M. Vanderkooi* Dept. of Biochem. and Biophys., School of Med., Univ.Pennsylvania, Philadelphia, PA 19104
The heme in Horseradish peroxidase (HRP) isoenzyme C was substituted by free base mesoporphyrin to make the system available for fluorescence techniques. The binding of the aromatic hydrogen donors, benzo- and naphthohydroxamic acids (BHA,NHA), resorcinol (RE), isomeric resorcyclic acids and hydroquinone (HQ) was studied at pH 5, 50mM ammonium acetate buffer, using conventional room and low ($77^{O}K$) temperature, and laser excited fluorescence spectroscopy at cryogenic temperatures (site selection spectroscopy). Conventional spectroscopy indicated different changes in the emission spectra for the hydroxamic acids (HA's) and resorcinols accompanied by fluorescence quenching effects with the exception of NHA. Dissociation constants estimated from the spectral changes were in agreement with literature data for the native enzyme as NHA < BHA << RE. The high resolution method makes possible to determine the vibrational energies for the first excited singlet state, the 0<-0 transition energies and the inhomogeneous site distribution of the chromophores. The vibronic energy feature for HRP/HA's was identical with HRP, for HRP/RE's transitions assignable to certain side chain vibrational modes were found strongly coupled to phonon vibrations. The 0<-0 transition is shifted to higher energies by 120 cm-1 and 200 cm-1 due to binding for HA's and RE's respectively. The width of site distribution is only slightly affected by binding.(Supported by NIH grant GM34448)

W-Pos279 MOLECULAR DYNAMICS SIMULATIONS OF METMYOGLOBIN IN WATER. Leonore A. Findsen, Shankar Subramaniam and B. Montgomery Pettitt, Dept. of Chemistry, Univ. of Houston, Houston, Texas 77204.

The results of a 100 ps molecular dynamics study of myoglobin with the water explicitly included is presented and compared with the experimental X-ray studies and other molecular dynamics simulations of myoglobin in vacuum. We find that there are significant differences in the motion of the protein atoms with and without solvent molecules. Our data shows that the system reaches an equilibrium state in approximately 50 ps. Also, we note that the external water molecules allow greater movement of the residues on the surface of the protein while reducing the motion around the heme site. The three-dimensional structure of the protein is well-preserved in the simulations as shown by a number of structural measures including a correlation analysis of helix-helix motions. The isotropic thermal B-factors are also well-reproduced. We also present an analysis of the hydrogen bond network between waters and the protein and the network inside the protein itself. The results highlight the advantages of carrying out molecular dynamics simulations of proteins and enzymes in water, rather than in vacuo, in order to reproduce physiological conditions.

Supported by grants from the NIH and the Robert A. Welch Foundation.

W-Pos280 **Do specific modes of protein motion control biochemical reaction rates?** José Nelson Onuchic* and William Bialek,** **Instituto de Física e Química de São Carlos, Universidade de São Paulo, 13560 São Carlos, SP Brazil.* ***Departments of Physics and Biophysics, University of California, Berkeley, CA 94720.*

Reactions in the interior of a protein involve coupling between local atomic rearrangements at the active site and more global motions distributed throughout the protein and solvent. This interaction between local and global dynamics appears in many different problems of chemical and solid state physics. Simple models for such coupled dynamics begin with the assumption that the global dynamics of the "environment" — protein and solvent in the biochemical case — can be described in terms of a small number of generalized coordinates which define the relevant modes of motion; the dynamics along these specific modes can then control the kinetics of the reaction. Recently we (1) have suggested that one should take these simple pictures more seriously, since several experiments provide suggestive evidence for coupling of specific protein vibrational modes to reactive dynamics. Here we develop in detail the criteria for validity of such a picture, emphasizing that many of the qualitative issues in protein dynamics can addressed by understanding the different time scales which control both the dynamics of a chemical reaction and the observable dynamics in spectroscopic experiments — although the dynamics on one time scale may appear quite complex, dynamics on the time scales relevant for a reaction may still be rather simple. We apply these ideas to electron transfer reactions and to ligand binding reactions in the heme proteins. One dramatic consequence of mode-specificity is that it should be possible to accelerate a biochemical reaction by pumping the relevant protein vibrationalmodes with infrared radiation. We discuss the possibility that Austin and co-workers may have observed this effect (2).

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2. R. H. Austin, B. S. Gerstman, P. A. Mansky, & M. W. Roberson, *Biophys. J.* **53**:280a (1988), and personal communication.
Work supported by the National Science Foundation, the Brazilian Agency CNPq, and the Department of Energy. We thank R. Austin for sharing his unpublished results.

W-Pos281 CALCULATED FREE ENERGY OF SALT BRIDGES IN METMYOGLOBIN. Leonore A. Findsen, Shankar Subramaniam and B. Montgomery Pettitt, Dept. of Chemistry, Univ. of Houston, Houston, Texas 77204.

Salt bridges in globular proteins play an important role in the structural stability of biological systems and govern the electrostatic environments of the interiors of proteins. Using free energy simulations, we probe the energetics of one such pair inside myoglobin. The amino acid pair, HIS(82) - ASP(141), forms a salt bridge between the H helix and the EF bend in myoglobin. We have calculated the free energy difference of the ionic pair of amino acids versus the neutral pair using the thermodynamic integration method to be approximately 80 KJ/mole. These two amino acids are charged even though they are in a hydrophobic environment in the interior of the protein. The calculated free energy difference is compared with experimental values in aqueous solutions to gain insight into the dielectric effect of the anisotropic hydrophobic interior of the protein on the charge response of the amino acid pair.

Supported by grants from the NIH and the Robert A. Welch Foundation.

W-Pos282 ELECTRON TRANSPORT INTO CHROMAFFIN-VESICLE GHOSTS. Gordon J. Harnadek and David Njus, Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202.

The membrane of the adrenal medullary chromaffin vesicle contains a transmembrane electron carrier (cytochrome b_{561}) that may import reducing equivalents for the intravesicular enzyme dopamine β-monooxygenase. This electron transfer system has been extensively studied by monitoring electron efflux from internal ascorbic acid to external electron acceptors. Recently, Cidon et al. (J. Biol. Chem. 258, 11684, 1983) found, using the optical probe acridine orange, that phenazine methosulfate and ascorbic acid added in combination dissipate the pH gradient across chromaffin-vesicle membranes. This phenomenon is attributable to electron flow from external ascorbic acid to internal phenazine methosulfate which, as a weak base, is accumulated within the acidic vesicles. Reduction of O_2 by phenazine methosulfate (PMS) consumes H^+ and raises the intravesicular pH. We have investigated this system as a model for inward electron transfer. The rate of electron transfer depends upon both the PMS and ascorbate concentrations. That this electron transfer is mediated by cytochrome b_{561} is confirmed by the fact that the cytochrome undergoes the expected spectral changes. The cytochrome becomes oxidized upon addition of PMS. Subsequent addition of ascorbic acid causes partial reduction of the cytochrome. The cytochrome reverts to the oxidized state if the concentration of ascorbate added is less than twice the concentration of dissolved O_2. If the amount of added ascorbate exceeds this, then the cytochrome becomes fully reduced when the medium turns anoxic. This indicates that PMS accepts electrons from cytochrome b_{561} at the internal surface of the membrane and establishes this as a good model for studying electron transfer from external ascorbic acid into the chromaffin vesicle. Supported by NIH Grant GM-30500.

W-Pos283 PROBING THE STRUCTURAL PATHWAY FOR COOPERATIVE FREE ENERGY TRANSDUCTION IN HUMAN HEMOGLOBIN AT INTERMEDIATE STATES OF LIGATION.
Vince J. LiCata and Gary K. Ackers, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Regulation of ligand binding affinity in human hemoglobin is due to free energy changes arising from interactions at the $\alpha^1\beta^2$ interface, including the $\alpha^1\beta^2$, $\alpha^2\beta^1$, and $\alpha^1\alpha^2$ intersubunit contacts (Pettigrew, et al. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*: 1849). During ligation tetrameric hemoglobin assumes ten combinatorially unique molecular species which are found to distribute into at least three cooperative free energy levels (Smith and Ackers (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*: 5347).

The intermediate ligation species [21], comprised of one unligated dimer and one dimer with two ligands, has been studied in combination with two interfacial perturbations: des-Arg hemoglobin where α141 has been deleted, and NES hemoglobin where the β93 cysteine has been reacted with N-ethylmaleimide. A novel thermodynamic technique, **cryogenic isoelectric focusing** (Perrella, et al. (1978) *Anal. Biochem. 88*: 212) is used to study these hybrid hemoglobins. Preliminary results suggest that the $\alpha^1\beta^2$ interface as a whole undergoes a conformational switch in this intermediate species, i.e., all three intersubunit contact regions are involved. A local pathway of free energy transduction cannot be ruled out until a larger number of interfacial perturbations is examined, however. The results also suggest a different structural state for the species [21] molecule relative to the fully ligated and fully unligated species. The results are incompatible with a two-state cooperative mechanism.

W-Pos284 ALLOSTERIC KINETICS OF TRILIGATED OXYHEMOGLOBIN Frank A. Ferrone, Anthony J. Martino*, Alison J. G. Murray**, and NianQiang Zhang, Department of Physics & Atmospheric Sicence, Drexel University, Philadelphia, PA 19104.

We have used modulated excitation to follow the rate of change between R and T structures in triligated oxyhemoglobin. The low quantum yield and rapid ligand binding rate necessitate high photon flux, which creates a temperature rise in the sample. This temperature rise is kept manageable by using a microscopic illumination system to maximize the surface to volume ratio. By direct control measurements the temperature rise for a 0.5% photolysis level is about 15 degrees. Signal to noise is excellent, and the rapid binding of oxygen further minimizes an associated problem of this technique, viz., multiple excitation induced out-of-phase signal. Ligand binding kinetics of the fourth oxygen agree with the rates measured by others. Compared to the allosteric kinetics with CO as a ligand, we find the R to T rate increases three fold, while the T to R rate drops slightly. This suggests a distal effect of ligands on the structural kinetics. From the rates determined here, we are forced to conclude that the equilibrium constant between R_3 and T_3 must be greater than unity. Implications of this finding will be discussed.

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W-Pos285 PHOSPHATES AFFECT THE RATE OF CHANGE AND EQUILIBRIUM BETWEEN QUATERNARY CONFORMATIONS OF HEMOGLOBIN Anthony J. Martino* and Frank A. Ferrone, Department of Physics and Atmospheric Science, Drexel University, Philadelphia, PA 19104.

The rates of quaternary conformational change in HbA with 3 ligands bound at pH 7.0 in 0.05 M bis-tris have been measured by using the fluorescent quenching of an analog of DPG, 8-hydroxy-1,3,6 pyrene trisulfonic acid (PTS), coupled with the method of modulated excitation, (Biophys. J. 1985, **48**, 269-282) to probe the movement of the subunits of the HbA molecule. The method (Biophys. J. 1988, **53**, 279a) allows the allosteric rates without PTS to be measured in the presence of PTS. The binding constant for the PTS-Hb complex is also determined, using DC photolysis to adjust the concentration of deoxy-Hb and is found to be different between deoxy Hb and the species with 3 ligands bound. Using this method, the rates and equilibrium constant are found to be: $k_{RT} = 3.4\pm1.0 \times 10^3\ s^{-1}$, $k_{TR} = 2.1\pm0.5 \times 10^4\ s^{-1}$ and $L_3 = 0.16 \pm 0.06$. PTS is found to be in rapid exchange with HbA with an on-rate of at least $4\times10^9\ M^{-1}\ s^{-1}$. The corresponding allosteric rates for HbA in phosphate buffer were found to be $k_{RT} = 1.0 \times 10^3$, $k_{TR} = 3.0 \times 10^3$ and $L_3 = 0.33$ (Biophys. J. ,1985, **48**, 269-282). As expected the addition of phosphates increases L_3 due to the stabilization of the T-state relative to the R-state. The concomitant decrease in the rates can be viewed as the narrowing of the free energy wells of both the T and R states, thus raising the free energy barrier between T and R. Modulated absorbance difference spectra show no difference in shape upon addition of PTS to the solution. The method of modulated fluorescence allows one to get the allosteric rates and equilibrium constant without *a priori* knowledge of the spectral signature. Comparison between modulated absorbance and modulated fluorescence measurements shows general aggreement, but small differences do exist, implying that the movement of the subunits may not be entirely correlated with the spectral event at the heme.
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W-Pos286 STABILIZATION OF HUMAN HEMOGLOBIN BY SINGLE AND DOUBLE CROSSLINKING. Thao Yang, Susan M. Bauer, Alicia J. West, and Kenneth W. Olsen; Department of Chemistry, Loyola University of Chicago, 6525 N. Sheridan Rd., Chicago, IL 60626.

Human hemoglobin can be stabilized by a variety of crosslinking reagents, including bis(3,5-dibromosalicyl) fumarate (diaspirin), dimethylpimelimidate (DMP), and diisothiocyanostilbene disulfonate (DIDS). The stability of these crosslinked proteins was demonstrated by thermal denaturation of methemoglobin in 0.01 M MOPS, pH 7, containing 0.9 M guanidine. The absorbance changes were monitored by a diode array spectrophotometer while heating at 0.3°C/min from 24-70°C. Hemoglobin crosslinked by diaspirin in the oxy form between the two Lys β82's (Walder et al. (1980) J. Mol. Biol. 141:195) or in the deoxy form between the two Lys α99's (Chatterjee et al. (1986) J. Biol. Chem. 261:9929) produced a markedly higher transition temperature (T_m) of 57°C, while the T_m of the uncrosslinked Hb was 42°C. Diaspirin crosslinked deoxy Hb yielded a second major product with a T_m of 59°C, which appeared to be β crosslinked Hb with additional modification sites as demonstrated by amino acid analysis and SDS electrophoresis on separated globin chains. Crosslinking of oxy Hb A with DMP was done in 0.1 M borate buffer, pH 9.5, and gave a product with a T_m of 59°C. The purified β82 crosslinked oxy Hb A was further treated with DMP to produce a double crosslinked hemoglobin, which had a T_m of 62°C. Thus, the second crosslinking only slightly improved the stability. DIDS reaction with deoxy Hb A in 0.01 M MOPS, pH 7, produced several crosslinked and modified species with different stabilities. These results should improve the design of stabilized crosslinked hemoglobins as blood substitutes. (Supported by a grant from the American Heart Association of Metropolitan Chicago.)

W-Pos287 POLYMERIZATION OF SICKLE CELL HEMOGLOBIN WITH CELL MEMBRANES AND CYTOSKELETONS, P.J. Baxter-Rahmoeller and H. Mizukami. Division of Regulatory Biology and Biophysics, Department of Biological Sciences, Wayne State University, Detroit, Michigan, 48202

The polymerization of sickle cell hemoglobin (HbS) in solution is a well studied phenomenon. However, information with regard to the intracellular processes is still scarce. Specifically, it is uncertain how the cytoskeletons and cell membranes might interact with polymerizing HbS. In this report we use a polarizing microscope to examine how the process of HbS polymerization is affected by cell membranes and cytoskeletons while the solution is being deoxygenated and reoxygenated.

Hemoglobin free inside-out membranes and cytoskeletons from the erythrocytes of normal donors and donors homozygous for the sickle cell trait were prepared in the usual manners. A chromatographically pure sickle cell hemoglobin solution of sufficiently high concentration was mixed with the membranes and cytoskeletons, then the suspensions were deoxygenated and reoxygenated in a sealed chamber during microscopic examination. A polarizing microscope was needed to locate and visualize the HbS polymers. In order to identify the location of the vesicles and skeletons, often it was necessary to adhere them to the coverslip prior to the addition of the HbS solution.

Our observations suggest that some of the polymer formations are dictated by the presence of these cell structures. How the rate of deoxygenation and the presence of hemichrome affect the polymerization will also be presented. (Supported in part by grants from NIH HL 16008)

W-Pos288 **OBSERVING the INTRACELLULAR POLYMERIZATION of HbS by DIFFERENTIAL POLARIZATION MICROSCOPY**

David A. Beach, Carlos Bustamante, Manuel Gurule, Hiroshi Mizukami † *and Clemensau Aquaye,*† Department of Chemistry, University of New Mexico, Albuquerque, NM 87131 and † Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202

Differential polarization imaging is a microscopic method that measures and displays the optical anisotropy at every pixel position on the object. With this technique, it is possible to space resolve domains of different molecular organization within cells and to follow the changes induced in these domains by changing external conditions. We have used this technique to establish the patterns of alignment of HbS polymers inside fixed sickle cells. These are in good agreement with those derived from electron microscope observations of cells of similar morphology. We have also followed the intracellular polymerization of hemoglobin S, induced in the erythrocytes of sickle cell anemia patients, as the cells are being deoxygenated and reoxygenated.

W-Pos289 DIFFUSION OF HEMOGLOBIN IN SICKLE HEMOGLOBIN POLYMER DOMAINS Michael R. Cho and Frank A. Ferrone, Department of Physics & Atmospheric Sicence, Drexel University, Philadelphia, PA 19104.

Using a unique instrument which can resolve the spatio-temporal evolution of polymer domains of sickle hemoglobin (Basak, Ferrone and Wang, 1988, Biophys. J. *in press*) we have observed the diffusion of hemoglobin into those domains. This is observed as an increase in the optical density at 430 or 420 nm which occurs as polymers form. It can be distinguished from other possible sources of increase, such as turbidity, by the lack of signal at 488 nm. The net monomer diffusion results from the relative immobility of polymerized HbS, allowing the total hemoglobin concentration (polymerized plus nonpolymerized) to rise above the initial polymer concentration. Comparison of the gradients of the monomer concentration with the intensity gradients seen in polymer light scattering permits us to explore the relationship between polymer mass and light scattering intensity, as well as the constancy of the diffusion constant. The eventual saturation of this increase in hemoglobin density, which we also observe, thus requires an additional mechanism, such as polymer-polymer exclusion, to terminate domain growth. Implications of these findings for the appearance of polymer gels will also be discussed.

W-Pos290 CALCULATIONS OF THE TRANSMISSIVITY AND REFLECTIVITY OF SOLUTIONS OF SICKLE HEMOGLOBIN POLYMERS AND MONOMERS. Marilyn F. Bishop, Department of Physics, Virginia Commonwealth University, 1020 West Main Street, Richmond, Virginia 23284-2000.

We have calculated the transmissivity and reflectivity for a solution of polymerizing sickle hemoglobin using a dielectric tensor obtained from calculations that employ Bruggeman's Symmetrical Effective Medium Approximation. In this theory, we assume that all polymers and monomers are embedded in an effective medium and that the solution surrounding them is represented by spherical water "particles" embedded in the same medium. The polymers are represented by infinitely long rigid cylindrical particles and the monomers by spherical particles, where the dielectric constant inside a monomer or polymer is the same. The dielectric tensor is obtained by requiring that the sum of the dipole moments of the monomers, of the polymers, and of the solution "particles" adds to zero. Because the polarizability per unit volume of a cylindrical particle is different from that of a spherical particle, the dielectric tensor for randomly oriented particles increases as the fraction of polymers increases, which has an effect on the reflectivity and transmissivity of the solution. In addition, if the polymers are aligned, the resultant dielectric tensor of the entire solution is anisotropic, which leads to a finite value of the transmissivity when viewed through crossed polarizers.

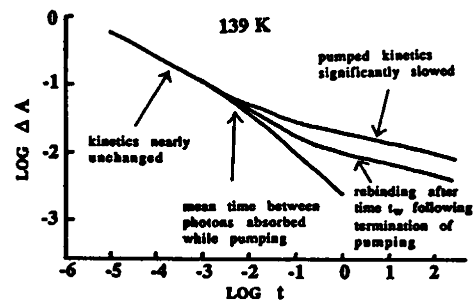
This work is supported by NIH Grant Number HL38614.

W-Pos291 SINGLE CRYSTAL OXYGEN BINDING CURVES OF HUMAN HEMOGLOBIN. Andrea Mozzarelli and Gian Luigi Rossi, Istituto di Scienze Biochimiche, Universita di Parma, Italy; and William A. Eaton and Eric R. Henry, Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, Maryland.

In order to determine the extent of cooperativity in oxygen binding to the T quaternary structure of human hemoglobin, we are investigating the binding of oxygen by single crystals grown from polyethylene glycol. X-ray diffraction studies show that the T quaternary structure is maintained in these crystals at atmospheric oxygen pressure (Brzozowski et al., Nature 307, 74 (1984); Liddington et al., Nature 331, 725 (1988)). The fractional extent of oxygen binding is determined from polarized absorption measurements on the (010) face of this orthorhombic crystal using a microspectrophotometer. Spectra from 450 nm to 700 nm are measured with the electric vector of the plane-polarized light parallel to the a- and c-crystal axes. Oxygen pressures are determined with a Clark electrode. The presence of nearly perfect isosbestic points in both the a-axis and c-axis polarizations indicates that the T quaternary structure is maintained at oxygen pressures up to 750 torr. In order to use all the information contained in the complete spectra for calculating a binding curve, we use the amplitude of the "average" oxy-minus-deoxy difference spectrum that is the best one component least-squares fit to the data. This amplitude as a function of pressure is obtained from the singular value decomposition of the difference spectra for each polarization. Hill plots of the binding curves measured on 5 different crystals yield $p50 = 98 \pm 12$ torr and $n = 1.07 \pm 0.07$, indicating that there is little or no cooperativity in the binding. The data also indicate that the affinity of the alpha and beta hemes differs by less than a factor of 3.

W-Pos292 **Long Lived States Induced by Extended Optical Irradiation of Carbonmonoxy Sperm Whale Myoglobin.**
Todd B. Sauke, Hans Frauenfelder, Stan Luck, Peter J. Steinbach, Aihua Xie and Robert D. Young, Department of Physics, Univ. of Illinois, Urbana, IL 61801

The kinetics of CO rebinding to sperm whale myoglobin after flash photolysis is inherently nonexponential in time below 160K. A multiple flash experiment in which rebinding kinetics is measured after repeated photolysis by brief light pulses (≤ 10 photons/protein) shows that the nonexponential kinetics is due predominantly to an inhomogeneous distribution of proteins among conformational substates having different barriers for rebinding.[1] Extended optical irradiation by >> 100 photons/protein (referred to as pumping), however, results in slowed kinetics, indicating a homogeneous contribution to the nonexponential kinetics. The slowing is not uniform across the distribution of substates: those proteins in substates with the smallest rebinding rates are most affected by the pumping (see figure). Photolysis at a time t_w after termination of pumping displays rebinding kinetics which has relaxed towards the original unpumped kinetics. We determine the preexponential factors and distributions of activation enthalpies for rebinding in the pumped and unpumped states. These phenomena are interpreted in terms of a hierarchical structure of conformational substates.



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W-Pos293 **CO Rebinding to Sperm Whale Myoglobin: Mapping the A Substates into the Soret**

Peter J. Steinbach, Hans Frauenfelder, J. Bruce Johnson, and Todd B. Sauke
Department of Physics, University of Illinois, Urbana, IL 61801

At temperatures below about 180K in a 75% glycerol/water solvent, the three bound-state conformations of MbCO (A_0, A_1, and A_3) each rebind the photolyzed ligand nonexponentially in time.[1] We interpret the nonexponential rebinding kinetics of MbCO as monitored at 440 nm as the sum of three rate distributions corresponding to these three conformational substates. We numerically invert the Soret rebinding kinetics from 100K to 140K using the Maximum Entropy Method[2] (MEM) to extract the underlying distribution of lifetimes, f(log τ). The f(log τ) distribution is then fit to three peaks. At all temperatures, the three peaks have areas consistent with the relative absorption of the A substates as measured by FTIR spectroscopy. Each of the three peak positions agrees well with the $\langle \log \tau \rangle$ predicted by the enthalpy distribution obtained from the IR rebinding kinetics of the corresponding substate.

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2. J. Skilling and R. K. Bryan, *Mon. Not. R. Astr. Soc.* **211:**111, 1984.

W-Pos294 **FTIR Studies of CO Orientation, and pH Effects in HisE7 Mutants of Carbonmonoxymyoglobin.**
David Bräunstein, Benjamin R.. Cowen, Karen D. Egeberg#, Hans Frauenfelder, Judy Mourant, Pal Ormos, Stephen G. Sligar#, Barry A. Springer#, and Robert D. Young, Departments of Physics, and #Biochemistry, Univ. of Illinois, Urbana, 61801 .*

The production of a variety of novel HisE7 mutants of sperm whale myoglobin (Mb) by Sligar and collaborators (1) has allowed us to probe the role of the HisE7 in determining the orientation of CO in the bound state. The CO adducts of seven mutants, four non-polar (His-> Gly, Val, Phe, Met), and three polar (His-> Tyr, Asp, and Arg) were prepared in a 70% glycerol-water solution at pH7. The ν(CO) bands of the bound and unbound CO were measured between $1900 cm^{-1}$ to $2200 cm^{-1}$ following flash-photolysis at 10K. The angle, α, between the CO dipole and the heme normal of CO bound, for each mutant, was obtained by measuring the linear dichroism of the ν(CO) bands after photoselection.(2). Native MbCO has an IR spectrum with three bound ν(CO) bands corresponding to three CO orientations, with the smallest α at the highest peak frequency (2). One might expect the IR spectrum of the mutants with non-polar or small distals residues to have single ν(CO) bands. No such simple behavior was observed: (i) The Gly and Met mutants each display two bands, whose relative intensities change with pH. Because of the absence of any nearby titratable distal residues, this behaviour might be due to the influence of residues on the proximal side, for instance the HisFG3 . (ii) The angle α of the seven mutants ranges from 20° to 33° and the peak frequencies of the stretch bands vary between $1962 cm^{-1}$ to $1988 cm^{-1}$. No clear correlations are apparent between angle and peak frequency for the seven proteins measured.

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2. Ormos, Pal, Braunstein, David, Frauenfelder, Hans, Lin, Shuo-Liang, Hong, Mi Kyung, Sauke, Todd B., Young, Robert D. (1988) **Proc. Natl. Acad. Sci USA** 85

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W-Pos295 A NEW ANIONS BINDING SITE IN RUMINANT HEMOGLOBIN. Clara Fronticelli, Anna Razynska & Enrico Bucci. Dep. Biochemistry. U. Md. Med. Sch. Baltimore MD 21201. Following the hypothesis that interaction with the solvent components plays a relevant role in the modulation of the oxygen affinity of hemoglobin, we have analyzed the hydropathy of the available amino acids sequences of the α and β chains of a group of hemoglobins with low oxygen affinity (ruminants) and high oxygen affinity (primates). The α chains of the two groups produced similar plots. In contrast when the β chains were compared three regions with different hydropathy scores were identified.

	Primates	Ruminants
β1-β10	-33.4	- 0.08
β64-β81	0.150	-92.3
β120-β130	-60.0	-12.4

In our laboratory we have shown that the low affinity of ruminant hemoglobins is consistent with the presence in the molecule of anions binding sites not present in primate hemoglobins (J.Mol.Biol.202:343,1988). In the β1-β10 region the different hydropathy results mainly from the presence in ruminants, of a methionine at the N-amino terminal residue. In the β64-β81 regions five hydrophobic residues found in primates are substituted with hydrophilic residues in ruminants; in particular a lysine replaces the β76 valine present in primates. We propose that these two regions of different hydrophobicity have a different folding in the two groups of hemoglobins. This would produce in ruminants hemoglobins a distorsion of the A and E helices which results in the formation of a positively charged pocket, defined by residues β7 lysine, β76 lysine and β77 hystidine with a high affinity for anions. Supported by NIH grants HL-332629 and HL 13164.

W-Pos296 HEMOGLOBIN MAY DELIVER SULFIDE TO INTRACELLULAR BACTERIAL SYMBIONTS. D.W. Kraus, J.E. Doeller, J.B. Wittenberg. Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Cytoplasmic hemoglobins (Hb) occur in symbiont-harboring gills of molluscs from environments where H_2S and O_2 coexist. The symbionts oxidize sulfide and fix CO_2. Spectra of living gills (*Solemya velum*, *Lucinoma aequizonata*) and of the bacteriocyte domain alone (*S. reidi*), exposed to H_2S at low P_{O2}, show about half the Hb reversibly converted to ferric Hb sulfide, the balance remaining HbO_2. Rapid formation and reduction of ferric Hb, in our experience, is unique to the symbiont-harboring gill. Optical spectra of non-bacteriocyte tissue: ciliated gill, pallial muscle, nerves and hypobranchial gland (*S. reidi*) never demonstrate ferric Hb sulfide formation. Three Hb's have been isolated from the gill of a related clam (*Lucina pectinata*). None form sulfhemoglobin. A "sulfide-reactive" Hb (Hb I) forms ferric Hb sulfide rapidly in the presence of H_2S at low P_{O2}. Ferrous Hb I combines with and dissociates O_2 rapidly (k_{on} = > 10^7 M^{-1} s^{-1}; k_{off} = 60 s^{-1}) The two "sulfide-unreactive" Hb's II and III do not form ferric Hb sulfide under the above conditions. Oxygen reactions are slow: k_{on} = 2 and 1.5 x 10^5 M^{-1} s^{-1}; k_{off} = 0.1 and 0.05 s^{-1}, respectively. Combination of ferric Hb II with sulfide is 1000-fold (acid limit) or 300,000-fold (alkaline limit) slower than combination of ferric Hb I with sulfide. Preliminary fractionation of Hb's from whole gills of *S. reidi* show 3 Hb's chromatographically similar to those of *L. pectinata*. In contrast, only two Hb's chromatographically similar to Hb II and III occur in pooled non-gill tissues. This suggests that "sulfide-reactive" Hb is found only in bacteriocytes. Perhaps "sulfide-reactive" hemoglobins deliver sulfide in the gill and "sulfide-unreactive" hemoglobins deliver solely oxygen. *S. reidi* and *L. aequizonata* were gifts of Drs. R. Reid and J. Childress.

W-Pos297 CARBON MONOXIDE DRIVEN REDUCTION OF OXIDIZED SCAPHARCA INAEQUIVALVIS DIMERIC HEMOGLOBIN. Alberto Boffi and Emilia Chiancone, C.S. Biologia Molecolare, University of Rome, Italy; Joseph and Celia Bonaventura, Duke University Marine Laboratory, Beaufort, North Carolina.

The homodimeric hemoglobin of the mollusc, Scapharca, forms a hemichrome when oxidized by ferricyanide. Surprisingly, when the hemichrome is exposed to CO its spectrum shifts to that of the ferrous CO derivative. CO removal by evacuation of N_2 flushing leads back to the hemichrome rather than to the ferrous deoxy spectrum. Tonometric CO binding studies reveal an apparent CO affinity whose $P_{1/2}$ is about 25 mm Hg at 20°C in 0.05 M Hepes buffer, pH 6.1. No cooperativity in CO binding is observed. Under pseudo-first order conditions, the time course of CO binding is monophasic at low protein concentrations where monomers predominate, and biphasic at higher concentrations where both monomers and dimers are present. The process requires several minutes at pH 6.1, but several hours at pH 8.5. Flash photolysis of the CO adduct shows much faster CO binding, with the deoxy ferrous species being the photodissociation product. Atomic absorption measurements indicate that the ferricyanide-oxidized material contains 1.8 ± 0.6 iron atoms per heme. We infer that ferrocyanide is tightly bound (even after chromatography on G-25 and amberlite mixed-bed ion exchange columns) and forms a complex in which its iron and that of the heme are in redox equilibrium. The ability of CO to shift the hemichrome spectrum to that of ferrous heme can thus be attributed to a mass-action effect on the redox equilibrium.

W-Pos298 **INFLUENCE OF NUCLEAR CODED SUBUNITS ON CYTOCHROME C OXIDASE ACTIVITY.**
A. Basu, R.W. Waterland, R.O. Poyton* and B. Chance. Dept. Biochem/Biophys, Univ. of Penn, Phila., PA 19104; and *Dept. Molecular,Cellular & Develop.Biol, Univ. Colorado, Boulder, CO 80309

It is already known that some of the nuclear coded subunits are essential for the assembly of cytochrome oxidase (1), while their regulatory role in protein function is still unknown.

In this work we found that the yeast (S. cerevisiae) cells maintain a ratio of a cytochrome oxidase concentration and a yeast hemoglobin like pigment. The activation energy of low temperature CO recombination of cytochrome oxidase is 5.5 K Cal Mol^{-1}. The co-recombination kinetics of cytochrome oxidase at -65° is identical in rate to that of hemoglobin at -80°C (pseudo-first order rate 10.5×10^{-2} sec^{-1}). The mutant strain GD5a contains low concentrations of oxidase (subunit 5b) with three times the concentration of hemoglobin.

(1) Poyton, R.O. et al in Cytochrome Systems: Molecular Biology & Bioenergetics (S. Papa, ed) Plenum Publ, NY (1978)

W-Pos299 **HIGH RESOLUTION, P31 NMR OF INTACT, SPLIT-THICKNESS, PIG SKIN.**
Henry Aceto, Jr., Elisa M. Donatelli, Patrick C. Cooney, Daniel J. Stralka, Peter F. Pond and Michael J. McCreery. Biophysical Research Division, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

High resolution nuclear magnetic resonance (NMR) represents a powerful tool in the study of cellular metabolism in isolated cell tissue and whole organ systems. The application of NMR to the augmentation of our understanding of metabolism in both normal and diseased conditions has enjoyed increased recognition. The conduct of such studies presumes the availability of model systems that conform to the geometric and logistic constrictions imposed by the NMR spectrometer. A model system has been developed to study cellular metabolism in skin. Skin was excised from the dorsal flank of weanling, Yorkshire pigs and dermatomed to thicknesses ranging from 200 um to 800 um. These sections, 12.5 cm^2 to 30 cm^2, were mounted on a specially constructed apparatus, inserted into a 16 mm NMR tube, and continuously perfused with Eagle's MEM modified with a nonphosphate buffer to maintain tissue viability for up to 16 hours during P31 NMR analysis. Spectra were obtained on a Varian XL-300 spectrometer operating at 121 MHz from 1200 transients using Ernst angle pulses and an interpulse interval of 1.75 sec. Consistent with literature reports from skin and other tissues, peaks were observed having chemical shifts derived from ATP, PCr, Pi, sugar phosphates, phosphodiesters, and NAD. Metabolite fluctuations, particularly PCr and sugar phosphates were observed while varying temperature from 7° to 25° C and oxygen tension from 50 to 800 mm Hg. This model system should prove useful in extending our undersanding of the energy metabolism in both normal skin, and skin compromised by such factors as aging, pathologic conditions, thermal burns, or chemical exposure.

W-Pos300 EXAFS STUDIES OF THE ISOLATED BOVINE HEART RIESKE IRON-SULFUR CLUSTER
T. Ohnishi[1], H. Schägger[2], G. Von Jagow[2], J. Smith[3], B. Chance[1,4], and L. Powers[5]. [1]Dept. Biochem.. & Biophys. University of Pennsylvania, Philadelphia, PA 19104-6089, [2]Abteilung fur Therapeutische Biochemie, Universität Frankfurt, 6000 Frankfurt/ M. 70, FRG, [3]Dept. Chemistry, Georgia State University, Atlanta, GA 30303, [4]ISFS Univ. City Science Center, Philadelphia, PA 19104, [5]Dept. of Chemistry, Utah State University, Logan, UT 84322-0300.

Recently involvement of one or, more likely, two nitrogen-ligands in the Rieske-type [2Fe-2S] cluster has been reported based on the chemical assay and various spectroscopic analyses, such as EPR, Mossbauer, ENDOR, and Resonance Raman, of isolated Thermus thermophilus HB-8 protein by Fee and his collaborators [Fee et al.(1984) J. Biol. Chem. 259, 124; Cline et al. J. Biol. Chem. 260, 3251; Kiula et al. (1987) J. Am. Chem. Soc., 109, 1559]. Similarly presence of at least one nitrogen-ligand was shown in the mitochondrial Rieske [2Fe-2S] cluster [Tesler et al. (1987) FEBS Lett. 185, 311]. We have conducted EXAFS studies of the Rieske [2Fe-2S] protein isolated from the cytochrome bc_1 complex of bovine heart mitochondria. Standard analysis could not distinguish one or two nitrogen ligands per cluster. However, one nitrogen and three cysteine ligands per cluster was found possibly to be a better solution in more comprehensive analysis procedures. [Powers and Kincaid (1988) Biochem. in press] (Supported by NSF DMB85-11160, Deutsche Forschungsgemeinschaft, NSF, Div. of Materials Research; NIH, Biotech. Res. Program, Div. of Research Resources)

W-Pos301 ADENINE NUCLEOTIDE THERMODYNAMIC OR KINETIC CONTROL OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION: A RECONCILIATION VIA EINSTEIN'S DIFFUSION EQUATION. **Erich Gnaiger and William E. Jacobus. Department of Zoology, University of Innsbruck, Austria and Division of Cardiology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205**

It is little appreciated that Einstein's diffusion equation provides us with a perspective into nonequilibrium thermodynamics which is unique from current theories. Intrinsic is the notion that chemical flow is regulated by both thermodynamic forces and chemical concentrations, or activities. In fact, this notion unifies thermodynamics and kinetics. Fick's first law of diffusion, combined with Einstein's and the universal gas law, has led to the development of the concept of chemical reaction pressure (RT times the concentration difference; units: Pascal) as a driving force for chemical flux. This theory has been successfully generalized for the adenine nucleotide control of both heart and liver mitochondrial oxidative phosphorylation. Steady-state rates of ADP production were induced by either creatine kinase, or hexokinase in the presence of creatine and glucose respectively. Two conditions were used, constant added ATP with variable enzyme, or constant enzyme with variable added adenine nucleotide. These conditions have previously led to paradoxical results. However, when transformed into flux-pressure plots, the conditions are indistinguishable, and the lines superimposable for the liver data. In heart, a unique line, with steeper slope, is observed for endogenous creatine kinase. Overall, the data support the notion that chemical reaction pressure is a useful concept for the description of flux control. Moreover, it extends the range of thermodynamic flux control analysis into the domain far from equilibrium, and thus more relevant to the living condition.

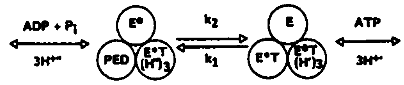
W-Pos302 EFFECT OF THREE STRUCTURALLY RELATED LIPOPHILIC-ANIONIC FLUORESCENT WHITENING AGENTS ON MITOCHONDRIAL AND PARACOCCUS DENITRIFICANS NADH-UBIQUINONE REDUCTASE: EVIDENCE FOR A NON-ROTENONE TYPE OF INHIBITION. W. Marshall Anderson, and Deborah L. Delinck. Ind. Univ. Sch. Med. NW Center for Med. Ed. 3400 Broadway, Gary, IN USA 46408

Bovine heart mitochondrial and Paracoccus denitrificans NADH oxidase is inhibited by three structurally related lipophilic-anionic fluorescent whitening agents (Tinopal AMS-GX, 5-BMGX, and UNPA). The $I_{0.5}$ ranged from 11 to 60 μM for the three compounds with mitochondrial NADH oxidase and from 35 to 45 μM with P. denitrificans NADH oxidase. Neither succinate nor cytochrome oxidase were significantly affected, localizing the site of inhibition to NADH-ubiquinone reductase. Studies with artificial electron acceptors such as 3-acetylpyridine adenine dinucleotide, ferricyanide, dichlorophenol indophenol (DCIP), juglone, menadione, and duroquinone, as well as coenzyme Q_1 revealed that all three Tinopals produced marked inhibition with both mmitochondrial and P. denitrificans NADH-ubiquinone reductase of all electron acceptors except DCIP. The Tinopals could be inhibiting NADH-ubiquinone reductase by (a) binding to the rotenone site, (b) binding to the rotenone site and to another site(s), or (c) binding to a non-rotenone site exclusively. Inhibition by binding to a non-rotenone site exclusively was confirmed as the mechanism of action by determining the inhibitory capacity of the three Tinopals on reduction of artificial electron acceptors and Q_1 using rotenone-insensitive preparations of mitochondrial NADH-ubiquinone reductase (complex I) and P. denitrificans membrane vesicles. In all cases except mitochondrial NADH-duroquinone reductase, the Tinopals inhibited the activities of the rotenone-insensitive preparations to the same extent or better than the sensitive preparations. Supported by Indiana Heart and NIH HL 32829.

W-Pos303 PROTON TRANSPORT CAUSED BY SALICYLATES IN PHOSPHOLIPID BILAYER MEMBRANES: A MODEL FOR SALICYLATE-INDUCED "LOOSE COUPLING" IN MITOCHONDRIA. John Gutknecht, Dept. of Cell Biology, Duke University and Duke Marine Laboratory, Beaufort, NC 28516.

Mechanisms of proton transport were investigated in phospholipid bilayer membranes exposed to salicylate and salicylic acid. Membranes were formed from diphytanoyl phosphatidylcholine in decane plus chlorodecane (usually 30%, v/v). Salicylates were added to the aqueous phase at concentrations ranging from 0.1 to 30 mM. Proton and salicylate conductances (G_H and G_A) were calculated from the steady-state total conductances and the H^+ or A^- diffusion potentials produced by transmembrane H^+ or A^- gradients. At low pH salicylate caused G_H to increase in proportion to the square of the total weak acid concentration, and G_H was maximum when pH = pK. At neutral to alkaline pH the membranes were salicylate selective, and G_A was proportional to the first power of the salicylate concentration. Both G_H and G_A were inhibited by phloretin, and the voltage dependencies were superlinear. The data suggest that salicylate is an HA_2^--type proton carrier, as exemplified previously by dinitrophenol. Benzoate and aspirin were about an order of magnitude less effective than salicylate. Salicylate has been implicated as a causal factor in Reye's syndrome and has been reported to increase the proton conductance in isolated mitochondria. My results suggest that in mitochondria salicylate does not increase proton conductance but does increase proton uptake by a combination of HA influx (driven by the pH gradient) and A^- efflux (driven by the voltage and concentration gradients). Model calculations suggest that within the range of therapeutic concentrations, salicylate causes net H^+ influx sufficient to explain the reported "loose coupling" and swelling in mitochondria. (Supported by NIH grant GM 28844.)

W-Pos304 ANALYSIS OF KINETIC MODELS FOR COUPLING OF PROTON TRANSPORT AND ATP SYNTHESIS BY THE F_0F_1-ATP SYNTHASE. W.D. Stein and P. Läuger, Dept. of Biological Chemistry, Life Sciences, Hebrew University, Jerusalem 91904, Israel, and Dept. of Biology, University of Konstanz, D-7750 Konstanz, F.R.G.
We present an analysis of models based on current structural concepts of the F_0F_1 synthases, accounting for coupling between proton transport and ATP synthesis. It is assumed that each of the three $\alpha\beta$ subunits of the synthase can exist in three different conformational states E, E^0 and E^*. Proton translocation is coupled to cyclic interconversion of the conformations of the $\alpha\beta$ subunits:



k_1 and k_2 refer to the rate-limiting conformational transitions; binding and release of ADP (≡D), ATP (≡T) and protons are assumed to be equilibrium steps. In one family of models, interconversion of the $\alpha\beta$ subunits of F_1 is coupled to the translocation event in F_0 acting as a proton carrier. In a second family of models, protons combine with F_0F_1, and are translocated during the interconversion step. Kinetic tests involving the mutual effects of [ATP], [ADP], [$H^{+\prime}$] and [$H^{+\prime\prime}$] are described which allow to distinguish between different models.

W-Pos305 **INHIBITION OF LYSOPHOSPHOLIPID ACYLATION BY AGENTS WHICH PROMOTE THE INNER MEMBRANE PERMEABILITY TRANSITION IN RAT LIVER MITOCHONDRIA. K.M. Broekemeier, M.E. Dempsey and D.R. Pfeiffer (Intr. by H.L. Brockman), Hormel Institute, University of Minnesota, Austin, MN 55912.**

Previously, we demonstrated that certain hypolipidemic drugs induce the inner membrane permeability transition and inhibit the acyl-coenzyme A (CoA):lysophospholipid acyltransferase activity of rat liver mitochondria (Riley, W. W., Jr., and Pfeiffer, D. R. (1986) J. Biol. Chem. 261, 14018-14024). Here, the effects of several other transition-inducing agents on this enzyme activity and on the activity of mitochondrial acyl-CoA synthetases are examined. N-ethylmaleimide inhibits both acyltransferase and acyl-CoA synthesis at concentrations which are effective in inducing the transition. WY-14643 and Hg^{2+} inhibit acyltransferase but do not prevent the production of acyl-CoA. Rhein, t-BOOH, and oxalacetate do not directly inhibit acyltransferase but shift intramitochondrial glutathione to the disulfide form. Disulfide glutathione inhibits acyltransferase although it is not clear whether this represents a direct effect on the enzyme or if a diminished availability of acyl-CoA is responsible. Finally, inorganic phosphate and phosphoenolpyruvate do not oxidize intramitochondrial glutathione or inhibit lysophospholipid acylation in broken mitochondria. The present results are consistent with earlier proposals on how "Ca^{2+}-releasing agents" cause the permeability transition in Ca^{2+}-loaded mitochondria. Specific activity for lysophospholipid acylation is 0.4 nmol/min/mg protein (25°C). Mitochondria purified by a Percoll gradient lose 80% of acylation activity but retain their sensitivity to Ca^{2+} plus "Ca^{2+}-releasing agent". The submitochondrial location of cycle enzymes is under investigation. (Supported by NIH grant HL08214 and the Louise Dosdall and Hormel Foundations)

W-Pos306 CHARACTERIZATION OF AN ADP-INDUCED SLIPPAGE OF THE MEMBRANE BOUND H^+ATP-ASE IN PHOTOSYNTHETIC CHROMATOPHORES. Rita Casadio, Dept.of Biology, V.Irnerio 42; I-40126 Bologna, Italy.

In photosynthetic chromatophores, the rate of ΔpH generation ($\dot{\Delta}$pH), coupled to ATP hydrolysis in the dark and in the presence of K^+-valinomycin, is related to the oligomycin-sensitive, Mg-dependent ATP-ase activity and to its modulators. Comparison of the kinetic analysis of ΔpH formation (as detected from the fluorescence quenching of ACMA) and the rate of ATP hydrolysis (v_p) shows that the H^+pumping activity is characterized by the same K_m for the substrate $MgATP^{--}$ as the hydrolytic activity(0.2mM). Free Mg ions act as competitive inhibitors with a K_i of 0.5 mM for both activities, and activators of the ATPase, such as sulfite, activate also the H^+pump to the same extent. In all these experimental conditions, the ratio of $\dot{\Delta}$pH to v_p is a constant value (30±10 ΔpH μmoles BChl/μmoles ATP hydrolyzed). From this figure, the buffer capacity of the vesicles is calculated to be 0.1-0.06 μmoles H^+/ΔpH μmoles BChl, by assuming a H^+/ATP stoichiometry of 2. However, in the presence of ADP (0.3mM), which acts as an allosteric regulator for both activities, $\dot{\Delta}$pH/v_p is decreased by a factor of two. Considering that neither the passive permeability nor the buffer capacity of the chromatophores are affected by addition of ADP as compared to control experiments, it can be concluded that less than 1 H^+ is pumped per ATP hydrolyzed under these conditions. This result is indicative of an ADP-induced slippage of the oligomycin-sensitive ATPase. Noticeably, the allosteric effect on both activities and the slippage are removed when sulfite is added above a 3:1 molar ratio with ADP.

W-Pos307 **MEASUREMENT OF COENZYME BINDING TO 3-HYDROXYBUTYRATE DEHYDROGENASE (BDH) BY ULTRAFILTRATION AND EPR SPECTROSCOPY.** J. Oliver McIntyre, Bernhard Rudy, Helmut Dubois, Reinhold Mink, Wolfgang E. Trommer and Sidney Fleischer. Dept. of Molecular Biol., Vanderbilt Univ., Nashville, TN 37235 USA and Dept. of Chem., Univ. of Kaiserslautern, Kaiserslautern, FRG.

An ultrafiltration binding assay was used to quantitate NAD(H) binding to BDH, a lipid-requiring membrane enzyme purified from bovine heart mitochondria. BDH is a tetramer in the membrane and has a specific requirement of phosphatidylcholine (PC) for optimal function. For BDH reconstituted into phospholipid (PL) vesicles, we find that: i) the binding stoichiometry for NADH or NAD is 0.5 moles/mole BDH monomer (2/tetramer); ii) NAD^+ and NADH binding are mutually exclusive; iii) NADH binding to BDH in the mixture of mitochondrial PL (K_D = 15 ± 3 µM) is similar to dioleoyl-PC (K_D = 12 ± 3 µM); iv) NAD^+ binding is weaker than that of NADH (K_D ~ 200 µM vs 15 µM) but is enhanced by forming a ternary complex with 2-methylmalonate (K_D = 1.1 ± 0.2 µM) or by sulfite which forms the NAD-SO_3^- adduct (K_D = 5 ± 1 µM); and v) the NADH binding stoichiometry is the same (0.5 moles/mole) for binary and ternary complexes. Binding studies by equilibrium dialysis or EPR spectroscopy were consistent with the ultrafiltration data. The nucleotide binding site is present in BDH which is inactive since: a) NAD^+, as the ternary complex with 2-methylmalonate, binds to BDH reconstituted with PL devoid of PC (K_D ~ 50 µM); and b) apoBDH, devoid of PL, binds NADH or NAD-SO_3^- weakly (half-max. binding at ~ 75 µM NAD-SO_3^-). We conclude that the role of PC for activation of BDH is related, at least in part, to enhancing binding to a pre-existing nucleotide binding site. Thus, PC appears to serve as an allosteric activator of BDH.

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W-Pos308 THE GIBBERELLINS: A PROPOSED MECHANISM FOR THEIR FUNCTIONING AS PLANT GROWTH HORMONES. Roger E. Clapp, The MITRE Corporation, Bedford, MA 01730.

Plant growth depends not only on effective utilization of solar photons, but also on fast and efficient disposal of the entropy accompanying those photons [Clapp, J. Theor. Biol. 92, 15-21 (1981)]. A plant is a heat engine working between 6000 K and 300 K. For entropy disposal near 300 K, there is a much faster mechanism than infrared emission or phonon transfer. There are appropriately placed vibrational resonances in gaseous CO_2 and H_2O; the most efficient (in the sense of carrying entropy at the least cost in energy) is a CO_2 vibration at 15 micrometers. A cell such as a chloroplast at a plant's surface is struck by thirty CO_2 molecules every picosecond, each of which can recoil vibrating, carrying an infrared quantum. To take best advantage of this entropy disposal mechanism, a plant should be able to focus sharply its disposable heat energy on a CO_2 or H_2O resonance.

There are at least 68 different gibberellins, growth hormones found in fungi and higher plants. Commercially, gibberellins are used to stimulate seedling growth. They all have the same basic skeleton, including an out-of-plane three-bond side branch that can serve as a locus where a pi-electron hole can have its phase reversed. Such a phase reversal, included in the path of an orbiting positive charge, results in a Möbius orbit and a Möbius excitation which is metastable since it is characterized by L=1/2 [Clapp, Biophys. J. 53, 535a (1988)]. The kinetic energy of the simplest Möbius excitation in a gibberellin molecule just equals the energy of the 15 micrometer resonance in the CO_2 molecule, permitting the gibberellin molecule to funnel the entropy-carrying heat quanta directly into the narrow channel represented by that CO_2 vibrational resonance.

W-Pos309 CHYMOTRYPSIN ACTIVATES CARDIAC MITOCHONDRIAL CARNITINE-ACYLCARNITINE TRANSLOCASE. Paul Wolkowicz, Daniel Pauly, Barry van Winkle, Jeanie McMillin, Dept. of Medicine, Div. of Cardiovasc. Diseases, Univ. of Ala. at Birmingham, Birmingham, Al. 35294

The carnitine acylcarnitine translocase(CTr) facilitates carnitine(carn) and acylcarn transport across the inner mitochondrial membrane. Incubation of beef heart sub-mitochondrial particles(SMPs) with chymotrypsin(1 unit/mg SMP) and increasing concentrations of carn produces a 7.5-fold increase in CTr mediated carn exchange(V_{max}= 0.16±0.033 to 1.19±0.10 nmol carn/min-mg; control and chymotrypsin treated) and a decrease in K_m for carn(2.24±0.069 to 1.0±0.125 mM; control and chymotrypsin treated). Chymotrypsin treatment produces an activation of palmitoylcarn for carn exchange by CTr (V_{max}= 0.17±0.046 to 1.89±0.89 nmol palmitoylcarn/min-mg; control and chymotrypsin treated). Chymotrypsin activation produces a 50 % decrease in the I_{50} for CTr inhibition by N-ethyl maleimide from 7 nmol NEM/mg to 3.2 nmol NEM/mg. Chymotrypsin activation of SMP CTr is strictly dependent on the presence of free carn or short chain acylcarn in the activation medium. Also, as the chain length of the acylcarn in the activation medium increases, chymotrypsin activation of CTr decreases. Replacement of free carn with palmitoylcarn in the activation medium results in less than a 1.5-fold stimulation of CTr activity. Incubation of SMPs with chymotrypsin and substrates for the carn-acyl-CoA transferase does not result in CTr activation. Chymotrypsin activation does not alter the passive permeability of the SMPs to carn, nor does it alter the NEM-insensitive component of SMP carn transport. These data suggest that carn binding produces conformational changes in CTr which allows subsequent chymotrypsin modification and activation of CTr. Supported by NIH HL 38455

W-Pos310 ADDITIVE EFFECTS OF THREE POLYNUCLEAR AROMATIC HYDROCARBONS ON MITOCHONDRIAL RESPIRATION.
Andrew C. Beach and H.J. Harmon (Intro. by R.A. Floyd). Department of Zoology, Oklahoma State Univ., Stillwater, OK 74078.

NADH oxidase activity in intact mitochondria is inhibited 50% by 3.9 ppm (25 μM) acenaphthene, 15 ppm (120 μM) naphthalene, or 525 ppm (6.7 μM) benzene. Inhibition by a mixture of these compounds represents simple additive inhibitions of each compound, the additive effect predictable by using simple linear regression analysis. NADH oxidase, NADH-Q_{10} reductase, and $QH_2 \rightarrow O_2$ activities are inhibited by all three compounds, while succinate-, cytochrome c-, and duroquinol oxidase activities are not inhibited. Inhibition of mitochondrial respiration occurs at the level of coenzyme Q for all three polynuclear aromatic hydrocarbons.

This research was supported by the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant number AFOSR 84-0264.

W-Pos311 DYNAMIC DISTRIBUTION OF FLUOROPHORE-LABELED HEXOKINASE IN LIVING CELLS. R. M. Lynch and F.S. Fay, Dept. Physiology, U. Mass. Medical Center, Worcester MA 01655.
Hexokinase (HK) isozyme I is proposed to bind to mitochondria, and this binding is thought to be regulated by insulin and/or the activity of the glycolytic pathway. In order to follow HK distribution during perturbations in metabolism using digital imaging microscopy, we have conjugated a fluorescent label to HK in such a way that it's biological activity is unaltered. Prior to incubation with tetramethylrhodamine succinimidyl ester, HK I was bound to rat liver mitochondria to protect the binding and catalytic sites from modification during conjugation. Competent enzyme was selected by sequential binding and release cycles using isolated mitochondria, and final purification was attained using DEAE Sephedex. The labeled enzyme has a specific activity (50 U/mg) and electrophoretic mobility identical to the unlabeled precursor, and a stoichiometry of labeling near 1:1. Immediately after microinjection (30 sec), the fluorophore was homogeneously distributed throughout 3T3-L1 cells. This distribution is similar to that of rhodamine-dextran of comparable molecular weight (100,000). After 2-5 minutes, the HK probe localized into discrete punctate regions, unlike the dextran or denatured labeled HK which remain dispersed. Many, but not all, of the punctate regions align with mitochondria as assessed by images of pyridine nucleotide or rhodamine 123 fluorescence. Nucleoli also appear to label specifically. Inhibition of glycolysis by addition of 10 mM lactate caused the disappearance of some, but not all of the localized regions. These observations support the notion that HK distribution is regulated in living cells. This work is supported by a Charles A. King Fellowship (RML) and NIH HL 14523.

W-Pos312 INTERACTION OF Mn^{2+} WITH THE Na^+-DEPENDENT AND Na^+-INDEPENDENT Ca^{2+} EFFLUX MECHANISMS OF LIVER AND BRAIN MITOCHONDRIA. K.K. Gunter, C.E. Gavin, and T.E. Gunter. Dept. of Biophysics, University of Rochester, Rochester, NY 14642.

The existence of a separate, mediated Na^+-independent mechanism of mitochondrial Ca^{2+} efflux has been brought into question (Crompton et al. in Intracellular Calcium Regulation. Manchester Univ. Press, 1986) in part because of the resistance of this mechanism to inhibition by most common Ca^{2+} transport inhibitors. In this view what has been reported as Na^+-independent mitochondrial Ca^{2+} efflux is nothing more than Ca^{2+} leakage through a permeabilized membrane. The above hypothesis is invalidated by the observations that both Na^+-independent and Na^+-dependent Ca^{2+} efflux mechanisms in liver mitochondria can be clearly distinguished from leakage through the permeabilized membrane, and that they each also show specific patterns of efflux kinetics and inhibitor kinetics characterizing them as mediated transport mechanisms (Wingrove and Gunter, J.B.C. 261:15159 and 15166, 1986). Here we further demonstrate the existence of this Na^+-independent mechanism through the observation that Mn^{2+} efflux from both liver and brain mitochondria takes place via the Na^+-independent efflux mechanism but not via the Na^+-dependent mechanism. Mn^{2+} and Ca^{2+} show competitive inhibition in transport over the Na^+-independent mechanism. Mn^{2+} inhibits Ca^{2+} transport over the Na^+-dependent efflux mechanism but is not itself transported over this mechanism. Supported by GM35550 and T32-ES07026.

W-Pos313 COOPERATIVE ACTION OF MAGAININS IN DISRUPTING MEMBRANE-LINKED FREE-ENERGY TRANSDUCTION. Davor Juretic, Richard W. Hendler, Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD 20892, Michael Zasloff, Department of Pediatrics and Genetics, Childrens Hospital of Philadelphia, Philadelphia, PA 19104, and Hans V. Westerhoff, Nederlands Kanker Instituut, 1066 CX Amsterdam, THE NETHERLANDS.

Recently, positively charged antimicrobial peptides have been isolated from the skin of Xenopus laevis and named magainins (Zasloff, PNAS 84, 5449 (1987)). We show here that magainin 2 and PGLa dissipate the membrane potential across various free-energy transducing membranes. Membrane potential changes in E. coli, rat liver mitochondria and cytochrome-oxidase vesicles were monitored with an extravesicular electrode selective for tetraphenyl phosphonium ion. Release of respiratory control in mitochondria and cytochrome-oxidase liposomes, by the peptides, was observed. The concentration dependence was suggestive of a multimeric active form of the magainins. In the liposomal system, Hill coefficients were 5 and 3 respectively for magainin 2 and PGLa. There is a cooperative interaction between the two peptides. Each peptide alone caused half maximal uncoupling of respiration when added at a molar ratio (peptide:phospholipid) of about 1:40 and 1:110 (for magainin 2 and PGLa respectively), but this ratio dropped to 1:290 and 1:720 when both peptides were present simultaneously. The cooperative effects on respiration uncoupling and membrane potential decrease, occurred at concentrations of peptides below 1 μM i.e. at less than 1/10th of their individual lethal concentrations for bacteria.

W-Pos314 ELECTRON-TRANSFER KINETICS OF SINGLY LABELED RUTHENIUM POLYPYRIDINE CYTOCHROME c DERIVATIVES. Bill Durham, Lian Ping Pan, Joan E. Hall, and Francis Millett. Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701.

A number of investigations of electron-transfer reactions of small metal complexes covalently linked to metalloproteins have appeared in the last decade. These investigations have been successful in probing some of the fundamental features of electron transfer over long distances. These studies, however, often suffer from a lack of strategically placed binding sites for the metal complexes. We have recently succeeded in attaching Ru(2,2'-bipyridine)2(4,4'-dicarboxy-2,2'-bipyridine)$^{2+}$ to specific lysine amino groups of cytochrome c. Derivatives at lysines 13,72,25,7, 39,86 and 87 have been isolated and electron-transfer between the iron center and the electronically excited Ru(II) have been observed. The rates of electron-transfer are sufficiently rapid (1.5-20x106 sec^{-1}) to dominant the decay of the luminescent excited state of the ruthenium complex. The rates of the back reactions of Ru(III) with Fe(II) appear to be several times greater than the reaction of the excited state. Electron transfer between the excited Ru(II) and the reduced form of the cytochrome c derivatives has also been observed. The observed rate constants (Ru(II)* + Fe(III) = Ru(III) + Fe(III) show an inverse relation with the distance to the Ru(II) label over the range of 7 to 18 A. Preliminary analysis indicates that the distance dependence is not as strong as that observed in previous examples where $Ru(NH_3)_5(his)^{2+}$ was the covalently bound redox partner.

W-Pos315 JUST WHAT ARE YOUR SINGLE-CHANNEL RECORDS TELLING YOU ABOUT THE UNDERLYING KINETIC NATURE OF YOUR SYSTEM?

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Application of classical stochastic theory to the problem of predicting single-channel kinetic behaviour has been well-described in the literature. Given a particular kinetic scheme it is a relatively straightforward matter to predict open- and closed-time histograms (or, more properly, probability density functions for the lifetimes in the open and closed conformations). What has not been well-described, or even discussed at all, for that matter, is the issue of how best to interpret single-channel data itself; i.e., how does one work backwards from actual data to uncover the underlying kinetic mechanism driving the system being studied? The lack of discussion concerning this topic is very unfortunate, not to mention misleading, for the following reason: We have been able to show, strictly on the basis of mathematical considerations, that it is highly unlikely that single-channel data alone will ever be able to provide sufficient information to allow a definitive selection from among possible competing schemes. In the present work we provide a careful description of the relationship between empirically-determined rate constants from open- and closed-time distributions, and the theoretical parameters of the stochastic matrices for possible competing mechanisms. We then show conclusively that for the general three-state problem it is mathematically not possible, no matter what the data look like, to select one from among the various competing choices.

W-Pos316 ROLE OF THE "VACUUM STRUCTURE" IN BIOLOGICAL INFORMATION PROCESSING.

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We describe a model of biological information processing with a vertical structure: macroscopic signals are transduced to less macroscopic intercellular signals, then into more microscopic intracellular messengers, and finally to a microscopic molecular level of organization. The classically unpicturable development of the prepared microstate in time corresponds to an inherently parallel mode of information processing that cannot be directly duplicated by a classical computing machine. The upward transduction of the unpicturable microstate into definite actions of the organism is reminiscent of the irreversible collapse of the wave function in measurement. Our hypothesis is that such irreversible processes are a fundamental feature of nature, but that they are masked in the simple systems ordinarily studied in the physics laboratory. It is possible to construct a model that displays such underlying irreversibility by assuming a Dirac type vacuum of negative energy fermions. The virtual bosons that mediate classical forces are identified with propagating chains of transient pair production processes, each with a fluctuation energy of $\hbar/\tau_K$ and a fluctuation momentum $\hbar/x_K$, where τ_K and x_K are time and space intervals determined by the density of vacuum particles. This density is influenced by positive energy mass and charge, and as a consequence the wave function of the vacuum must enter into a classical description of the force. If this wave function changes significantly the underlying irreversibility will be unmasked. A contraction-expansion density wave accompanies these propagating chains, and real bosons can be identified with chains in which the accompanying wave carries real energy.

W-Pos317 LIFE STAGES AND AGING AS A MORPHOGENETIC PROBLEM

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P. Kloeden, Department of Mathematics, Murdoch University, Perth, Australia. (Intr. by O.E. Rossler.)

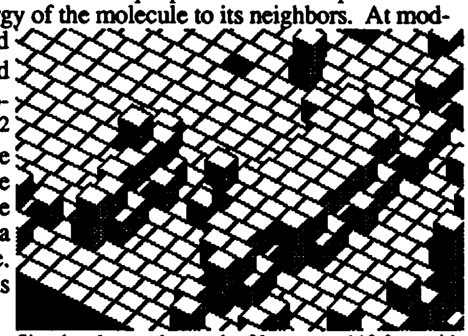
The central problem of morphogenesis is what has been called "positional information" by Wolpert. While in principle a purely "local" model is a possible solution to this problem, with cell-specific gene products controlling the differentiation of the subsequently produced cells, the preferred view todate regarding higher organisms is a "global" one. In this Rashevsky-Turing-Gierer-Meinhardt model, controlling substances ("morphogens") that interact in a pattern-generating dynamical manner across many cells are postulated. The resulting spatial gradients then secondarily tell the genes of the involved cells into what state to lock. Several such patterns are thought to follow each other in succession, each time after the preceding wave got fixated so to speak, until organogenesis is finished. (See H. Meinhardt's 1983 book with Academic.) Thereafter - and this is the new point - only positional information about position in time remains to be gauged by each cell. If one again sticks to the global alternative, it suffices to assume that the preceding "waves" simply go on, only that the variables involved are of the "lumped" (purely temporally rather than spatio-temporally distributed) type this time. The involved substances cease to be combined "morphochronogens" and become pure "chronogens." Thus, a temporal sequence of life-stage specific, life-stage terminating hormones are postulated in effect. This hypothesis can be tested. A Loewi-type experiment will involve genetically identical individuals of different ages and will require a sufficiently long-term coupling. Both macroscopic (parabiotic embedding) and microscopic (coupled tissue cultures) experiments are possible.

W-Pos318 A DETAILED STUDY OF THE NETWORK THERMODYNAMIC ANALYSIS OF COUPLED PROCESSES:THE METRIC, THE DEGREE OF COUPLING AND THE REFERENCE STATE. D. C. Mikulecky, Dept. Physiology and Biophysics and The Biomedical Engineering Program, Med. Col. of Va. Commonwealth Univ., Richmond, VA 23298-0551.

Peusner(Studies in Network Thermodynamics,Elsevier,1986) has developed an extensive analysis of the metric structure underlying nonequilibrium thermodynamics using network thermodynamics. The canonical representation of any energy conversion process is its network. The degree of coupling which determines the system's maximum efficiency (Kedem and Caplan, Trans. Faraday Soc. 61:1897,1965) also determines the geometry of the embedding of the affine coordinate system defined by nonequilibrium thermodynamics into the orthogonal coordinates defined by the network representation. This embedding established the degree of coupling of any energy conversion process as a geometric invariant. Using the convection-diffusion 2-port as an example of an intrinsically nonlinear system which has a convenient and often used linearization for low values of the Pecelet number, the dependence of the degree of coupling and the metric structure on the choice of reference state (Mikulecky and Sauer, J. Math. Chem. 2:171-196,1988) are examined in detail. This illustrates the value of the reference state in defining the relation between flows and forces in nonlinear systems. The results of this study are general and apply to all energy conversion processes in biophysical systems.

W-Pos319 SIMULATION OF LYSOZYME CRYSTAL GROWTH BY THE MONTE-CARLO METHOD* S. D. Durbin and G. Feher, Physics Department, University of California San Diego, La Jolla, CA 92093

Protein crystallization can be viewed and modeled as a molecular assembly process. We have reproduced the main features of lysozyme crystal growth by a model in which the free parameters are the strengths of the three bonds (A,B,C) occurring in the $P4_32_12$ lattice of the tetragonal form of lysozyme and the absolute time scale of molecular collisions with the lattice. In Monte-Carlo simulations with this model, the probability of attachment of a molecule is proportional to the protein concentration c and that of detachment to $\exp(-E_b/kT)$, where E_b is the binding energy of the molecule to its neighbors. At moderate and high supersaturation c/s, where s is the solubility, both simulated and real crystals grow at rates that are a highly nonlinear function of c/s,[1] and display complex surface structures.[2] These are consequences of the two-dimensional nucleation growth mechanism which dominates in this regime.[1,2] Varying the bond strengths and simulating growth for different values of the driving force $\Delta\mu = kT \ln(c/s)$, we find that the observed crossover in relative growth rate of the 101 and 110 faces (and resulting habit change) and the double-layer structure of islands on the 110 face (see Fig.) both arise from a difference in the strength of bonding to the crystal of alternate 110 layers (i.e. ABAB...and CCCC...). By contrast, the elongated shape of the 110 islands is due to the lattice structure and is not very dependent on relative bond strengths.



Simulated crystal growth of lysozyme 110 face with bond strengths A=kT, B=2kT, C=3kT; c/s~1.6. Note the elongated double-layer islands.

1. S. D. Durbin and G. Feher, *J. Crystal Growth* **76**, 583 (1986).
2. S. D. Durbin and G. Feher, *Biophys. J. (Abstracts)* **53**, 300a (1988).
* Work supported by NIH.

W-Pos320 HIGH PROTEIN CONCENTRATIONS RETARD SHORT-RANGE LATERAL DIFFUSION IN MOLECULAR DYNAMICS SIMULATIONS OF MEMBRANES. Patricia J. Donaldson and Watt W. Webb, Applied Physics, Cornell University, Ithaca, NY 14853

In previous monte-carlo simulations we observed protein concentration inhibition of long-range lateral diffusion for both lipids and proteins (Biophys. J. 53:121a, 1988). These simulations assumed that the short-range diffusion coefficient is independent of protein concentration. Our recent molecular dynamics simulations contradict this assumption.

Proteins and lipids, modeled as large and small hard disks, were placed in a box at low density, then squeezed to a target pressure. Diffusion was followed through 10^6-10^7 particle collisions. The variable simulation parameters were: the large disk (protein) area fraction, the large/small disk diameter ratio, and the lateral pressure. At a given pressure the small disk diffusion coefficient decreased with increasing large disk area fraction. Diffusion of large disks was retarded if either the area fraction or the diameter ratio increased. Particle tracking in a simulation at high pressure (63 dynes/cm) showed that most small (lipid) disks crystallized into patches, while those small disks near large disks remained quite mobile. At the best theoretical pressure estimate of 70 dynes/cm, we predict that the segregation induced by the two particle sizes will inhibit long-range protein diffusion, while preventing complete lipid crystallization and immobilization.

This research was sponsored by NSF DMB 8609084 and NSF DMR 8414796, using the Cornell National Supercomputer Facility, which is jointly funded by New York State, the Corporate Research Institute, the National Science Foundation and IBM corporation.

W-Pos321 **FRACTAL ANALYSIS OF HUMAN RETINAL VESSELS**

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We have demonstrated that the morphology of retinal vessels can be described by fractal concepts. The arteries and veins in the retina have a characteristic branching pattern which resembles many two-dimensional fractal structures in nature. Retinal photographs of humans, age 15-50, were digitized and the length of the vessels, $L(r)$, within concentric circles of radius r were measured. The fractal dimension D, was determined from the relation $L(r) \sim r^D$. We have also determined the two-point correlation function $C(r)$, which gives the probability that a pair of points at a distance r belong to the pattern. It is expected that, $C(r) \sim r^{D-d}$, where d is the dimension of space. For retinal vessels, $d=2$. We find that both methods give a fractal dimension of about 1.7. This supports the hypothesis that the patterns of retinal vessels is controlled by a diffusion-limited process. We propose that oxygen gradients in the retina during early development are the determining factor in the formation of the global pattern of retinal vessels.
This work was supported by the National Eye Institute, the Office of Naval Research and the Petroleum Research Fund, administered by the American Chemical Society.

W-Pos322 THE EFFECT OF AN ELECTRONEUTRAL COPORT ON THE STEADY STATE OF AN EXCITABLE CELL.
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I have simulated the effects that an electroneutral Na-K-Cl coport has upon the steady state, using a computer model of an excitable cell. The model cell includes voltage-dependent Na and K fluxes, a concentration dependent Na-K pump, osmotically driven water fluxes, and a passive Cl current. The equation for the coport was written so that the sensitivity to changes in the internal concentrations could be modified by a parameter, Dco. The larger Dco, the greater the changes in the coport current for a given change in the internal concentrations.

Increasing the coport current always depolarizes the steady state membrane potential (SSMP). However, as Dco is increased, the relationship between the SSMP and the coport current becomes progressively flatter. Paradoxically, increasing the coport sensitivity causes the the changes in cell volume to become larger. The effects of increasing the Na-K pump current are also modulated by the coport sensitivity. With a constant coport (Dco = 0), increasing the Na-K pump current causes the SSMP to hyperpolarize. Increasing Dco gradually abolishes this hyperpolarization. When Dco is sufficiently large, increasing the Na-K pump current will depolarize the SSMP. All of the above results hold independent of whether the coport transport ratio (Cl:Na:K) is 3:2:1 or 2:1:1.

W-Pos323 REASONABLY DIRECT METHOD FOR MODELING MEMBRANE BILAYER STRUCTURES USING STEP FUNCTIONS
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Small angle x-ray diffraction from biological membranes usually yields a small number of diffraction peaks, and the electron density profile calculated therefrom is of low resolution. It is therefore common to construct a step function model to represent the electron density of the sample. If there are n orders of diffraction, the first n terms of a trial step function model are used to calculate an electron density profile which is then compared to that of the data. A common procedure is to adjust the widths and amplitudes of the step function components until visual agreement is achieved between the model and the data. An algorithm has been developed which results in a more accurate solution in considerably less time. A symmetric step function model, comprising an amplitude y1 between x=0 and x=x1, y2 between x1 and x2, etc., is chosen, and its Fourier series is written. The first n amplitudes of the Fourier series are then equated to the n amplitudes from the data. n=0 corresponds to the background density. The simultaneous solution of this set of equations can often be obtained by use of commercial least-squares computer programs in a matter of minutes. The solution obtained is the best possible model of the chosen form to the diffraction data. Supported by NIAAA center grant #03510 and RJR Nabisco, Inc.

W-Pos324 MATHEMATICAL MODELING OF GAS TRAPPING AND HYSTERESIS DURING INFLATION/DEFLATION CYCLES OF EXCISED LUNGS. Gunter N. Franz and David G. Frazer, Department of Physiology, West Virginia University School of Medicine, Health Sciences Center North, Morgantown, WV 26506 and Division of Respiratory Diseases, NIOSH, Morgantown, WV 26505

Frazer and co-workers have presented evidence for the sequential opening and closing of lung units during inflation/deflation cycles in excised rat lungs (Respiration Physiol. 61: 277-288, 1985). Recruitment (opening of closed lung units), uniform expansion and contraction of open lung units, and derecruitment (closing of open lung units) in a pressure-dependent manner provide an explanation for the gas trapping and hysteretic behavior observed with quasistatic volume-pressure curves (e.g., Respiration Physiol. 46: 237-246, 1981). Derecruitment of lung units at positive transpulmonary pressures is assumed to occur because menisci form and functionally close airways, and thus trap gas acquired during inflation.

This study models the behavior of a population of lung units subject to volume perturbations similar to those used in the experiments of Frazer and co-workers. Once open, these model lung units have similar volume-pressure characteristics, but opening pressures and closing pressures are assumed to vary according to normal density curves of different mean and standard deviation, respectively. There is qualitative similarity with essential features of the experimental results.

W-Pos325 REMODELING WITH AGING: USE OF BODY MASS INDEX (WEIGHT/HEIGHT2) FOR COMPARISON OF VARIOUS POPULATION GROUPS. Richard P. Spencer. Department of Nuclear Medicine, University of Connecticut Health Center, Farmington, CT 06032.

With the aging process, there is a remodeling or change in body composition and an alteration in the size relationship of several body parts. While no single relationship can define this complex time-related event, BMI:the body mass index (weight divided by the square of height) provides a useful first approach when plotted as a function of the age of the population. An immediate problem is that there has been an intergenerational increase in size in most groups (see for example, Garn, J. Nutr. 117:817, 1987); hence a reanalysis is needed for one age cohort followed for a long period of time. We start with healthy black Americans, using data in Hargreaves et al (Nutr. Res. 8:875, 1988). The black population has less bone loss with age (less osteoporosis and a lower incidence of vertebral fracures). For ages 20, 25, 35, 45, 55 years, log BMI can be given as a function of log Age: log BMI = 1.09 + 0.216 log Age (corr. coeff. = over 0.98). Between ages 55 and 65 years, BMI falls for this black population. The equations closely mimic weight changes, as the height alters but little. In a Japanese population (Inoue et al, Acta Path. Jpn. 37:343, 1987), BMI is the same in males in 65 and 75 year old groups and then falls in the 85 and 95 year old cohorts(again paralleling weight). A rather similar finding is noted in Japanese females. By way of contrast, in a small number of patients (57) from New Zealand, studied by Larking et al (Biochem. Med. & Metab. Biol. 37:246, 1987), in groups of mean ages 35, 55 and over 65 years, there was no change in BMI with aging. The BMI will have to be combined with other indices, such as total height divided by umbilical height, to provide a group by group comparison of the effects of aging on remodeling of the body.

W-Pos326 A DYNAMICAL MODEL OF OSCILLATIONS IN TUBULAR PRESSURE, FLOW AND DISTAL CHLORIDE ACTIVITY IN RAT KIDNEYS. N-H Holstein-Rathlou and D.J. Marsh. Dept. of Physiol. and Biophys., Univ. of So. Calif., Los Angeles, CA.

Recent experiments have shown stable oscillations in proximal and distal tubular pressure, in tubular flow rate, and in distal tubular chloride activity in Halothane anesthetized rats. The oscillations are generated by a feedback system operating at the level of the individual nephron, the tubuloglomerular feedback system, which uses distal tubular chloride activity as a signal. There are significant phase shifts among the oscillating variables. We have developed detailed mathematical models describing pressure and flow in a glomerulus and in a nephron, coupled by a tubuloglomerular feedback function to simulate the dynamical behavior of this system. The nephron model consists of three partial differential equations with non-linear boundary conditions describing pressure, and flow, and NaCl concentration in the ascending limb of Henle's loop; the glomerular model consists of one ordinary differential equation describing pressure and flow rate in the attached glomerulus. The function coupling the nephron and the glomerular models used early distal NaCl concentration as input and afferent arteriolar vascular resistance as output. The solutions show oscillations in the different variables for realistic parameter values. Furthermore, the phase differences between the oscillating variables are accurately described by the model. To further validate the dynamic characteristics of the model, it was exercised using a white noise forcing of the arterial pressure as input and the resulting renal blood flow as output. The model closely reproduced results obtained in rat experiments using white noise forcing of the arterial pressure. It is concluded that the model gives an accurate description of the dynamics of the tubuloglomerular feedback system, and that it faithfully predicts the dynamics of vascular autoregulation.

W-Pos327 QUANTUM SIMULATION OF FERROCYTOCHROME-C

Chong Zheng, Chung F. Wong & J.A. McCammon, Department of Chemistry, University of Houston, Houston, Texas 77204-5641, and Peter G. Wolynes, Noyes Laboratory, University of Illinois, Urbana, Illinois 61801.

The dramatic progress in the understanding of the dynamics of biomolecules has been largely fuelled by computer simulations based on the law of classical mechanics. However in some respects biomolecules are at the borders of the domain of applicability of classical mechanics. The role of quantum mechanical effects in biomolecular structure and function is therefore worth investigating. Here we present preliminary results from a quantum simulation of a protein and contrast them with results from full classical simulations. The most significant differences are found in motions of high frequency, such as bond stretching or the torsional oscillation of groups that bear hydrogen atoms. The amplitudes of such motions are significantly increased by the penetration of atoms into classically forbidden regions. These differences will directly influence the rates of such processes as proton and electron transfer.

W-Pos328 ELECTRONIC TRANSITIONS IN HYDRATED INDOLE: A MD-INDO/S STUDY.

Predrag Ilich, Christopher Haydock and Franklyn G. Prendergast,
Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, Minnesota 55905.

Low singlet absorption bands in indole were simulated by coupled molecular dynamics - electronic structure calculations. The trajectories of one indole and 233 water molecules (0.24 molal solution), subjected to spherical periodic boundary conditions, were simulated over a 130 ps time period. Electronic transitions in hydrated indole, calculated by INDO/S-CISD method, are compared with those in isolated chromophore. Specific indole-water interactions, shown to be largely of one-electron contribution, demonstrate an average slight red shift in both lowest singlet absorption bands, $S_{1\leftarrow 0}$ and $S_{2\leftarrow 0}$. Transition electric dipole moments are decreased ($mu_{1\leftarrow 0}$) or approximately unchanged ($mu_{2\leftarrow 0}$) while both excited state moments exibit a significant increase in modulus. These integral characteristics of the medium-induced perturbation show pronounced oscillations in both directions with a periodicity of several hundred femtoseconds. The latter results, possibly indicating coarse grain dynamics of available electronic states in hydrated indole, are discussed in view of experimental spectroscopic measurements approaching the time resolution of calculations.
Supported by grant GM34847.

W-Pos329 THE SIEVING OF ROD-SHAPED VIRUSES DURING AGAROSE GEL ELECTROPHORESIS. Gary A. Griess, Richard Herrmann, Elena T. Moreno, and Philip Serwer, The Univ. of Texas Health Science Center, San Antonio, TX 78284-7760.

By measurement of electrophoretic mobility (μ) as a function of agarose percentage ($\underline{A}$) for a sphere of known radius (R), the effective pore radius (P_E) of an agarose gel has been determined as a function of $\underline{A}$ (Griess, G. A. et al. [1988] Biopolymers, in press). From knowledge of P_E ($\underline{A}$), the $\mu(\underline{A})$ of any particle can be used to determine its effective $R(\underline{A})$, constant in the case of a spherical particle. In the case of the rod-shaped viruses fd, fd length variants, tobacco mosaic virus (TMV) and TMV length variants, $R(\underline{A})$ remains constant at the lower $\underline{A}$ values and then progressively decreases as $\underline{A}$ increases. In the region of constant effective R, the effective R for monomeric fd and TMV is indistinguishable from that of a sphere whose surface area is equal to the surface area of the rod. The volume of this sphere is significantly greater than that of the rod. The $\underline{A}$ value at which the effective R of a rod begins to decrease is the $\underline{A}$ value of the gel with a 2 x P_E that is 0.53-0.21 times the length of the rod. This ratio decreases as the rod's length increases. An interpretation of the above regions is: (a) In the region of constant R, the rod has a random orientation. (b) At higher $\underline{A}$ values, the rod is oriented preferentially in the direction of electrophoresis (reptation). Supported by NSF (DMB-8701379).

W-Pos330 ACCELERATION OF DNA IN GELS DURING ELECTRIC-FIELD INVERSION. G. Holzwarth, Kevin J. Platt, and Richard W. Whitcomb, Dept. of Physics, Wake Forest University, Winston-Salem, NC 27109.

In order to understand the DNA motions which are exploited in DNA separations by pulsed-field gel electrophoresis, we have measured the instantaneous velocity of T4, T5, and λ DNA in 1% agarose gels during the first 20 s after field inversion. Immediately after a 10 V/cm field is inverted, the velocity of T4 DNA changes from -5 μm/s to +10 μm/s in less than 50 ms. The velocity then declines to a broad undershoot of 2 μm/s at about 3 s, peaks again to 7 μm/s at 8 s, and finally reaches a plateau of 5 μm/s. At lower molecular weights, and at higher values of E, the oscillations occur more rapidly. The large, rapid initial change in velocity is distinctly different from the slow acceleration observed when a field is first applied to a randomly coiled chain and does not correlate with alignment of the DNA. The measured velocity data explain the "antiresonance", or mobility minimum, previously observed in FIGE separations. Current theoretical models predict some aspects of this complex behavior.

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W-Pos331 ORIENTATION OF DNA IN AGAROSE GELS. Julian Borejdo, Cardiovascular Research Institute, University of California, San Francisco, Ca. 94143

Orientation of the lambda DNA during the electrophoresis in agarose gels was measured by a microscopic linear dichroism technique. The method involved staining DNA with the dye ethidium bromide and measuring under the microscope the polarization properties of the fluorescence field around the electrophoretic band containing the nucleic acid. It was first established that the fluorescence properties of the ethidium bromide-DNA complex were the same in agarose gel and in a solution. Then the linear dichroism method was used to measure the dichroism of the absorption dipole of EB dye bound to lambda DNA. In a typical experiment the orientation of two-tenth of a picogram (2×10^{-13}g) of DNA was measured. When the electric field was turned on, the dichroism developed rapidly and assumed a steady state value which depended on the strength of the field and on the size of DNA. When the field was turned off, the dichroism decayed more slowly. A linear dichroism equation related the measured dichroism of fluorescence to the mean orientation of the absorption dipole of ethidium bromide and to an extent to which the orientation of this dipole deviated from the mean. The observed development of dichroism in the presence of an electric field was interpreted as an alignment of DNA along the direction of the field. The increase in the steady state value of dichroism with the rise in the strength of the field and with the increase of the size of DNA was interpreted as a better alignment of DNA along the direction of the field and as a smaller deviation from its mean orientation.

W-Pos332 ORIENTATION OF THE AGAROSE GEL MATRIX IN PULSED ELECTRIC FIELDS. John Stellwagen and Nancy C. Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

When pulsed electric fields are applied to agarose gels (in the absence of DNA), the gel matrix becomes oriented and gives rise to an electric birefringence signal. If the applied electric fields are 1-10 kV/cm in amplitude and 0.01-3 msec in duration, the sign of the birefringence is positive, indicating that the agarose chains are being oriented parallel to the electric field. The rise and decay of the birefringence are characterized by time constants ∿10-100 μsec.

When smaller electric fields, 10-100 V/cm are applied to the gel for durations of 100-2000 msec, complicated orientation patterns are observed. The birefringence signal, after an initial lag period, becomes positive and/or negative during the pulse. After removal of the electric field the birefringence continues to increase in absolute amplitude, passing through an extremum after about 5 seconds before returning to zero. These unusual results indicate that domains or large clusters of the matrix are oriented by the longer pulses used at lower electric field strengths.

The sign of the birefringence observed at low field strengths was randomly positive or negative for different gels, indicating that the direction of orientation of individual clusters or domains of the matrix depended on the microstructure of the gel. The sign of the birefringence was reversed by reversing the direction of the electric field, indicating that the domains of the agarose matrix, once oriented in the electric field, tend to orient in the perpendicular direction upon field reversal. Supported by Grant No. 29690 from the National Institutes of General Medical Sciences.

W-Pos333 OBSERVATION OF INDIVIDUAL DNA MACROMOLECULES UNDERGOING ELECTROPHORESIS. S. B. Smith, *P. K. Aldridge and *J. B. Callis, Dept. of Genetics, *Dept. of Chemistry, University of Washington, Seattle, Washington 98195

Individual stained DNA macromolecules undergoing agarose gel electrophoresis are viewed with the aid of a fluorescence microscope and a low-light television system. It is observed that (a) the molecules advance lengthwise through the gel in an extended configuration, (b) the molecules alternately contract and lengthen as they move, (c) the molecules often become hooked around obstacles in a "u" shape for extended periods and (d) molecules display elasticity as they extend from both ends at once. The molecular motions involved in field inversion gel electrophoresis (FIGE) and orthagonal field gel electrophoresis (OFAGE) are observed. A computer model is demonstrated which simulates the molecules' migration behavior in OFAGE conditions. A video tape of individual molecules undergoing electrophoresis will be shown.

W-Pos334 MODIFIED SIEVING OF OPEN CIRCULAR DNA AND BACTERIOPHAGE CAPSID-DNA COMPLEXES DURING AGAROSE GEL ELECTROPHORESIS. Philip Serwer and Shirley J. Hayes, The Univ. of Texas Health Science Center, San Antonio, TX 78284-7760

Progressively elevating voltage gradient during the electrophoresis of linear DNA causes electrophoretic mobility (μ) to increase in magnitude (reptation; reviewed in Stellwagen, N. C. [1987] Adv. Electrophoresis 1, 177-228). Although reptation sometimes also occurs for open circular DNA (Serwer, P. and Allen, J. L. [1984] Biochemistry 23, 922-927), eventually elevating voltage gradient arrests the DNA. In the present study, elevated voltage gradient-induced arrest also is demonstrated for a complex of the linear, double-stranded bacteriophage T7 DNA (40 Kb) with its capsid (radius = 30 nm); the DNA is outside of the capsid. By use of pulsed-field gel (PFG) electrophoresis, arrest is prevented for both the capsid-DNA complexes and open circular DNA. At the lower pulse frequencies (<0.1 sec^{-1}) and 3 V/cm, sieving of 48.5 Kb open circles sometimes increases as agarose percentage (A) decreases. This phenomenon was not observed for capsid-DNA complexes and apparently has never been observed for any other molecule. In contrast, at either (a) 1 V/cm or (b) 3 V/cm and higher pulse frequency (approx. 10 sec^{-1}), the sieving of 48.5 Kb open circles monotonically decreases with decreasing A. At the higher pulse frequency and lower A values, 48.5 Kb open circular DNA migrates more rapidly than linear DNA of the same length. Supported by NIH (GM-24365).

W-Pos335 DIRECT DETERMINATION OF APPARENT CHARGE ON MACROMOLECULES THROUGH EQUILIBRIUM ELECTROPHORESIS.
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A fundamental property of macromolecules is their charge. We have developed theory, apparatus and methods to directly determine the apparent charge on a macromolecule, regardless of size or shape. The method uses equilibrium electrophoresis, a procedure in which opposing solute flows from electrophoresis and from diffusion become equal and opposite as the system reaches a steady state distribution. At equilibrium, the solute concentration increases exponentially. The exponent is proportional to the product of apparent charge and of position. Further interpretation of the apparent charge yields estimates of the titrable charge. The method uses only small quantities of materials, is nondestructive to the sample and requires only simple, inexpensive instrumentation. Experimental examples of the procedure will be presented.

W-Pos336 SDS-PROTEIN DISCONTINUOUS GEL ELECTROPHORESIS IN AGAROSE
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A discontinuous electrophoresis system in which agarose is used for both stacking and separating gels has been developed. Preparative electrophoresis of SDS-denatured proteins can be conducted in standard vertical electrophoresis cells, using polyester support film. Denatured peptides can be resolved in the range of 10,000 to 200,000 daltons; larger peptides can be separated at lower concentrations. Either a customized buffer system or standard Laemmli buffers can be used.

The denatured proteins can be recovered from the system by excising the separated band and melting the agarose at 65°C. Peptides can be recovered from the melt by blotting onto nitrocellulose or other supports, and detected with colloidal gold. Gels may also be stained with Coomassie blue or agarose compatible silver stain (e.g., Peats method), or directly electroblotted. Separations equivalent to Laemmli gels can be obtained with whole E Coli extracts, as well as with standard proteins. Gel components are stable and nontoxic..

W-Pos337 RAPID PULSE FIELD AND CONTINUOUS ELECTROPHORESIS WITH LOW ELECTROENDOSMOSIS AGAROSES
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We have shown elsewhere that alteration of the EEO (electroendosmosis) of agarose changes the speed at which separation occurs, without altering the sieving power of the medium (Kirkpatrick and Duggan, ms submitted). We show here that dramatic reductions of electrophoresis time are possible by further reducing the EEO of the agarose. Experimental agaroses can have running times up to 50% faster than conventional "low endosmosis" agaroses, when run in the proper buffers. These effects are obtained in all electrophoresis experiments, but are particularly dramatic in pulse field gel electrophoresis. It is possible to separate S. Pombe chromosomes in less than 48 hrs using the new system; S. Cerevisiae can be run overnight. Adjustment of pulse times is required for these separations, and optimization of angle using a Serwer rotating gel system is also useful.