M-Pos193

INTRAOCULAR PRESSURE COMPRESSION OF RABBIT CORNEAL EPITHELIUM.

L. Stephen Kwok. *Lions Eye Research Laboratories, LSU Eye Center, New Orleans LA.*

Elevation of the intraocular pressure (δ) beyond normal (>2.5 kPa) can lead to visual impairment if left untreated. At equilibrium, an increased δ will lead to a re-distribution of corneal tissue stresses - but the temporal and spatial characteristics are unknown. Intact rabbit corneas were mounted atraumatically by the method of Dikstein & Maurice (1972), and perfused on both sides with Ringer's solution at 37°C with δ = 2 kPa (20 cm H_2O) initially. After 1-2 hr, hydrostatic pressure on the endothelial side was increased to δ = 4.7 kPa (48 cm H_2O). Epithelial thickness, usually 40-50 μm, transiently decreased by up to 30%, and then recovered within 2 hr. Stromal thickness was increased. This suggests significant tangential stresses on the rabbit corneal epithelium arising from radial distension of the underlying stroma due to elevated intraocular pressure. Apparently, the true corneal effect of δ *in vitro* depends on the presence of an intact and healthy epithelium. The δ effect in de-epithelialized corneas needs careful interpretation. (U.S. PHS grants EY03311 and EY02377.)

M-Pos194

AN INWARD RECTIFYING K^+ CHANNEL IN THE BASOLATERAL MEMBRANE OF NECTURUS OXYNTIC CELLS.

S. Supplisson, D.D.F. Loo, & G. Sachs. Dept of Physiology, UCLA and CURE, VA Wadsworth, Los Angeles CA 90024.

The basolateral membrane of Oxyntic cells from Necturus gastric mucosa in resting condition (not secreting acid) contains a conductance predominant for K^+. In this membrane, two K^+ channels, one activated by cAMP and the other by $[Ca]_i$ have been described but from their low open probability (P_o) they cannot account for the resting K^+ conductance. We have found a K^+ channel normally open under resting conditions and is most likely responsible for this conductance. In cell-attached patches (pip=110mM KCl) the IV relationship rectified inwards. Channel conductance was 25 pS at positive pipette potentials (V_p=40 mV) and decreased to 2.5 pS at negative V_p (-60 mV). At the resting potential P_o was 0.92 and decreased to 0.1 and 0.08 at V_p=+20 and +40mV. This channel may also maintain the resting potential.
(NIH DK40615 & USVA SMI).

M-Pos195

SEROTONIN REGULATES Cl CHANNELS IN MOUSE CHOROID PLEXUS.

B.C.P. Hung, D.D.F. Loo and E.M. Wright. Dept. of Physiology, UCLA, Los Angeles, Ca, 90024-1751.

The highest density of serotonin receptors (5HT-1c) in the brain is in the choroid plexus, and it is proposed that they play a role in the modulation of cerebrospinal fluid (c.s.f.) secretion. We have examined this hypothesis by testing the effect of serotonin on ion channels in the apical membrane of mouse choroid plexus. When the intact choroidal epithelium was bathed in Ringer's solution and the pipette solution contained 140 mM KCl, cell-attached patches on the apical membrane revealed Cl channels in 70% of the patches. The channels had linear I-V relations with mean conductances of 12 $\pm$ 2 pS. Addition of 10^{-6} M serotonin to the bath solution increased channel open probability as well as activating channels in silent patches. We conclude that serotonin may affect c.s.f. secretion in choroid plexus via modulation of apical Cl channels. (NIH NS09666)

M-Pos196

ANALYSIS OF IONIC MOVEMENTS IN AIRWAY EPITHELIAL CELLS.

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A new mathematical model of ion transport in airway epithelia is presented, which allows both time-varying and steady-state predictions of ionic fluxes, membrane potentials and ion concentrations. The model includes sodium and chloride channels in the apical membrane, a Na^+/K^+ pump and a co-transport system for Cl^- with stoichiometry $Na^+:K^+:2Cl^-$ or $Na^+:Cl^-$. Potassium channels are located in the basolateral membrane.

Membrane potentials and intracellular ion concentration are calculated as a function of medium composition on both sides, the pump current and the co-transport parameters. Theoretical predictions from the model are compared with the available experimental data from airway epithelia.

Supported by the Alberta Heritage Foundation for Medical Research and the Canadian Medical Research Council.

M-Pos197

RELAXATION OF THE Na^+/GLUCOSE COTRANSPORTER.

L. Parent, S. Supplisson, D.D.F. Loo, E.M. Wright. Depart. of Physiology, UCLA-School of Medicine, L.A., CA 90024-1751.

Currents associated to the cloned Na^+-glucose cotransporter were studied in cRNA injected Xenopus oocytes using the two-electrodes voltage-clamp technique. Steady-state I/V relationships were measured between -150 to 90 mV. In pre-steady-state conditions (t < 75 ms) and in absence of sugar, an outward transient current is superimposed to the steady-state I/V relationship. This outward current is present only for depolarizing pulses (at Vm > -30 mV). $t_{½}$ is voltage-dependent increasing from 15 to 23 ms between 30 to -20 mV and is 20 times slower than the capacitive transient. The transient current is reversibly blocked by 100 μM phloridzin; is Na-dependent; and is absent in H_2O-injected oocytes. Moreover, addition of 1 mM α-MDGlucose (100 mM Na) while increasing steady-state I_{max} to -250 nA at -150 mV, decreases the transient current with $t_{½}$ to 5ms at 0 mV. We believe this transient is a direct measure of the transporter relaxation properties.

This project is supported by USPHS DK 19567.
L. Parent is a CMRC post-doctoral fellow.

M-Pos198

TWO [^{3}H]BUMETANIDE BINDING SITES ON MOUSE KIDNEY MEMBRANES: IDENTIFICATION OF CORRESPONDING PROTEINS BY PHOTOAFFINITY LABELLING.

Mark Haas and Bliss Forbush III, Yale Univ. School of Medicine, New Haven, CT 06510

Bumetanide is a potent inhibitor of Na-K-Cl cotransport in many tissues, and [^{3}H]bumetanide binding correlates well with inhibition of transport. We examined [^{3}H]bumetanide binding to crude microsomal membranes isolated from whole kidneys of CD-1 mice, and also photolabelled these membranes with the bumetanide analog [^{3}H]BSTBA, which we have used to identify 150 kDa Na-K-Cl cotransport proteins in dog kidney and duck red cells (Am. J. Physiol. 253: C243-52, 1987). In mouse kidney membranes we identify two distinct classes of [^{3}H]bumetanide binding sites: one with high affinity ($K_{1/2} \cong 0.05$ μM, $B_{max} \cong 2$ pmol/mg prot.) similar to sites identified on dog kidney membranes (Forbush & Palfrey, JBC 258: 11787-92, 1983), and low-affinity sites ($K_{1/2} = 2$-3 μM, $B_{max} \cong 20$ pmol/mg) not seen with dog kidney. When mouse kidney membranes are incubated with [^{3}H]BSTBA and then photolyzed, two proteins incorporate the label in a saturable manner. As with dog kidney, a ~150 kDa protein is labelled with high affinity ($K_{1/2} \cong 0.07$ μM), apparently corresponding to the high-affinity [^{3}H]bumetanide binding site which is the Na-K-Cl cotransporter. A second protein of ~75 kDa is photolabelled by [^{3}H]BSTBA but with much lower affinity ($K_{1/2} = 2$-3 μM); the maximal amount of label incorporated into this protein is 5-10 times higher than that for the ~150 kDa protein. The ~75kDa protein thus appears to correspond to the low-affinity [^{3}H]bumetanide binding site. In the thick ascending limb of Henle's loop of mouse kidney, it was recently proposed that both Na-Cl and Na-K-Cl cotransporters may be present in the apical membrane (Grossman et al., Kidney Int. 35: 480, 1989). As a putative Na-Cl cotransport protein of ~80 kDa was isolated from mouse Ehrlich ascites cells using a bumetanide affinity gel (Feit et al., J. Membr. Biol. 103: 135-47, 1988), and as the affinity of this Na-Cl system for bumetanide is lower than with most Na-K-Cl cotransporters, we suggest that the ~75 kDa protein identified by [^{3}H]BSTBA photolabelling of mouse kidney membranes may correspond to a Na-Cl cotransport system related to, but distinct from the ~150 kDa Na-K-Cl cotransport protein. (Supported by NIH grant DK17433).

M-Pos199

EFFECT OF AMILORIDE ON ADRIAMYCIN-INDUCED UNIDIRECTIONAL FLUXES OF Ca^{2+} AND WATER IN THE TOAD BLADDER.

J.S. Chen, and R.A. Sjodin, Biophysics Dept., University of Maryland School of Medicine, Baltimore, MD 21201.

We have recently shown that exposure of mucosal surface of toad bladder to 52 μM of the antitumor antibiotic, adriamycin, caused a marked increase in short circuit current, with concomitant increases in net Ca^{2+} efflux and net water absorption, but did not alter Na^+ pump activity in the tissue (Ann. N.Y. Acad. Sci. 433, 530, 1984). Our early study seemed to suggest that Na^+ channels at the apical membrane of the toad bladder epithelial cells may be the limiting step in the control of mode of action and cytotoxicity of adriamycin. In the present study, we examined the effect of amiloride, a specific inhibitor of apical Na^+ channels in the toad bladder, on unidirectional fluxes of Ca^{2+} ($_{ca}J_{sm}$ and $_{ca}J_{ms}$; s=serosa and m=mucosa) and water ($_wJ_{sm}$ and $_wJ_{ms}$) in adriamycin-treated tissues that are responsive to the drug treatment. Our data show that in several individual experiments, mucosal treatment of adriamycin-exposed tissue with 1 μM amiloride completely blocks the effect of adriamycin not only on $_{ca}J_{sm}$ and $_{ca}J_{ms}$, but also on $_wJ_{sm}$ and $_wJ_{ms}$; 1 μM amiloride alone does not alter any of these fluxes in the toad bladder exposed to PBS (phosphate-buffered saline, pH 7.4). The results of this study appear to indicate that adriamycin acts on the amiloride-sensitive sodium channel.

M-Pos200

SINGLE K CHANNEL AT THE BASOLATERAL MEMBRANE OF FROG SKIN EPITHELIUM.

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On-cell single channel currents were recorded from isolated cells kept in primary cultures up to 4 days. The patch pipette was filled with KCl. The event most often observed was an inward current of amplitude -3.0±0.9 pA (SD, n=10) at a pipette voltage (V_p) of zero. The I-V relation showed inward rectification with a slope conductance, at V_p=0, of 73±10 pS (SD, n=8) and a reversal potential in the range V_p= -40 to -70 mV. Cell depolarization by substitution of K for Na in the bath shifted the I-V relation, decreasing the reversal potential to a value of less than -8 mV, as expected for a K-selective channel. Opening of the channel occurred in bursts separated by silent periods of up to several hundred ms which were more frequent after hyperpolarization. However, neither the closed nor the open time distributions within bursts were significantly altered by voltage changes. Closed time distributions showed a single exponential of τ_c=1.3±0.6 ms (SD, n=10). In 5 cases an additional exponential (6.5±2.2 ms) was required for the fitting. Likewise, the open time distributions showed a single exponential of τ_o=11.4±5.8 ms (SD, n=10), but a second exponential with shorter time constant (2.1±1.4 ms) was required in 4 of the experiments. Supported by USPHS DK 39214.

M-Pos201

CALCIUM CHANNEL BLOCKERS INHIBIT AMILORIDE RESPONSE IN LARVAL FROG SKIN EPITHELIUM. Thomas C. Cox, Department of Physiology, Southern Illinois University, Carbondale, IL 62901.

The apical membrane of the skin of the frog tadpole (Rana catesbeiana) contains a relatively nonselective cation channel that is activated by amiloride. Since the inward current has some characteristics in common with cation currents through calcium channels in the absence of calcium, we examined the effects of several calcium channel blockers on the amiloride (10^{-4}M) induced current. Millimolar concentrations of multivalents (Ca, Ba, La) completely inhibited the response. Ruthenium red (1mM), nitrendipine (50μM), verapamil (1mM), and W-7 (0.1mm) inhibited the amiloride response to 105, 72, 21, and 8% of control. Bay K 8644 (1μM) also caused inhibition to 36% of control. We are currently using patch clamp techniques to examine the effects of these reagents on amiloride activated single channel currents. We will establish which characteristics the larval cation channels have in common with calcium channels and adult Na selective channels.

M-Pos202

CHARACTERIZATION OF K AND CL CHANNELS IN APICAL MEMBRANE VESICLES FROM RABBIT OXYNTIC CELLS. W. W. Reenstra and J. G. Forte, Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA, 94720.

Apical membrane vesicles from stimulated oxyntic cells contain the H,K-ATPase and channels for K and Cl. As ATP-dependent pH gradient (ΔpH) formation, assayed by acridine orange fluorescence quenching, requires internal K, ΔpH formation can be used to assay K and (due to electroneutrality) anion entry into vesicles. At 75 mM KCl the Cl-channel blocker NPPB does not inhibit ΔpH formation, but at 1 mM Cl, 75 mM K, NBBP inhibits, K_i=50 uM. The K-channel blocker Ba also inhibits ΔpH formation, at 75 mM KCl K_i=3.6 mM. K_i is insensitive to the [Cl] but has a third order dependence on the [K]. As this could be an effect on the H,K-ATPase or a KCl symporter rates of ΔpH dissipation were measured. Glucose and hexokinase were added to stop ΔpH formation; whereupon addition of the protonophore TCS increased the rate of ΔpH dissipation. Since at low [Cl] Ba blocked the TCS-induced increase in the rate of ΔpH dissipation, Ba must inhibit an electrogenic K pathway. The K_i for ΔpH dissipation is similar to the K_i for ΔpH formation and the K-ionophore, valinomycin, reversed the Ba inhibition. A transient ΔpH can be formed without ATP by adding an impermeant base to vesicles that have been equilibrated at a low pH. In KCl or K gluconate media TCS increases the rate of ΔpH dissipation. In the absence of K (N-methylglucamine or sucrose) TCS has no effect on the rate of ΔpH dissipation. These results show that the H,K-ATPase containing membrane of the stimulated oxyntic cell has a Cl-channel that is weakly inhibited by NPPB and a Ba sensitive K-channel. (DK10141)

M-Pos203

Adrenergic Release of VIP from Local Neurones during Regulation of Bicarbonate Secretion in the Turtle Urinary Bladder. Brodsky, W.A., Durham, J.H., *Paul, S., Mt. Sinai Med. Ctr., NY, N.Y., and * Univ. Nebraska, Omaha, Neb.

We have previously reported porcine derived vasoactive intestinal peptide, pVIP, or cyclic AMP, induces an isobutyl methyl xanthine (IBMX)-potentiated primary active electrogenic secretion of HCO_3 in isolated short-circuited urinary bladders from alkalotic and euhydric (post-prandial) turtles, but not from acidotic turtles (Durham et al; 1987). Since then, with the help of radioimmunoassays and immunohistochemical techniques, we have obtained the following findings. (i) An endogenously formed reptilian (r) type of VIP (r-VIP) is stored in sub-mucosal neuronal structures of the turtle bladder. (ii) Norepinephrine (NE), an alpha-2-adrenergic agonist, apparently acts primarily to release r-VIP from its sub-mucosal storage sites, as indicated by the NE-induced, IBMX-potentiated secretion of alkali in alkalotic or euhydric turtle bladders. Tentative Conclusions. During alkalotic or post-prandial states in the turtle, NE- stimulated the release of r-VIP from its neuronal storage sites. Once released, r-VIP acts on nearby alkali excreting epithelial cells to trigger an adenylate cyclase-initiated, cyclic AMP-mediated activation of the alkali secretory pump mechanism.

M-Pos204

CATION CHANNELS OF INSECT MIDGUT GOBLET CELLS: CONDUCTANCE DIVERSITY AND Ba^{+2} ACTIVATION. D.F. Moffett and S.A. Lewis, Dept. Physiol. & Biophys. UTMB, Galveston, TX 77550.

Goblet cells of the tobacco hornworm midgut epithelium actively transport K^+ from the plasma to the lumen. Patch clamping the basal membrane of freshly dissociated goblet cells revealed (based on conductance) 4 types of channels in symmetric 90 mM KCl: small (20 pS), medium (40 pS), large (120 pS) and maxi (220 pS). The medium and maxi channels were studied in detail and showed a 20:1 selectivity of K:Cl and an insensitivity to bath Ca^{2+}. Most importantly, the channels were activated by $BaCl_2$ (2-12 mM). This Ba^{2+} activation is consistent with previous reports of a Ba^{2+} induced increase in both transepithelial current noise and K^+ transport in high (K^+) bathing solutions. The medium and maxi cation channels may mediate K^+ entry into the midgut's transepithelial transport system.
(NSF DCB 8811354 and NIH DK33243).

M-Pos205

VOLUME-ACTIVATION OF A QUINIDINE-SENSITIVE BASOLATERAL K CONDUCTANCE IN TURTLE COLON EPITHELIUM IS REVERSIBLE. K. Backman, B. Harrison, M. Meysenburg, C. Schwartz, and W. Germann, Dept. of Biology, University of Dallas, Irving, TX, 75062

Epithelia were mounted in Ussing chambers and short-circuited. Transcellular I_K were measured following permeabilization of the apical membrane by amphotericin B in the presence of a K gradient. In previous work (J.Gen.Physiol., 88:237, 1986), a quinidine-sensitive K conductance (QSC) activated by cell volume increases was identified. In the present study, quinidine-sensitive I_K were generated in the presence of Cl-containing mucosal solutions. (The QSC is activated when the mucosal bath contains ampho-permeant anions-e.g., Cl.) After attainment of a steady I_K, symmetrical addition of sucrose (final conc. 200mM) caused QSC to fall rapidly to near zero. Similar results were observed when SO_4 (an impermeant ion) was substituted iso-osmotically for Cl. Inactivation was abolished following 1 hr incubation with metabolic inhibitors (1mM KCN + 1mM iodoAc$^-$). It was clear that inactivation, but not activation, of QSC was blocked, since replacement of mucosal SO_4 by Cl led to induction of the QSC when inhibitors were present. (Supported by O'Hara Chemical Institute, University of Dallas)

M-Pos206

INTERACTIONS BETWEEN Ca^{2+}, H^+ AND Na^+ IN RABBIT SALIVARY GLAND ACINAR CELLS P.D. BROWN, A.C. ELLIOTT and K.R. LAU (Introduced by G.F. ELLIOTT). Dept. of Physiological Sciences, University of Manchester, Manchester M13 9PT, England. Removal of extracellular Na^+ abolishes fluid and electrolyte secretion in salivary glands, perhaps through altering pH_i or $[Ca^{2+}]_i$. We have used fluorescent dyes to investigate the changes in pH_i and $[Ca^{2+}]_i$ which result from Na^+ removal in rabbit salivary acinar cells. Replacing Na^+ with N-methyl-D-glucamine (NMDG) acidified the cell and increased resting $[Ca^{2+}]_i$. Re-addition of Na^+ or Li^+ produced an alkalinization which could be blocked by amiloride derivatives, consistent with the presence of Na^+-H^+ exchange. Neither $[Ca^{2+}]_i$ or pH_i was altered if Na^+ was replaced with Li^+. Stimulation with the secretory agonist acetylcholine (ACh) evoked a sustained increase in $[Ca^{2+}]_i$ and a fall in pH_i under both control and Na^+-free conditions. In ACh-stimulated cells in Na^+-free (NMDG) medium re-addition of either Na^+ or Li^+ decreased $[Ca^{2+}]_i$. However, a similar decrease in $[Ca^{2+}]_i$ could be produced by alkalinizing the cell with weak bases, suggesting that the effect of Na^+ or Li^+ re-addition on $[Ca^{2+}]_i$ was mediated via a change in pH_i.

M-Pos207

MODEL OF ION TRANSPORT IN Cl-SECRETING AIRWAY CELLS: INTEGRATED DESCRIPTION OF ELECTRICAL AND CHEMICAL MEASUREMENTS. T. Hartmann & A.S. Verkman (Intr. A.C. Chao). CVRI, UCSF.

An electrokinetic model was developed to calculate electrical parameters, ion fluxes and activities in airway epithelial cells. Model variables included [Na], [K], [Cl], volume, and membrane potentials. The model contained apical Cl, Na and K conductances, basolateral K conductance, Na/K/2Cl and Na/Cl symport, and 3Na/2K ATPase, and a paracellular conductance. Permeabilities were determined from ion flux data and potentials in canine tracheal epithelia. The model predicted accurately the short-circuit current (I_{sc}), conductances, voltage-divider ratios, open-circuit potentials and time course of cell ion composition in ion substitution experiments. The model was used to examine: (1) effect of transport inhibitors on I_{sc} and potentials, (2) the dual role of apical Cl and basolateral K conductance in secretion, (3) the K requirement of basolateral symport, and (4) the regulation of apical Cl conductance by cAMP and Ca-dependent signaling pathways. The model made predictions subjectable to experimental verification. This model has application to kidney thick ascending limb, sweat duct and intestinal secretory cells in normal and pathological states including cystic fibrosis and cholera.

M-Pos208

REGULATION OF THE FORMATION AND WATER PERMEABILITY OF ENDOSOMES CONTAINING THE VASOPRESSIN (VP)-SENSITIVE WATER CHANNEL FROM TOAD BLADDER. Lan-Bo Shi, Yong-Xiong Wang & A.S. Verkman. CVRI, UC San Francisco, CA.

Osmotic water permeability (P_f) in toad bladder is regulated by the VP-dependent movement of vesicles between cytoplasm and apical membrane of granular cells. Endosomes formed with serosal VP have very high P_f (Shi and Verkman, 1989, J Gen Physiol, in press). We examine here: 1) the influence of protein kinase A and C (PKC) on transepithelial P_f (P_f^{te}) in intact bladders and on the number and P_f of labeled endosomes, and 2) whether endosome P_f can be modified physically or biochemically. In paired hemibladders, P_f^{te} and endosome P_f induced by maximal 8-Br-cAMP forskolin were the same. PKC activation by 1uM PMA induced submaximal P_f^{te} (0.015cm/s) and endosome P_f (0.022 cm/s); PMA increased 3-fold the number of apical endosomes with high P_f formed with serosal VP. Endosome P_f decreased 4-fold by increasing membrane fluidity with hexanol or chloroform. We conclude: 1) water channels in endocytic vesicles are not subject to physiological regulation, 2) VP and forskolin do not have cAMP-independent actions, 3) PKC stimulates trafficking of water channels, and 4) water channel function is sensitive to membrane fluidity.

M-Pos209

cAMP MODULATES NaF INDUCED Ca++ TRANSIENTS IN RAT PANCREATIC ACINI WITH A CHOLERA TOXIN INSENSITIVE MECHANISM. V.O. Palmieri, G. Palasciano(1), A. Scarpa. Dept. Physiol. Biophys. CWRU, Cleveland, OH 44106 (1)Dept.Int.Med., Bari Univ., Italy. Intro. by S. Papa.

We have previously shown that cAMP analogues and, with lower potency, forskolin can modulate intracellular Ca++ mobilization induced by cholecystokinin (CCK-8) but not by carbamylcholine (CCH) in rat pancreatic acini. To further characterize this effect, we have investigated the action of cAMP analogues on intracellular Ca++ mobilization induced by NaF, with and without pretreatment with cholera toxin, using the Ca++ fluorescent probe fura2. We found that: 1) NaF promotes intracellular Ca++ mobilization after a lag period of 60-90sec and decreases CCK and CCH induced Ca++ transients; 2) pretreatment of the acini with cholera toxin had no effect on Ca++ transients induced either by CCK, CCH or NaF; 3) addition of the cAMP analogues after NaF causes a transient (30-60sec) and dose dependent decrease of the Ca^{++}_{in} and enhances the effect of both CCK and CCH administered during this phase; the effect was also cholera toxin insensitive.Therefore: 1) cAMP analogues effect on NaF induced Ca++ mobilization suggests the existence of a Ca++ mobilization pathway common to CCK and NaF(G protein NaF sensitive?) but not to CCH; 2) the lack of effect of cholera toxin seems to exclude the involvement of a G protein to this toxin sensitive.

M-Pos210

EVIDENCE FOR A SIMPLE Cl CONDUCTANCE PATHWAY IN NUTRIENT MEMBRANE OF FROG STOMACH, M. Schwartz, T.L. Holloman, G. Carrasquer and W.S. Rehm, Depts. of Physics, Applied Mathematics and Medicine, University of Louisville Louisville, KY 40292

The question arose whether the decrease in PD of about 19 mV due to a tenfold decrease in nutrient (N) Cl conc. might be attributed to a neutral mechanism involving Cl in N membrane and a simple Cl conduct. in the secretory (S) membrane. Expts. were done in 1) HCO_3-free solns. and 2)HCO_3-free, Na-free solns. on both sides of the gastric mucosa. The HCO_3-free solns. were gassed with 100% O_2 on both sides and N pH was kept at 7.2. In 1) ΔPD = -13.5 mV and in 2) ΔPD = -6.7 mV per tenfold decrease in N Cl. These expts. indicate that the residual PD of 6.7 mV was not due to a NaCl symport or a HCO_3-Cl exchanger in N membr. Expts. in HCO_3-free, Na -free and K -free N and S solns. gave ΔPD = -11 mV per tenfold decrease in N Cl. The latter eliminated a KCl symport. The residual PD could best be explained by a simple Cl conductance pathway in the N membrane.

M-Pos211

SINGLE CHANNEL ACTIVITY BY THE AMILORIDE BINDING SUBUNIT OF THE EPITHELIAL NA+ CHANNEL. S. Sariban-Sohraby, Université Libre de Bruxelles and R.S. Fisher, WRAIR, Washington, D.C.

The apical Na+ channel of high resistance epithelia was studied using the patch-clamp technique in 3 different types of preparations: 1)intact amphibian renal(A6) cells in culture grown as monolayers on translucent collagen-coated supports; 2)detergent-solubilized apical membranes of bovine renal papillae reconstituted into liposomes; 3) purified 150 kDa subunit of the Na+ channel protein. In cell-attached patches of A6 monolayers unitary conductance averaged 5pS. Solubilized apical membranes (purified 10X) were incorporated into freeze-thaw liposomes. Observed unitary conductance jumps averaged 10pS. The 150 kDa subunit was separated by HPLC after reduction of the purified Na+ channel protein with DTT. Unitary conductance jumps observed averaged 8pS. For all 3 types of preparations, open times ranged from 50msec to several sec. The observed single channel behavior is consistent with apical Na+ channel activity and indicates that the 150 kDa subunit, which binds amiloride, may contribute to the apical Na+ current in epithelia.

We thank Dr. E. Carmeliet, Dept Fysiologie, KULeuven, Belgium, for his support.

M-Pos212

A VISIBLE SUBSTRATE ANALOGUE FOR THE INTESTINAL NA/GLUCOSE COTRANSPORTER

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A glucose analogue, 2,2,6,6-tetramethylpiperidine -1- oxyl glucose (TEMPO - glucose) was synthesized and examined for its ability to mimic glucose as a substrate of the intestinal Na/glucose cotransporter. Three measures of transport were examined including competition with [^{3}H] glucose for Na-dependent glucose uptake, relief of Trans Na inhibition, and protection against Na-dependent phlorizin binding. Using these measures of substrate competence, TEMPO-glucose is transported by the intestinal Na/glucose cotransporter with approximately 60% - 75% of the efficiency of glucose.

M-Pos213

THE "SUPER-NERNSTIAN" RESPONSE OF Ca-SELECTIVE MICROELECTRODES. T.L. Croxton, J.A. Tanner, and W.M. Armstrong. Dept. Physiol./ Biophys., Indiana Univ. Sch. of Medicine, Indianapolis.

Fine tip Ca-selective microelectrodes often show "super-Nernstian" behavior, i.e. their calibration curves contain regions where the slope of potential vs. log activity exceeds the limit imposed by the Nernst equation. These deviations can be associated with decreased sensitivity and altered selectivity. Tsien and Rink (J. Neurosci. Meth. 4: 73, 1981) showed that the super-Nernstian response of Ca-selective microelectrodes can be reduced by coating their tips with polyvinylchloride and concluded that this response results from leakage of ions through the glass adjacent to the tip. In normal recording, such a leakage current is balanced by an equal and opposite current through the ion-selective cocktail in the tip region. We found the following evidence that electrical current through the ion-selective phase is directly responsible for the super-Nernstian effect: 1) a theoretical model based on an approximate solution of the one-dimensional Nernst-Planck equation with net flux predicts super-Nernstian calibration curves similar to those observed experimentally; 2) super-Nernstian calibration curves were obtained with a large tip K-selective microelectrode when an inward current was passed through it; 3) a compensatory current eliminated the super-Nernstian response of a Ca-selective microelectrode. Current injection appears to be a simple, effective, and theoretically justified method for correcting the super-Nernstian effect in Ca-selective microelectrodes. Supported by USPHS grants DK 12715 and DK 36575.

M-Pos214

COMPARTMENTAL MECHANISM FOR SHORT-CIRCUIT-CURRENT OVERSHOOT IN FROG SKIN RESOLVES PARADOX. Stefan Machlup and T. Hoshiko, Dept. of Physics and Dept. of Physiology & Biophysics, Case Western Reserve University, Cleveland, OH 44106

The rapid decay from the peak current following a jump in $[Na]_{apical}$ is conventionally explained in terms of a shutdown of Na channels. The current falls with a time constant circa 2 seconds; the paradox is that the channel-closing rate constants are an order of magnitude slower, as reflected in the sodium noise spectrum. We postulate a subcellular compartment at the end of the Na channel: As inward Na current fills this subcompartment with Na^+, the Nernst potential across the apical channel falls off.

Na leaking diffusively from the compartment into the adjacent intracellular space introduces another rate constant $1/\tau$. The decay of the concentration ratio

$$c(t) = [Na]_{subcompartment}/[Na]_{apical}$$

is governed by the two time constants σ and τ:

$$-dc/dt = (1/\sigma)\log c + (1/\tau)(c - c_{intracellular})$$

The sodium current is proportional (Ohm's Law) to log c. The rise of the current as the high-Na front diffuses through the stratum corneum with characteristic risetime ρ is included by multiplying log c by $\mathrm{erfc}(\rho^2/t^2)$.

M-Pos215

ARACHIDONIC ACID BLOCK OF EPITHELIAL Cl^- CHANNELS IN TRACHEAL AIRWAY CELLS.
T. C. Hwang, S. E. Guggino, and W. B. Guggino. Departments of Physiology and Medicine, Johns Hopkins University, School of Medicine.

Opening of epithelial Cl^- channels regulates transepithelial Cl^- secretion. Using single channel recordings of excised inside-out patches, we find dose-dependent block of Cl^- channels by arachidonic acid. Kinetic analysis shows the mean open time is decreased 10 fold at 25 μM. There is a linear relationship between mean open time and blocker concentration within the range of 1 to 25 μM. Mean block time does not change with arachidonic acid concentration. Other cis-unsaturated fatty acids, like linoleic and oleic acids, demonstrate similar block. Trans-unsaturated fatty acids (20 μM), like linolaidic acid, and saturated fatty acids (20 μM) do not block the channel. Finally, arachidonyl alcohol (20 μM), i.e. replacement of the carboxyl group with an alcohol group, has no effect on the channel. Our results suggest that arachidonic acid may play a negative role in the regulation of Cl^- channel activity by direct inhibition of channel current.

M-Pos216

^{13}C-UREA EXCHANGE ACROSS THE RBC MEMBRANE DETERMINED BY NMR

Robert Macey and Daniel Karan, University of California, Berkeley

The temperature and concentration dependence of ^{13}C-urea self-exchange across the human red cell membrane has been determined by NMR measurements of T1 (spin-lattice) relaxation times. T1 for intracellular label is 17 sec, which is much longer than the urea exchange time across the cell membrane (about 0.5 sec). T1 for urea in extracellular solution is quenched with 17 mM of impermeable Mn^{2+} in less than two msec. Hence the observed T1 (corrected for intracellular decay) is a measure of urea exchange across the cell membrane. The method is tested by showing both PCMBS and increasing concentrations of urea lengthen T1. Studies of temperature dependence showed that gross activation energies were strongly dependent on both temperature and concentration. However, this apparently anomalous behavior can be resolved into two well behaved functions, K_m and V_{max}, with linear Arrhenius plots and apparent "activation energies" of 15.5 and 12.4 kcal/mol resp. Assuming a simple asymmetric channel model with single binding, $K_{1/2}$ becomes the dissoc. equilibrium constant for the site with $\Delta H°=15.5$ kcal/mol and $\Delta S°=51.8$ cal/(mol-deg); dissociation is entropically driven. Supported by NIH: GM18819, HL20985

M-Pos217

MOLECULAR MOTIONS IN SPECTRIN MODIFIED BY GLUTARALDEHYDE: A SPIN LABEL EPR STUDY

Benito O. Kalaw and L. W.-M. Fung, Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626

Spectrin, a major human erythrocyte membrane skeletal protein, was spin labeled with N-(1-oxy-2,2,6,6-tetramethyl-4-piperidinyl)maleimide. Our earlier results indicated that spectrin exhibited at least three types of segmental motions. To study spectrin's motional responses to chemical perturbations, we modified spectrin with glutaraldehyde and followed the spin label mobility with EPR. The EPR data of purified spectrin showed that glutaraldehyde modification led to a slight increase in the W/S spectral parameter from that of un-modified. The W/S parameter is an amplitude ratio of strongly immobilized and weakly immobilized signal components. SDS gel electrophoresis patterns on modified proteins were also obtained, and showed substantial cross-linkage of spectrin hetero-dimer to higher molecular weight species. Our results indicated that cross-linking of spectrin introduced only small changes in the segmental motions of spectrin molecules.

(*Supported in part by funding from NIH and Loyola University of Chicago.*)

M-Pos218

EVIDENCE FROM pH EFFECTS THAT AMINO ACIDS OTHER THAN ARGININE AFFECT SUBSTRATE AFFINITY OF THE EXTERNAL-FACING TRANSPORT SITE OF HUMAN ERYTHROCYTE BAND 3.

Si-qiong Liu and Philip A. Knauf. U. Rochester Sch. Med., Rochester, NY 14642.

The effects of external pH (at constant internal pH) on K_o^I, the dissociation constant for iodide binding to the form of band 3 (Eo) with the transport site facing outward, were measured. K_o^I increases from 0.14 mM at pH 6.4 to 28.7 mM at pH 11.6.

pHo:	6.4	7.2	8.5	9.9	10.8	11.6
K_o^I:	0.14	0.63	1.13	5.98	16.6	28.7

This titration takes place at pH values well below the pK of the putative arginine residue whose protonation is essential to transport, suggesting that amino acids other than arginine affect iodide affinity either directly or by changing the local concentration of iodide near the transport site. A model based on the latter hypothesis gives a pK of 8.7 for the titratable site, compared to 11.8 for the essential arginine. The apparent half-saturation concentration for external Cl, $K_{1/2}^o$, also increases with pHo. Treatment with the amino-reactive cross-linker BSSS increases $K_{1/2}^o$ at pH<10, and the response to pHo is greatly altered, showing effects of Lys reaction or cross-linking on the response of $K_{1/2}^o$ to pHo. (Supported by NIH Grant DK27495.)

M-Pos219

A PREDICTION OF THE THREE DIMENSIONAL STRUCTURE OF THE SPECTRIN 106-AMINO ACID REPEAT SEGMENT

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Spectrin is a major structural protein in the membrane skeleton of the human red blood cell. The basic unit of the molecule consists of repeat segments of 106 amino acid residues. We have attempted to build a three dimensional model of the protein from the amino acid sequence, with CD data providing the percentage of helix, and EM data supplying the general dimensions of the dimer. The earlier triple helical model failed to account for the propensity of the spectrin sequence to have a larger number of turns. Chou-Fasman analysis of the available spectrin sequences leads to a predicted α-β-α-α-β-α arrangement, with five major segments connected by type IV β turns. Cohen's pattern search method served to limit the length of secondary structures in our approach. We then close packed the secondary structures for consistency with the dimensions derived from EM data, and also to have continuity of the repetitive structures. Our prediction scheme leads to side-by-side and cylindrically inter-packed dimer models. Molecular mechanics - dynamics calculations were used at each stage to anneal the structures. (*Supported in part by funding from NIH and American Heart Association of Metropolitan Chicago.*)

M-Pos220

A METHOD TO EVALUATE THE EFFICIENCY OF THE ANTIOXIDANT SYSTEM FOR RADICALS IN MEMBRANES OF INTACT ERYTHROCYTE, Yin Zhang and L. W.-M. Fung, Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626

The antioxidant system in human red blood cells provides efficient means for protecting cellular components against oxygen-radical damage. However, radicals in membranes are sequestered from the cellular antioxidant system, and are removed less efficiently by the system. We have developed a method to evaluate the efficiency of radical reduction in membranes by introducing fatty acid spin labels, as radicals, into the membranes of intact cells and measuring the signal reduction by EPR methods. The cells were under CO atmosphere to ensure no re-oxidation by atmospheric oxygen. The signal reduction over a period of two hours provides a pseudo first order reduction reaction rate constant of about 5 x 10^{-3}/min. The uncertainty of the rate constant measured by this method is less than 10%. Among the normal adult blood samples (n=32) we measured, we found that the rate constants range from 1.7 to 8.4 x 10^{-3}/min, with a mean of 5.1 x 10^{-3}/min, and a standard deviation of 1.7 x 10^{-3}/min.

(*Supported in part by funding from NIH and Loyola University of Chicago.*)

M-Pos221

CHLORIDE TRANSPORT IN DICTYOSTELIUM DISCOIDEUM. R. B. Gunn and C. A. Bender. Department of Physiology, Emory University School of Medicine. Atlanta, GA 30322

The AX3 strain of slime mold was grown in axenic medium to a density of 5 x 10^6 cells/ml. The cells were washed in a 13-mM Cl solution and equilibrated with tracer, then packed in nylon tubes at 2,000 xg for 5 min. The trapped space measured with ^{22}Na was 8.6% and with ^{14}C-PEG was 9.4%. The intracellular cell water was 8.4 gr/gr cell solids. The chloride concentration ratio was 0.1 in 13 mM or 0.3 in 115 mM Cl_o. Each cell has 0.145 ng of protein by Lowry assay. The chloride fluxes at 0° and 20° were 0.01 and 0.30 mmoles/lit cell•min with an activation energy of 27 kcal/mole. Human erythrocytes at the same chloride concentration at 0°C have a flux of 90 and E_a of 30 kcal/mole. Efflux into 400 µM DNDS solutions or Cl-free citrate/sucrose were not inhibited. These cells have a very low intrinsic chloride transport, making them more suitable as an expression system than oocytes or cultured CHO or 293 cells. Supported in part by PHS grant HL28674.

M-Pos222

EFFECTS OF LIPIDS ON SICKLE HEMOGLOBIN OXIDATION, C. A. LaBrake and L. W.-M. Fung, Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626

It has been found that deoxygenated sickle hemoglobin (HbS) has abnormal solubility, and oxygenated (oxy) HbS looses its heme and oxidizes faster than normal hemoglobin (HbA). Our study involved HbS oxidation in the presence of small unilamellar vesicles (SUVs) of bovine brain phosphatidylserine (BPS), using HbA as a control. The hemoglobin was purified to remove metal contaminants and all traces of enzyme activity of catalase and superoxide dismutase. In the early stages of the oxidation reaction, in the presence of BPS SUVs at 37 °C, methemoglobin was formed at a rate 128 times faster than that in the absence of BPS for HbS, and 83 times faster for HbA. In the second stage of the oxidation reaction, hemichrome concentrations were about 30 % for HbS and 25 % for HbA. In the third stage of the reaction, the hemichrome concentrations were about 60 % for both HbS and HbA, and the lipid vesicles began to aggregate. It also appeared that HbS enhanced lipid vesicle aggregation at a faster rate than HbA. We believe that these findings may provide some insights into the understanding of membrane abnormalities in sickle erythrocytes.

(*Supported in part by funding from NIH and Loyola University of Chicago.*)

M-Pos223

ESTIMATION OF THE PORE SIZE FORMED BY A NON-PROTEIN HEMOLYTIC AGENT FROM SCHISTOSOMA MANSONI. M.R. Kasschau, M.P. Byam-Smith, F.N. Watson and D.L. Gentry. University of Houston-Clear Lake, Houston, TX 77058.

We have indentified a hemolytic agent from the adult Schistosoma mansoni which is found in the pellet fraction of whole adult worm homogenates (Kasschau and Dresden, 1986, Exp. Parasitol. 61:201). Hemolysis produced by schistosome extracts appears to have different mechanisms of action at pH 5.1 and pH 7.5. At low pH, a single-hit detergent like lysis occurs, while at pH 7.5 a multi-hit mechanism requiring 3 units of lytic agent per cell is involved. At pH 7.5 a membrane pore of definitive size appears to be formed. Hemolysis at pH 7.5 was completely prevented by the addition of dextran 40 or polyethylene glycols (PEG) with mol. wt. greater than 20,000 to the extracellular medium. Dextran 20 and PEG 8000 each gave partial protection with PEG 8000 reducing hemolysis by more than 60%. These data suggest that the pore formed is approximately 6.4 nm in diameter. To verify pore size we are studying the uptake of various sized ^{14}C labeled dextrans and proteins into schistosome treated RBCs stabilized with dextran 40. (Funded by NSF DCB-8517512)

M-Pos224

HEMISODIUM INCREASES Na AND WATER CONTENT OF NORMAL AND Hb SS ERYTHROCYTES (RBCs). D. Kaji and A. Malik. VA Medical Center, Bronx, NY 10468 and Mount Sinai School of Medicine, New York, NY 10029.

The probability of Hb polymerization is inversely related to the water content of RBCs. Therefore, agents that increase RBC water content may be useful in preventing sickling. We investigated the effect of hemisodium, a cryptand, on RBC Na, K and water content from 3 normal subjects and 2 subjects with Hb SS. Hemisodium (1 mM) increased RBC Na content in mM/kg dry cell solid (dcs) from 33 to 348 in 4 hrs. In Cl (but not in NO_3 media), the RBC K content (mmol/kg dcs) decreased slightly from 263 to 240, probably consequent to the activation of swelling-activated K:Cl cotransport. In Hb SS RBCs, the increase in RBC Na content was similar, but the fall in K content was somewhat greater. Water content (liter/kg) increased in both normal RBC (from 1.79 to 3.7) and in SS RBC from (1.82 to 3.5) at 4 hrs. The increase in Na transport was conductive as membrane potential (by dye fluorescence) changed from -9 to +20 mv with hemisodium. Hemisodium produces a marked isosmotic persistent swelling of normal (Hb AA) and SS RBCs.

M-Pos225

THE EFFECT OF INSERTED OXIDIZED STEROLS ON SICKLE RED BLOOD CELL MEMBRANES. R. Szostek, and J.W. Kauffman, Northwestern University, Evanston, IL, and O. Kucuk, R. Mata, T. Dey, M.P. Westerman and L.J. Lis, The Chicago Medical School and VA Medical Center, N. Chicago, IL.

Since lipid peroxidation occurs in sickle red cells and oxidized sterols could have a significant effect on cell membrane function, we have examined the significance of this abnormality by inserting oxidized sterols into the membrane of sickle red cells. Known quantities of a number of oxidized sterol compounds were incorporated into the cell membranes of sickle and normal red blood cells. Membrane ghosts of these cell membranes were subsequently prepared and examined using Fourier Transform Infrared spectroscopy. The spectroscopic results infer that there is less pertubation of lipid membrane organization when oxidized sterols are incorporated into sickle red cell membranes than in normal red cell membranes. These data support a role for oxidized sterols in the differences in lipid organization within the membranes of sickle and normal red cells.

M-Pos226

EVIDENCE FOR THE PRESENCE OF OXIDIZED STEROL COMPOUNDS IN SICKLE RED BLOOD CELL MEMBRANES. O. Kucuk, T. Dey, R. Mata, M.P. Westerman, and L.J. Lis, The Chicago Medical School and VA Medical Center, N. Chicago, IL, and R. Szostek and J.W. Kauffman, Northwestern University, Evanston, IL.

Although lipid peroxidation is known to occur in sickle red cells, the extent to which cholesterol oxidation occurs is unknown. Since oxysterols could have significant effects on the cell membrane, we have examined sickle red cells for the presence of oxysterols. Membrane ghosts and lipid extracts of sickle and normal red blood cell membranes were prepared and examined using thin layer chromatographys (TLC) and Fourier Transform Infra-red(FTIR) spectroscopy. The presence of at least six different TLC spots have been observed in sickle RBC membrane lipid extracts that can be correlated with the presence of oxidized sterol compounds. Normal RBC membrane lipid extracts do not show these spots. FTIR spectroscopic data from head group vibrations also imply the presence of oxidized sterols in sickle RBC membranes. These data suggest that oxidized sterols are present in sickle red cell membranes which could contribute to morphological and functional changes in the sickle cell.

M-Pos227

ACETAMIDE CROSSES THE HUMAN ERYTHROCYTE MEMBRANE VIA A LARGE BILAYER PERMEABILITY AND A CARRIER. *Linda Staffero and Lenore W. Yousef, Biology Department, California State University, Fresno, CA 93740*

Permeability coefficients for C^{14} acetamide were measured under equilibrium exchange conditions from 0.01 mM acetamide to 2060 mM acetamide at pH 6.0. All these data are consistent with acetamide using a carrier and a lipid pathway to cross the membrane. The permeability coefficient for the lipid path is between 0.24×10^{-4} cm/sec and 0.37×10^{-4} cm/sec. Transport parameters calculated from the acetamide data depend on the bilayer permeability chosen: for $P_L = 0.37 \times 10^{-4}$ cm/sec, $K_{1/2} = 67$ mM and $V_{max} = 2.0 \times 10^{-5}$ mmol/(cm^{2}sec); for $P_L = 0.24 \times 10^{-4}$ cm/sec, $K_{1/2} = 126$ mM and $V_{max} = 3.2 \times 10^{-5}$ mmol/(cm^{2}sec). (Urea transport parameters measured on the same blood at pH 6.0 were $K_{1/2} = 208$ mM and $V_{max} = 1.1 \times 10^{-4}$ mmol/(cm^{2}sec).)

Acetamide competitively inhibits the urea transport system: in the presence of 103 mM acetamide, $K_i = 107$ mM and in the presence of 206 mM acetamide, $K_i = 123$ mM. Urea also competitively inhibits acetamide transport.

Thus, acetamide moves across the human cell membrane via the urea carrier and a large bilayer permeability.

M-Pos228

COMPARATIVE STUDIES OF RED CELL SWELLING BY PHENOTHIAZINE DERIVATIVES.
Thompson A, Reilly MP, Asakura T, Horiuchi K. Children's Hosp. of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

Previously we reported that chlorpromazine (CPZ) induced swelling in normal erythrocytes and that this effect was hematocrit- and pH-dependent (Fed. Proc, 46: 2111a,1987). Such membrane active substances may prevent polymerization of Hb S and thus sickling by reducing the intracellular hemoglobin concentration. We compared three structurally similar phenothiazine derivatives - CPZ, trifluoropromazine (TFPZ) and trifluoperazine (TFP). Swelling (as measured by % increase in MCV) with all three derivatives occurred in a dose-dependent fashion and was also hematocrit- and pH-dependent. Red cell suspensions at a 10% hematocrit were incubated for one hour at 20°C and pH 7.4 with each compound over a range of concentrations that induced swelling without producing excessive hemolysis (50-200μM). Our results show that at any drug concentration, TFP (>TFPZ>CPZ) caused more swelling. Properties of the chemicals which correlated with relative potency included the degree of ionization, calmodulin binding affinity, and chemical structure. Preliminary studies on net intracellular ion changes show that Na^+ increase and K^+ decrease associated with increased cell water content are balanced. Morphologic studies performed on reversibly sickled cells show that these phenothiazine derivatives inhibited sickle formation and induced cell swelling with the same tendencies as normal erythrocytes.

M-Pos230

ACTIVATION OF K:Cl COTRANSPORT BY HYDROXYLAMINE IS PREVENTED BY CARBETHOXYLATION. P.K. LAUF (Introd. by N.C. Adragna) Physiol. & Biophys., Wright State Univ., Dayton, OH

Hydroxylamine (HOA), a potent oxidizing agent, activated K:Cl cotransport of low K (LK) sheep red cells (src) almost 6 fold. When K:Cl cotransport was already stimulated by N-ethylmaleimide (NEM) HOA caused an additional 2 fold activation. Moreover, the HOA effect was also additive in LK src treated with diamide known to reversibly activate K:Cl cotransport. Dithiothreitol reversed the diamide effect but had little impact on the HOA stimulation. The HOA effect was associated with a disappearance of glutathione suggesting oxidation of SH groups. Diethylpyrocarbonate (DEPC), known to modify an anion-sensitive setpoint site through carbethoxylation of an imidazole or an amino-group, completely abolished the HOA effect independent of the order of treatment as it has been reported for the combined treatment with NEM and DEPC (Lauf, 1989). Hence HOA oxidizes chemical groups different from those modified by thiol alkylation or oxidation. Carbethoxylation, on the other hand, reveals a chemical group essential for the stimulation of K:Cl cotransport through both HOA induced oxidation and thiol group alkylation or oxidation. The HOA effects could be separated from those due to methemoglobin formation since the latter was Cl-independent.

M-Pos229

CHEMICAL EVIDENCE FOR AN ANION-SENSITIVE SETPOINT SITE OF K:Cl COTRANSPORT. P.K. Lauf, (Introduced by T. Sernka) Dept. Physiol. & Biophys., Wright State Univ., Dayton, OH.

Preincubation of low K (LK) sheep red cells (src) at 37°C in isosmotic media with Cl replaced by halides and semihalides determined the magnitude of K:Cl cotransport with an anion sequence of the following decreasing efficiency: $SCN>I>NO_3>Cl>Br$ which is opposite to that required for K transport through the system ($Br>Cl>I=NO_3>SCN$). The activating effect of SCN was volume dependent. The osmolarity at which K:Cl cotransport was zero (setpoint) was around 280 mOsM in Cl but 370 mOsM in SCN-pretreated cells. The apparent affinity for furosemide inhibition was increased by SCN four-fold. The effects of the non-Cl anions were reversible and temperature dependent, and were not seen in high K (HK) src. Treatment of src with diethylpyrocarbonate (DEPC) abolished the anion-activatory effects on K:Cl cotransport an effect also occurring in swollen and NEM-treated LK src. Hence, a carbethoxylated chemical group (imidazole, ε or α amino groups) must be crucial for the anion-modulatory effect. Since HK src failed to respond to these anions in a similar manner as LK src they must lack the anion-sensitive setpoint site(s) placing the genetic basis of the cation dimorphism in src into the regulatory domain of cation transport.

M-Pos231

ASYMMETRY IN THE BINDING MODE OF K AND Cl ON BOTH SIDES OF THE SHEEP RED BLOOD CELL COTRANSPORTER

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Dayton, Ohio 45401-0927.

The binding mode of K and Cl was determined on both sides of the cotransporter of hypotonically swollen sheep red blood cells by measurements of unidirectional fluxes under zero-K-trans conditions and varying the K and Cl concentrations at the cis side. Inside, binding of K and Cl occurred randomly while ordered externally, with chloride binding first. As the mode of binding yields information about the modulation of unidirectional fluxes by ions, the kinetic asymmetry observed cannot account for the functional differences between efflux and influx. Indeed we also demonstrated an asymmetry in the Km/Vmax ratios of K influx and efflux, suggesting the existence of a net flux at equilibrium (when $[K]_i x [Cl]_i = [K]_o x [Cl]_o$). Our kinetic results, obtained in swollen sheep red blood cells, are consistent with the non-diffusive nature of K:Cl cotransport as previously suggested under alkylation stimulation (Lauf, Mol. Cell. Biochem. 82, 97-106, 1988).

M-Pos232

NMR MEASUREMENT OF THE MEMBRANE POTENTIAL IN ERYTHROCYTES FROM THE PARTITION OF TETRAPHENYLPHOSPHONIUM. H. Blum, J. Szklaruk, D. J. Wang, and R. G. Johnson, Jr., HHMI, Univ of Penna Med Center. Phila, PA 19104.

We have adapted the technique [1] of assessing transmembrane potential in isolated cells from the partition of radiolabelled tetraphenylphosphonium (TPP^+) by monitoring the ^{31}P-NMR spectrum of this lipophilic cation in erythrocytes. Intra- and extracellular compartments were distinguished by use of the shift reagent $Dy(DPA)_3^{-3}$ (DPA=2,6-Pyridine-dicarboxylic acid) [2]. Although the movement of TPP^+ across the membrane would tend to collapse the transmembrane potential, we find that the erythrocyte can compensate for this without loss of high energy phosphates (ATP). The calculated transmembrane potential in rat erythrocytes is -8 +/- 2 mV.

The NMR signal is increased by 19% after hemolysis, presumably releasing membrane-bound intracellular TPP^+. This nonspecific binding, unlike the similar application of radiolabelled TPP^+, does not cause errors in the estimation of membrane potential since it is not observed in the NMR signal. This noninvasive and dynamic technique has the possibility of application to more complex tissues.

[1] Ritchie RJ. *Prog Biophys molec Biol* 43: 1-32, 1984.
[2] Pike MM, et al. *Inorg Chem* 22: 2388-2392, 1983.

M-Pos233

SWELLING-ACTIVATED POTASSIUM TRANSPORT IN RED BLOOD CELLS: LACK OF EFFECT OF CELL SHAPE. M.L. Jennings and R.K. Schulz. University of Texas Medical Branch Galveston, TX 77550.

Hypotonic or isotonic swelling activates a KCl cotransport system in the red blood cells of several species. We have used rabbit red cells to examine the relationship between cell shape and the swelling-activated flux, measured as ouabain-insensitive ^{86}Rb influx. The least dense third of the cells were used to eliminate older cells, which have a smaller volume-sensitive flux. In young cells of normal shape a 25% increase in cell volume causes a 10-fold increase in the flux. Cells were converted to echinocytes by addition of low concentrations of detergents, dipyridamole, or trypsin treatment. The shape change by itself activates the flux only very slightly, and the flux activates normally in swollen echinocytes. In chlorpromazine-induced stomatocytes of normal volume the flux is not activated, and in swollen stomatocytes the flux is activated to slightly higher levels than in swollen discocytes. These results indicate that conditions can be found in which swelling-stimulated transport is nearly unaffected by cell shape. Supported by NIH Grant HL37479.

M-Pos234

STRESSES IN A SICKLE CELL. Sunday O. Fadulu and H. Richard Leuchtag, Department of Biology, Texas Southern Univ., Houston, TX 77004.

The flexibility of a normal erythrocyte derives from the absence of a cytoskeleton, but in a sickled cell the structure of crystallized HbS fibers acts as a pathological cytoskeleton. The shape of the sickle cell results from the interaction of the HbS cytoskeleton with the cell membrane. Under equilibrium conditions the membrane configuration depends on hydrostatic pressure difference ΔP, contact forces **F** from the HbS cytoskeleton and surface stress **T** in the membrane. The membrane is a complex of bilayer and membrane skeleton; if it is assumed for simplicity to act as a simple elastic sheet, **T** is purely tensile and acts tangentially. An equilibrium relation between the forces on the membrane is obtained by setting to zero the resultant force across an arbitrary cross section of the cell.

Supported by DRR/NIH RCMI award RR-0345.

M-Pos235

ORIENTATION OF INTACT HUMAN ERYTHROCYTES IN A NARROW GAP EPR FLAT CELL. J.E. Nieves, A.H. Beth, & J.V. Staros, Dept. of Biochemistry and Dept. of Physiology & Biophysics, Vanderbilt Univ., School of Medicine, Nashville, TN 37232. It has been observed that intact human erythrocytes suspended in an EPR quartz flat cell tend to spontaneously align with the greater part of the membrane surface parallel to the plane of the flat cell windows. This alignment can be measured using the fatty acid spin label 5-doxyl stearic acid (5-NS). 5-NS is known to poses its major magnetic axis parallel to the molecular axis and when intercalated in the erythrocyte membrane results in EPR spectra that show orientation dependence relative to the external magnetic field (i.e. parallel and perpendicular). The extent of orientation of the cells can then be calculated by correcting for the distribution of the spin labels in the surface of the biconcave discoid cells and an additional correction assuming a gaussian distribution of cells about an orientation perfectly parallel to the windows of the flat cell. These studies will set the basis for calibrating the orientation of spin labels bound to specific sites in erythrocyte membrane proteins (Band 3) where the geometry of the spin label relative to the membrane plane is unknown. Supported by NIH DK31880, HL34737, & DK07061.

M-Pos236

THE GLUCOSE TRANSPORTER SERVES AS A WATER CHANNEL IN J774 MACROPHAGES. J. Fischbarg, K. Kuang, L. Rogozinski, S. C. Silverstein, and J. Loike, Depts. of Physiol.& Cell. Biophys., Ophthalmol., and Medicine, Coll. of Physicians and Surg., Columbia Univ., New York, N.Y. 10021.

Since the route of water transport across plasma membranes is unclear, we have investigated a possible role of the glucose transporter as a water channel. We monitored volume changes of cells of the J774 murine macrophage-like cell line by recording the intensity of light scattered by them, and we investigated the effects of several inhibitors of glucose transport on cell membrane osmotic water permeability. Cytochalasin B (2.5 μg/ml), phloretin (20 μM) and tomatine (3 μM) reversibly blocked glucose uptake into these cells. All three inhibitors reversibly decreased the osmotic water permeability of J774 cells from 89.6±3.2 to 27.2±1.4 μm/sec. We conclude that a major component of the osmotic water flow across the plasma membranes of these cells traverses the glucose transporter.

M-Pos237

19F NMR MEASUREMENT OF INTRACELLULAR Ca2+ IN OLD AND YOUNG HUMAN ERYTHROCYTES. *Nanci R. Aiken*, W.R. Galey* & J.D. Satterlee#.* *University of New Mexico, Albuquerque, NM 87131; #Washington State University, Pullman, WA 99164. Elevated cell calcium has been implicated in functional changes with erythrocyte aging. However, conflicting reports exist as to the free ionic [Ca2+] in red cells. We have made use of the calcium chelator probe 5,5'-difluoroBAPTA and 19F nuclear magnetic resonance (NMR) techniques *(Murphy et al., 1986)* to measure changes of intracellular Ca2+ concentrations with cell aging. Binding of BAPTA to Ca2+ results in a shift of the 19F NMR signal relative to the signal arising from unbound BAPTA. The lightest (young) and heaviest (old) 10% of density fractionated cells were loaded with BAPTA-AM, the 19F NMR experiment performed, and [Ca2+]i calculated from peak heights and Kd for Ca2+ binding. Unseparated RBCs were found to contain approximately 70 nM Ca2+, while density separated young and old fractions contain 50 and 350 nM, respectively. These changes in intracellular Ca2+ correlate with an age-related increase in anion transport and a left shift in hemoglobin-oxygen dissociation. The increase in free ionic calcium with cell age suggests that either the [Ca2+] regulatory system is defective, or that the membrane of old cells is more permeable to the ion. *Supported by NIH grants DK30912 & HL01758 (JDS), and Amer. Heart Assoc. grant NM02183(WRG).*

M-Pos238

A COMPARISON OF THE EFFECTS OF TRANSPORT INHIBITORS ON ANION EXCHANGE AND CONDUCTANCE IN HUMAN RED BLOOD CELLS. O. Fröhlich and V. K. Gottipaty. Dept. Physiol., Emory University Sch. Med. Atlanta, GA 30322.

From the quantitative agreement between anion exchange and net efflux (conductance) with respect to the binding/inhibition parameters of Cl_o and the anion transport inhibitors DNDS and phloretin, it had previously appeared that a common set of binding sites existed for these ligands, as if the same band 3 molecule mediated both transport modes. In contrast, we had also found that sulfate, a competitive Cl exchange inhibitor, had no effect on anion net efflux. To explore this discrepancy further, we examined the effect of several more transport inhibitors. Representative are the effects found for the irreversibly acting, arginine reagent phenylglyoxal (PG) and dipyridamole (DIP). We found that exposure of red cells to PG inhibited anion exchange by 90%, in agreement with findings by other laboratories; however, PG had essentially no effect on anion net efflux. The exchange inhibitor DIP exhibited a peculiar dependence on extracellular chloride (Cl_o) for its inhibitory effect on the conductance: at Cl_o=0 it had no effect on Cl net efflux, but at high Cl_o it inhibited net efflux to the same extent as DNDS. This Cl dependence is strongly reminiscent of the Cl dependence for inhibiting sulfate flux that reported previously by other laboratories. The simplest explanation for these observations is that the anion conductance is mediated by band 3, but to mediate anion net flux, band 3 needs to be in a different conformational state from that mediating exchange.

M-Pos239

REGULATION OF Na/K/Cl-COTRANSPORT BY CHANGES IN VOLUME AND MAGNESIUM OF HUMAN RED CELLS. H.Mairbäurl and J.F.Hoffman. Department of Cellular and Molecular Physiology, Yale Medical School, New Haven, CONN.

The relation of Na/K/Cl-cotransport (CT) to cell volume (MCV) and to the cell Mg concentration (Mg_i) was studied in red cells after treatment with nystatin to elevate Na_i. Cotransport was measured as bumetanide-sensitive Na-efflux in the presence of ouabain. There was an inverse relation between CT and MCV in red cells from different donors. MCV was directly related to 2,3-DPG, but no difference in total Mg was found indicating a possibly inverse relation of free-Mg_i with MCV. Altering Mg_i with A23187 revealed a high Mg_i-sensitivity of CT in the physiological range. Depletion of Mg_i to below 50 μM with 5 mM EDTA inhibited about 50% of the normal CT activity, while an elevation of Mg_i activated CT about 4-fold. The sensitivity of CT to acute MCV changes was measured by suspension of high Na_i-cells in anisotonic media. Cotransport was stimulated by cell shrinkage and inhibited by cell swelling. Also shrinkage elevated CT at any Na_i (2 to 80 mM, reciprocally varied with K_i) and shifted V_{max} towards higher Na_i. Neither Mg_i-depletion nor varying Mg_i eliminated the volume sensitivity of CT at V_{max}. These results indicate that the effects of Mg_i and cell volume can be dissociated. (Work supported by NIH grant HL09906)

M-Pos240

HPLC GEL FILTRATION ANALYSIS OF THE OLIGOMERIC STRUCTURE OF BAND 3, THE ANION TRANSPORT PROTEIN OF THE ERYTHROCYTE MEMBRANE. Joseph R. Casey and Reinhart A. F. Reithmeier, MRC Group in Membrane Biology, Department of Medicine and Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Oligomers of human erythrocyte Band 3 were separated by HPLC gel filtration using a TSK 4000SW column in the presence of the non-ionic detergent, octaethylene glycol n-dodecyl ether ($C_{12}E_8$). The protein eluted as a major peak with a Stokes radius = 79 Å which corresponds to Band 3 dimer. Variable amounts of larger Band 3 species were found in some preparations, while little or no Band 3 monomer was detected. The oligomeric state was not changed by addition of 2-mercaptoethanol, NaCl (0.1-1.0 M), $C_{12}E_8$ (0.01- 10%) or by changes of protein concentration (.015-15 mg/ml). Band 3 is aggregated below pH 6.0 and at detergent concentrations below 0.01%. No equilibration between oligomeric states of Band 3 could be seen by either rechromatographing separated oligomers or by large-zone gel filtration. Band 3 labeled with the anion exchange inhibitor H_2DIDS showed the same oligomeric distribution as control Band 3, although the higher oligomeric form was usually better resolved from the dimer peak. Band 3 deglycosylated with endoglycosidase-F remained dimeric with a Stokes radius = 77 Å. The 55 kDa membrane domain of Band 3 labeled with DIDS eluted as a dimer (Stokes radius = 57 Å). Control 55 kDa domain eluted as a broad peak, with a similar Stokes radius. We conclude that Band 3 in $C_{12}E_8$ solution exists as a stable dimer, that can form higher order irreversible aggregates but can not be readily dissociated into monomers. (Supported by Medical Research Council of Canada.)

M-Pos241

THE INFLUENCE OF POLYMER FORMATION ON THE RHEOLOGICAL PROPERTIES OF SICKLE ERYTHROCYTES Linda H. Mackie Dept. of Mech. Egr. The Catholic Univ. of Amer. Wash. DC

Micromanipulation techniques were used to measure the shear modulus or rigidity, μ, the recovery time constant, t_c, and the unfolding time constant, t_f, for individual sickle cells. The rheological properties were analyzed by varying the oxygen tensions, temperature and cell densities. The oxygen tension was varied from 37 to 157 mmHg and the temperature was controlled at 25°C or 37°C. Three mean cell hemoglobin concentrations were studied: 29g/dl, 33g/dl and 46g/dl.

Using the data of Noguchi and Schechter and others to calculate the amount of polymer at different densities, temperatures and oxygen tensions, it was found that the values for μ, t_c and t_f increased linearly between 0 and 60% polymer. Above 60% polymer the cells became quite rigid. Cells at 37°C, 38 mmHg pO_2 and a density greater than or equal to 46g/dl(80% polymer) exhibited a rigidity that was sixteen times greater than SS cells with no polymer and a density of 33g/dl, which indicated that the densest 3% (MCHC≥46g/dl) of a patient's cells probably contribute most to sickle crises.

M-Pos242

NMR BINDING STUDIES SUPPORT THE CARRIER MODEL FOR ERYTHROCYTE GLUCOSE TRANSPORT. Scott A. Ross and Sunney I Chan, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125.

NMR measurements of ligand-binding to transport proteins provide a useful complement to classical transport studies of these systems. We previously developed an ^1H NMR method to measure binding of sugars to the human erythrocyte glucose transporter,based on sugar proton transferred NOEs (TRNOEs) (Proc. Natl. Acad. Sci. USA 83, 1985, 3277-3281). Glucose-binding to the transporter has been examined in three preparations of erythrocyte ghosts which differ in their exposure of the intra- and extracellular faces of the membrane to the bulk solution. In all three preparations, the observed TRNOE was eliminated by a saturating concentration of cytochalasin B. No evidence was found for the existence of a ternary complex of transporter, glucose and cytochalasin B. Furthermore, the dependence of the TRNOE on glucose concentration was found to be consistent with the presence of only two classes of binding sites, one on either side of the membrane. These NMR results support the adequacy of the simple carrier model to account for glucose transport by the human erythrocyte transporter.

M-Pos243

EFFECTS OF LIPID ORDER ON DYNAMICS AND FUNCTION OF BAND 3 IN HUMAN ERYTHROCYTES. C.E. Cobb, S.F. Juliao, J.V. Staros, & A.H. Beth, Mol. Physiol. & Biophysics, & Biochemistry, Vanderbilt Univ., Nashville, TN 37232. Band 3, the anion exchange protein of human erythrocytes, is an integral membrane protein which passes through the lipid bilayer multiple times. The anion exchange function of band 3 could potentially be dependent on the ordering of membrane lipids. Introduction of a spin-labeled fatty acid into the membranes of *intact* erythrocytes has allowed us to examine the effects of temperature, diethyl ether, and glycerol on membrane lipid ordering by EPR spectroscopy. We have also used saturation transfer EPR to investigate the effect of these agents on the rotational dynamics of band 3 labeled with the membrane impermeant bifunctional spin label BSSDA. The ordering of membrane lipids decreases, the rotational motion of band 3 increases, and the $SO_4^=$ uptake V_{max} increases with increasing temperature (from 0 to 37° C). Diethyl ether decreases the ordering of membrane lipids, increases the rotational motion of band 3, but has no effect on the $SO_4^=$ uptake V_{max} (20° C). Glycerol increases the ordering of membrane lipids, decreases the rotational motion of band 3, but has little effect on the $SO_4^=$ uptake V_{max} (20° C). These data suggest that though the rotational dynamics of band 3 are dependent on the ordering of membrane lipids, the rate limiting step for $SO_4^=$ uptake is not strongly influenced by the ordering of membrane lipids and that the activation energy for uptake (~30 kcal/mole) is not dominated by a conformational rearrangement of the protein which requires displacement of lipids. Supported by HL 34737 & DK 31880.

M-Pos244

IDENTIFICATION OF THE EOSIN-5-MALEIMIDE & EOSIN-5-IODOACETAMIDE REACTION SITES ON HUMAN ERYTHROCYTE BAND 3. C.E. Cobb, H. Lin, & A.H. Beth, Mol. Physiol. & Biophys., Vanderbilt Univ., Nashville, TN 37232.

Eosin-5-maleimide (EMA) and eosin-5-iodoacetamide (EIA) are fluorescent probes which affinity label band 3 in *intact* human erythrocytes. We have identified lys-430 of the 17 kDa transmembrane segment to be the primary reaction site for EMA on band 3 by direct sequencing of HPLC purified EMA labeled peptides. We have also characterized the inhibition of EMA labeling of band 3 in *intact* erythrocytes by H_2-DIDS and the membrane-impermeant bifunctional spin probe bis(sulfo-succinimidyl)-4-doxyl azelate (BSSDA). Under conditions which result in labeling of 95-100% of the band 3 population of control erythrocytes for each reagent, pre-labeling of cells with H_2-DIDS or BSSDA reduces EMA labeling by 80% or 55%, respectively. Pre-labeling with EMA reduces H_2-DIDS labeling at neutral pH by about 55% and BSSDA labeling by 85%. These data suggest that although the covalent reaction sites for EMA and the other two reagents may be different, the sites are close in spatial distribution. We have also identified lys-430 to be the primary amino acid residue covalently labeled with EIA. It is noteworthy that although there is a cysteine residue present in the 17 kDa peptide (cys-479), apparently it is not reactive with either of these eosin derivatives. Supported by HL34737.

M-Pos245

WW781 NONCOMPETITIVELY INHIBITS RED BLOOD CELL Cl^- EXCHANGE BY A 2-STEP MECHANISM WHICH IS SENSITIVE TO SUBSTRATE BINDING AND TRANSPORT SITE REORIENTATION. Nancy S. Mendoza, Laurie J. Spinelli, and Philip A. Knauf. Dept. of Biophysics, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

In red blood cells with 5 mM Cl^- inside and outside (Cli=o), the membrane potential-sensing fluorescent dye WW781 inhibits Cl^- exchange by 50% at 0.83 ± 0.14 (SE) μM (the ID50). Inhibition increases with time of exposure to WW781, but can be completely reversed after washing with solutions containing albumin. If cells with 5 Cli=o are pretreated for 10 min with WW781 and then suspended in media with the same WW781 concentration, the ID50 decreases to 0.063 ± 0.001 μM. These data suggest a two-step binding:

$$b3 + W \overset{K_1}{\longleftrightarrow} b3{\cdot}W \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} b3\text{-}W$$

where formation of the b3·W complex is fast and conversion to the b3-W complex is slow. With 150 mM Cli=o, K_1 is 0.63 ± 0.08 μM, k_{-2} is 0.15 ± 0.03 min^{-1}, and k_2 is 1.1 ± 0.3 min^{-1}. Increases in Cli=o do not increase K_1, showing that the inhibition is not competitive. Binding has a 1:1 stoichiometry with band 3 and is strongly affected by band 3 conformation. (Supp. by NIH Grant DK27495 and U of R DeKiewet Summer Fellowship.)

M-Pos246

PROPOSED MODEL OF AQUEOUS CHANNEL FOR PERMEATION OF NONELECTROLYTES ACROSS THE HUMAN RED CELL MEMBRANE. Michael R. Toon and A. K. Solomon, Biophys. Lab., Harvard Medical School, Boston, MA 02115.

Our model of the red cell aqueous channel is a tripartite assembly, comprising H-bond-specific regions at both membrane faces, joined by a narrow sieve-specific region. Small hydrophilic nonelectrolytes first bind to the H-bond-specific region to exchange their solvation shell for channel H-bonds, consistent with our finding that urea, amide and ethylene glycol (eg) transport is a saturable process. The sieve-specific regions account for the observation that the solute/membrane friction is much larger than the solute/water friction; the reflection coef. decreases linearly with increased solute diameter. We find that the permeability coefs., ω_{urea} and ω_{eg} are correlated with the hydraulic conductivity; and that ω_{urea} is correlated with ω_{eg} in cells treated with BS^3 (bis(succinimidyl suberate)) a lysine-reactive anion transport inhibitor, showing that urea, eg and water enter the red cell through the same channel and suggesting that the channel is associated with band 3. (Supported by Council for Tobacco Res. and Squibb Inst. for Med. Res.)

M-Pos247

INTERACTION BETWEEN RED CELL GLUCOSE TRANSPORT PROTEIN AND BAND 3. Agnes Janoshazi and A. K. Solomon, Biophys. Lab., Harvard Med. School, Boston, MA 02115

Previous studies (J Memb Biol in press) show cytochalasin B modulates kinetics of DBDS (4,4'-dibenzamido-2,2'-disulfonic stilbene) binding to band 3. We now show that DBDS modulates kinetics of maltose binding to the glucose transporter, confirming the band 3/glucose transporter linkage. Carruthers et al (1986,87) showed that outside maltose, but not D-glucose, displaces inside cytochalasin B from the glucose transporter in stripped ghosts. D-glucose, but not L-glucose, quenches tryptophan (trp) fluorescence in purified transporter. Our stopped-flow studies with fresh red cells show that maltose binding consists of an immediate adsorption followed by a biphasic conformation change (first rise then fall in trp fluor) with a 100-150 sec time course. 2 μM DBDS produces a time dependent change in the maltose induced trp fluor, consistent with the band 3/glucose transporter link. Controls show: DBDS decreases trp fluor with no time dependence; D-glucose causes a linear rise in trp fluor, unaffected by DBDS; L-glucose has no effect on trp fluor. Supported by Council for Tobacco Res. and Squibb Inst. for Med. Res.

M-Pos248

TWO MECHANISMS BY WHICH FLUORESCENT OXONOLS INDICATE MEMBRANE POTENTIAL IN HUMAN RED BLOOD CELLS. P.R. Pratap, T.S. Novak, and J.C. Freedman, Dept. of Physiology, SUNY-HSC, Syracuse, NY 13210. Potentiometric dyes have been used to monitor transmembrane electrical potentials (E_m) of many cells and organelles. An understanding of dye response is needed for the design of dyes with improved responses and for unambiguous interpretation of experimental results. This work describe the responses to ΔE_m of 20 impermeant anionic oxonols in human red blood cells. Fluorescence (F) of 15 oxonols decreased with hyperpolarization ($\leq$ -10%ΔF for WW781), consistent with an "on-off" mechanism, while 5 oxonols unexpectedly showed E_m-dependent increases in fluorescence at < 2μM [dye] (> +30%ΔF for RGA451). Binding curves for WW781 and RGA451 at 1 mM $[K]_o$ (-ΔE_m with gramicidin) and at 90 mM $[K]_o$ (ΔE_m=0 with gramicidin) showed E_m-dependent changes in binding. %ΔF of cell suspensions correlated with %$\Delta[dye]_{bound}$ for WW781, in accordance with the "on-off" mechanism, but not for RGA451. A model is suggested in which RGA451 moves between two states of different quantum efficiencies within the membrane. NIH grant GM28839 for JCF, and AHA-NY Affiliate post-doctoral fellowship for PRP.

M-Pos249

ELECTRO-OSMOTIC TRANSDUCTION BY HUMAN RED BLOOD CELLS. John D. Bisognano, James A. Dix, and Jeffrey C. Freedman, Dept. of Chemistry, SUNY, Binghamton, NY 13901, and Dept. of Physiology, SUNY Health Science Center, Syracuse, NY 13210.

The fluorescence of diS-C3(5) indicates that the membrane potential of human red blood cells depolarizes upon exposure to hypertonic sucrose--an example of electroosmotic transduction. In addition to dye fluorescence, $H^+(OH^-)$ flux was measured by a pH stat, cell volume was monitored by light scattering, and cell electrolytes were directly measured when cells were shrunken either with NaCl or with sucrose. Cells shrunken with sucrose exhibit a rapid depolarization followed by a slow hyperpolarization, an initial H^+ efflux or OH^- influx of 0.7 Eq/g Hb/min and a Cl^- shift of 7 mEq/L cell water in 15 minutes. With hypertonic NaCl, the cells are initially close to Donnan equilibrium but with sucrose, a outwardly-directed Cl gradient is slowly dissipated either as a net HCl efflux or by Cl^-/OH^- exchange. The data agree with predictions of equations describing nonideal osmotic and ionic equilibria. (Supported by NIH grant GM 28839.)

M-Pos250

GAPS IN THE ERYTHROCYTE MEMBRANE SKELETON: A STRETCHED NET MODEL. Michael J. Saxton, Plant Growth Laboratory, University of California, Davis, California 95616, and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, Berkeley, California 94720.

The geometry of spectrin-free regions in the erythrocyte membrane skeleton is modeled in terms of an incomplete triangular lattice of entropy springs under tension. Intact springs correspond to normal spectrin molecules; cut springs correspond to spectrin that is missing or unable to associate normally. As springs are cut and the network is allowed to relax to mechanical equilibrium, gaps in the network appear. Geometrical properties of these gaps are obtained as a function of the fraction of springs cut. The most important property modeled is the area of the largest spectrin-free region; this area increases approximately exponentially as the fraction of normal spectrin decreases from 100% to ~50%. The effect of these gaps on lateral diffusion, membrane fusion, exocytosis, and endocytosis is discussed. Supported by NIH grant GM38133.

M-Pos251

MOLECULAR DYNAMICS STUDIES OF ION MOVEMENT IN GRAMICIDIN CHANNELS. See-Wing Chiu and Eric Jakobsson. Department of Physiology and Biophysics and Program in Bioengineering, University of Illinois, Urbana, IL 61801

Molecular dynamics computations have been done on a system of the gramicidin channel containing univalent cations (Na^+, K^+, Cs^+) deep in the channel plus water, plus a cap of water on each end of the channel. Time-correlation analysis is used to estimate mobilities from short-time fluctuations in the ion and water positions. A pronounced distortion of the channel in the vicinity of the ion is observed, including a time-averaged bending of some peptide bonds by more than twenty degrees out of a planar configuration. The mobility of the ion-water chain in the channel is less than that of a chain of waters without ions. Further analysis of the computed trajectories will be done to determine whether there are periodic motions of the ion-water chain, similar to periodic motions observed for the chain of waters without ions. Additional computations will be done with ions near the channel mouth, to determine whether there are functionally significant special interactions involving ions and the tail end of the channel polypeptide.

M-Pos252

TIME-CORRELATION ANALYSIS OF SIMULATED WATER MOVEMENTS IN THE GRAMICIDIN CHANNEL. See-Wing Chiu[#], Shankar Subramaniam[*], Eric Jakobsson[#,+], and J. Andrew McCammon[*,@]. [#]Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801; [*]Dept. of Chemistry, University of Houston, Houston, TX 77204; [+]Bioengineering Program, University of Illinois, Urbana, IL 61801; [@]Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030. We have done molecular dynamics simulations on a system consisting of a gramicidin A channel containing water molecules and with caps of waters on each end representing the bulk water. In the absence of the explicit inclusion of the phospholipid membrane, artificial restraints are included on the motions of the waters and the polypeptide backbone. Several different types of motion are seen. For the chain of waters in the channel, one sees diffusive motions along the channel axis and also coordinated periodic motions in a direction perpendicular to the channel axis. The distribution of frequencies of the coordinated periodic motions is a systematic function of the strength of the artificial restraints. We will present a variety of correlation functions calculated from the results of our dynamics simulations and discuss their significance.

M-Pos253

PROTON MOBILITY IN THE GRAMICIDIN CHANNEL IS EQUAL TO OR GREATER THAN PROTON MOBILITY IN ICE. M. Akeson & D.W. Deamer, Zoology Dept., University of Calif., Davis, CA. Proton conductance in the gramicidin channel is anomalously high, presumably due to movement of H^+ along H-bonded waters(Hladky & Haydon, 1972). We are interested in rate-limiting steps for this process, and in the maximum rate at which H^+ moves along the putative single water strand. We measured H^+ currents in gramicidin D channels embedded in glycerol monoolein/decane bilayers. At 60 mV applied potential, single channel currents increased with HCl concentrations to 1.0 M, as seen previously (Eisenman & coworkers, 1980). This suggests that at 1.0M HCl, diffusion to the channel mouth in the aqueous phase is rate limiting. To test this prediction, we measured single channel H^+ currents in 1.0 M HCl + 1.5M sucrose, wherein the diffusion coefficient of H^+ is halved. As expected for an aqueous-diffusion limited process, the single channel current at 60 mV was reduced from 40 pA (- sucrose) to 14 pA (+sucrose). Furthermore, the current in 1.0 M HCl was equal to that predicted by aqueous diffusion to the channel mouth assuming a 0.9 Å capture radius (Decker & Levitt, 1988). On this basis we calculate that H^+ mobility in the gramicidin channel is at least 1.3E-3 cm^2/ (V-s) (similar to ice) and probably much greater. ONR Contract N00014-85-0231.

M-Pos254

AMINO ACID SEQUENCE MODULATION OF GRAMICIDIN SINGLE CHANNEL DURATIONS. M.D. Becker, D.B. Sawyer, R.E. Koeppe II[*], and O.S. Andersen. Department of Physiology and Biophysics, Cornell University Medical College and [*]Department of Chemistry, University of Arkansas.

The four tryptophanyls at positions 9, 11, 13 and 15 in Gramicidin A influence the average channel duration. In order to study the positional dependence of Trp upon channel function, we examined seven sequence substituted gramicidins with Trp --> Phe substitutions: single substitutions, Phe-9, Phe-11, Phe-13 and Phe-15; a double substitution, Phe-9,15; and triple substitutions, Phe-9,13,15 and Phe-11,13,15. In DPhPC bilayers, 1.0 M NaCl, all seven peptides form channels that are structurally equivalent to Gramicidin A because they form "hybrid" channels with Gramicidin A. Trp --> Phe substitutions at positions 9, 13 or 15 do not substantially alter average channel duration: 740, 500, 750, 500,and 800 ms for Phe-9, Phe-13, Phe-15, Phe-9,15, and Gramicidin A, respectively. A Trp --> Phe substitution at position 11 causes a net stabilization of channels as Phe-11 and Phe-11,13,15 form channels with average durations of 2400 and 1360 ms, respectively, and Phe-9,13,15 channels have an average duration of only ~5 ms. A Trp <--> Phe interchange between positions 9 and 11 thus results in a large change in channel stability, the magnitude of this effect depends on the residues at positions 13 and 15, which suggests an underlying cooperative influence of the four Trps upon channel behavior.

M-Pos255

NMR STUDIES OF TYR-1 GRAMICIDIN A.
M. J. Taylor and Roger E. Koeppe II
Dept. Chem. Biochem., University of Arkansas, Fayetteville, AR 72701.

Analogues in which the formyl-Val-1 of gramicidin A (GA) has been substituted by another formyl-amino acid generally form dimer channels that are structurally equivalent to GA channels [J. T. Durkin et al., J. Mol. Biol., in press]. For side chains as diverse as Gly to o-, m-, and p-F-Phe at position #1 of GA, there is no energetic cost for channel formation and no energetic destabilization of hybrid dimers relative to symmetric dimers. Either Tyr-1 or hexafluoroVal-1 GA, however, forms hybrid channels with Val-1 GA that have unusually short durations and low conductances. These properties may reflect subtle conformational differences between these analogues and Val-1 GA. We are using 2-D NMR at 500 MHz to investigate the conformations of these analogues, initially in d6-DMSO solution and later in deuterated micelles or membranes, using Val-1 and Phe-1 GA as reference compounds.

M-Pos256

LASER DOPPLER SCATTERING AND ION VELOCITY DISTRIBUTIONS IN CHANNNELS AND MEMBRANES—
M. E. Starzak and F. Macias, Laser Doppler velocimetry, which uses the Doppler shift of scattered light to probe particle velocity, is applied to a study of ion motions in membrane channels and bilayers. While scattering from individual permeant ions such as Tl(I) is small, the directed motion of an ensemble of these ions in a permeation region small with respect to the probe laser wavelength permits constructive multiple ion scattering from ions at all locations in the membrane. Extraction of the Doppler difference frequency with a non-linear detector produces a frequency spectrum which can be converted to a velocity spectrum to reflect the relative number of ions at each local velocity. This generates a velocity distribution for these ions. Since the local velocities are the intrachannel rate parameters, the distribution can be used to eludicate the permeation mechanism. To test potential permeation models, a new deconvolution technique, which permits the determination of velocity distributions from the net membrane flux is introduced and applied to some basic models for permeation in the gramicidin channel.
Supported by the Office of Naval Research.

M-Pos257

THE THALLIUM ION DISTRIBUTION IN THE GRAMICIDIN CHANNEL BY X-RAY DIFFRACTION.
Glenn A. Olah, Huey W. Huang and Wenhan Liu, Physics Department, Rice University, Houston, TX 77251

Highly aligned dilauroylphosphatidylcholine multilayer samples containing gramicidin and various ions were prepared between a polished Be window and silica plate. These samples allowed x-ray diffraction to yield the 1-D electron density profiles at 4Å resolution. By comparing the density profiles of samples with different ions, we are able to determine the thallium ion distribution in the gramicidin channel. We also found that the effects of the channel on the lipid headgroup and hydrocarbon chains are different with different ions. These samples were further charaterized by circular dichroism (CD) studies. The CD results suggest a slight disordering of the TRP side chains, when the channel binds Tl^+.

M-Pos258

COOH-TERMINAL SUBSTITUTIONS IN GRAMICIDIN CHANNEL AFFECT CONDUCTANCE HETEROGENEITY.
V. Fonseca[1], D. B. Sawyer[1], R. E. Koeppe II[2], F. Heitz[3], R. Lazaro[3], Y. Trudelle[3], and O. S. Andersen[1]; [1]Cornell Univ. Med. Coll., New York, NY; [2]University of Arkansas, Fayetteville, AR; and [3]CNRS, Montpellier and Orleans, France.

The presence of minute concentrations of detergents increases the conductance heterogeneity in channels formed by gramicidin (Sawyer et al. Biochemistry 28: 6571, 1989). These detergent effects depend on the structure of the COOH-terminal end of the channel-forming peptides. When the four tryptophans of gramicidin A are replaced by phenylalanines or tyrosines, 8 μM Triton X-100 increases the conductance heterogeneity similar to that seen with gramicidin A. When the tryptophans are replaced by tyrosine-O-benzyl ether or naphtylalanine, Triton has no effect upon the conductance heterogeneity. Replacement of the COOH-terminal ethanolamine by methylamine has no effect on the conductance heterogeneity, whereas the acylation of a fatty acid to the ethanolamine increases the conductance heterogeneity in the absence of Triton while diminishing the effect of Triton. The differential effect of these COOH-terminal substitutions on the conductance heterogeneity suggests that the heterogeneity arises from an alterred COOH-terminal conformation in the abberant channels, possibly mediated through alterred gramicidin/lipid interactions that are sensitive to the primary channel structure.

M-Pos259

SINGLE CHANNELS FORMED BY GRAMICIDIN A IN "SOLVENT-FREE" BILAYERS FORMED FROM SURFACE MONOLAYERS USING A TIP-DIP TECHNIQUE. D. B. Sawyer, S. Oiki, and O. S. Andersen. Cornell University Medical College, New York, NY.

In order to examine the behavior of channels formed by gramicidin in stable "solvent free" bilayers we have used the tip-dip technique of Hanke et al. (Biochim. Biophys. Acta, 727: 108, 1983) in monolayers made from a solution of diphytanoylphosphatidylcholine (DPhPC) in squalane at the air/electrolyte interface. Squalane is a linear saturated hydrocarbon that like squalene should be virtually insoluble in lipid bilayers (Simon et al., Biophys. J. 19:83, 1977). Membranes were formed by the repeated dipping of a 10-15 μm diameter glass pipette through the monolayer. The pipet and membrane has a capacitance of ~6 pF, a background resistance of $\sim 10^{12}$ Ω and a RMS noise of ~0.5 pA (0-5 kHz). These membranes provide stable recordings of gramicidin channels over periods of more than 24 hours, allowing one to examine events lasting from $\sim 10^{-5}$ to $\sim 10^{5}$ s. In 1 M CsCl, gramicidin A channels have a most probable conductance of ~50 pS, which is indistinguishable from their conductance in DPhPC/decane membranes, and a mean duration of ~300 s, about 500-fold longer than the duration in DPhPC/decane membranes. The increased stability of the gramicidin dimer allows one to study extremely short-lived gramicidin analogs that cannot be resolved in solvent-containing membranes.

M-Pos260

GRAMICIDIN CHANNELS ARE RIGHT-HANDED β-HELICAL DIMERS. O.S. Andersen, L. L. Providence, and R. E. Koeppe II. Cornell Univ. Med. Coll., New York, N. Y., and Univ Arkansas, Fayetteville, AR.

Gramicidin channels are β-helical dimers, but the helix handedness is at dispute (cf. Urry et al., Science 221:1064 [1983] and Arseniev et al., FEBS Lett. 186:168 [1985]). We addressed this question using two enantiomeric gramicidin analogues: gramicidin A^-, a mirror image of gramicidin A that in DPhPC bilayers forms channels with properties similar to those of gramicidin A channels; and gramicidin M^-, in which the Trp residues were replaced by Phe such that the CD spectrum reflects almost exclusively backbone contributions with no Trp interference. Based on CD measurements in DMPC vesicles gramicidin M^- forms left-handed channels (Heitz et al., Biophys. Chem. 24:149, 1986). Gramicidin A^- and M^- channels are structurally equivalent based on hybrid channel experiments. Both peptides thus form left-handed helices, and we conclude that gramicidin A channels in phospholipid bilayers are right-handed β-helical dimers.

M-Pos261

CONDUCTANCE OF [TAU^{16}]GRAMICIDIN. Xian-Zheng Jin and David Busath, Division of Biology and Medicine, Brown University, Providence, RI 02912.

Des(ethanolamine)-taurine^{16}-gramicidin A (TG) forms channels with a compact, negatively charged sulfate at each end. From an electrostatic point of view, the ionic conductance should be increased at low concentrations as a result of an enhanced cation concentration near the entrance caused by the negative charge. At the same time, the negative charge should increase the binding affinity of the cation binding site at the channel entrance which would be reflected as a deeper free energy well in rate-theory modeling. Single channel conductances were studied for [NaCl] ranging between 0.05 and 2.0 M. The single-channel Na+ conductance is higher than that of the normal gramicidin channel (G) at low concentrations but are virtually the same at high concentrations. In terms of Michaelis-Menten parameters, Km(TG)=0.12 mol/liter vs. Km(G) =0.41 mol/liter; Gmax(TG)= 30.2 pS vs. Gmax(G)=33.5 pS. This result is in discord with the predictions of a rate theory model with a fixed central barrier, (Gmax should decrease if the entry-well deepens locally), but consistent with the enhanced-cation-concentration model. A new theory is needed that synthesizes the electrostatic and rate theory approaches.

M-Pos262

DETERMINATION OF TORSION ANGLES IN THE BACKBONE OF THE GRAMICIDIN CATION CHANNEL BY SOLID STATE NMR. Q. Teng, S.M. Pascal, and T.A. Cross. Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306.

Structure determination in hydrated lipid bilayers is being performed by solid state NMR spectroscopy while, the structure in organic solvents is being determined from solution NMR techniques. The structural comparisons are very important to studies of the incorporation of the polypeptide into a lipid environment, since the bilayer samples are prepared from dried organic solutions of gramicidin and lipid. The solid state approach utilizes uniformly aligned samples of single and multiple site labeled gramicidins. These peptides have been prepared by solid phase synthesis. The torsion angles are determined from a set of orientational constraints developed from data that provides the orientation of the nuclear spin interactions with respect to the magnetic field. The orientations of many of the ^{15}N and ^{13}C chemical shift tensors with respect to the molecular bonds in the gramicidin backbone have been determined and the orientation of the dipolar interactions with respect to the molecular frame is known. The analysis of these orientational constraints is very similar to the analysis of distance constraints being used to determine the solution structures from standard 2D high resolution NMR data.

M-Pos263

MOLECULAR DYNAMICS COMPUTATIONS AND SOLID STATE NMR OF THE GRAMICIDIN CATION CHANNEL. S.-W. Chiu*, L.K. Nicholson◊, M.T. Brenneman*, S. Subramaniam#, Q. Teng◊, C. North◊, J.A. McCammon‡, T.A. Cross◊, and E. Jakobsson+. *Dept. of Physiol. and Biophys., Univ. of Illinois, Urbana, IL 61801, ◊Dept. of Chem. and Inst. of Mol. Biophys., Florida State Univ., Tallahassee, Fl 32306, #Dept. of Chem., Univ. of Houston, Houston, TX 77204, ‡Dept. of Physiol. and Mol. Biophys., Baylor College of Medicine, Houston, TX 77030, +Bioengineering Program, Univ. of Illinois, Urbana, IL 61801.

The refinement of macromolecular structures requires both detailed structural data and sophisticated computational techniques. For the transmembrane channel form of the polypeptide gramicidin A an atomic resolution structure has not yet been achieved due to difficulties in preparing peptide/lipid cocrystals that diffract to high resolution. Solid state ^{15}N NMR of single-site isotopically labeled gramicidin A aligned with respect to the magnetic field has recently been used to show that this channel is a right-handed β-helix. Although the ^{15}N chemical shift resonance does not yield a unique orientation for a given site, the frequency defines an allowed orientational space to which various model structures may be compared. Molecular dynamics (MD) computations produce total molecular structures from an approximate starting point structure. It is shown here that the MD structure is in excellent agreement with the atomic resolution solid state NMR data, provided that the MD is started with a right-handed helix. The information provided by NMR and MD are complementary to each other and when applied in a coordinated manner they provide a powerful approach to structure determination in molecular systems that are not amenable to x-ray diffraction.

M-Pos265

TIME-RESOLVED FLUORESCENCE STUDIES OF DIFFERENT GRAMICIDIN CONFORMATIONS USING INTRINSIC TRYPTOPHAN AND LIPID BILAYER FLUOROPHORES

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PA19107

Gramicidin D was used a model protein in which to assess the possibility of using the fluorescence properties of the tryptophans and DPH type lipid bilayer fluorophores to determine conformational responses of the protein to changes in the lipid bilayer environment. After reconstitution as channel and non-channel forms differences in the emission spectra and steady state fluorescence anisotropies of the tryptophans were found reflecting the different environments of the two forms. The fluorescence anisotropy of the tryptophans was insensitive to lipid bilayer properties such as the phase transition (eg DMPC) or different lipid types. Perturbation of the lipid bilayer by ethanol addition was, however, reflected in a decrease in the fluorescence anisotropy paralleling effects on the lipid acyl chains as assessed by DPH.

M-Pos264

A COMPUTATIONAL METHOD FOR DETERMINING THE SIDE CHAIN CONFORMATIONAL STATES OF THE PEPTIDE GRAMICIDIN A. M.T. Brenneman, S.W. Chiu, E. Jakobsson. University of Illinois, Dept. of Physiology and Biophysics, Urbana, IL 61801

We outline here a computational method for predicting the equilibrium distribution of side chain conformers for a protein with a known backbone structure and present preliminary results obtained with this method for the ion channel gramicidin A. This approach takes advantage of a simple analogy suggested by a spin-glass model for proteins which appears to provide an efficient means for searching the entire conformational space of the side chains. Preliminary results indicate that many nearly isoenergetic rotational isomers exist, which we believe may represent conformational sub-states of gramicidin. Several of these side chain conformers are selected to study the interaction between the backbone and side chains with and without a sodium ion at the channel binding site to examine the hypothesis that such rotational isomers can represent states with different functional activity (the "mini-channels").

M-Pos266

FERROELECTRIC-SUPERIONIC ELECTRODIFFUSION MODEL OF THE Na CHANNEL. H. Richard Leuchtag, Department of Biology, Texas Southern Univ., Houston, TX 77004. **Model:** Electrodiffusion modified by nonlinear dielectric equation of state, applied to ion-conducting pathway. Equations imply ferroelectric and paraelectric states. Paraelectric phase assumed to be a superionic conductor of Na^+. **Function in action-potential initiation:** At rest potential, pathway is in ferroelectric state. Threshold depolarization shifts Curie point downward, below temperature, initiating phase transition to superionic state. In this state pathway has conductivity of order 1 S/m, thus is "open." Restoration of rest potential raises Curie point, triggering return to ferroelectric, "closed," state. **Implications:** Hysteresis, seen in axons and Na channels; upper and lower temperature limits to excitability, seen as heat and cold block; others. **TTX block** explained as locking in of ferroelectric phase by TTX. Ferroel. **86**:105-113 (1988).

M-Pos267

NA CHANNEL GATING AND PERMEATION MODELED IN TERMS OF A PHASE TRANSITION OF A S4 TRANSMEMBRANE PEPTIDE CHAIN

K. Benndorf, (Intr. by C. Methfessel), Institute of Vegetative Physiology, University Cologne, F.R.G.

The transmembrane S4/IV alpha-helix of the Na channel protein is treated as thermodynamically independent peptide chain which can transform to an unfolded structure in analogy to the well known helix-coil transition in solution. As special unfolded structure a channel-helix is proposed which forms together with a single file of water molecules plus ion(s) a double-helix while half of the former transmembrane amino acids are moved to the outside. The channel-helix is further stabilized by a nearly perfect fit of the positively charged side chains to polarized regions in the surrounding protein as copies of previous positions of other positively charged side chains. Similar to the gramicidin A channel, the axial dipole moment of the channel-helix is nearby 0 Debye and the water molecules and ions are coordinated by carbonyl oxygens and imine hydrogens. The voltage dependence of such a gating results from the break down of the alpha-helix macrodipole and from the shift of the positively charged side chains in the electric field of the membrane yielding a net gating charge of 2.74 elementary charges per opening which is well in line with recent experimental findings.

M-Pos268

PROPERTIES OF SQUID SINGLE Na CHANNELS IN AN EXTENDED VOLTAGE RANGE

Ana M. Correa and Francisco Bezanilla. Dept. of Physiology, UCLA, Los Angeles, CA

The activity of single sodium channels from the squid giant axon was studied at potentials that include the voltage range at which the activation of macroscopic Na currents becomes maximal. Single channel currents were recorded from outside-out patches, excised from the internal surface of a cut-open axon. To increase the resolution, recordings were made in high external Na, 2 or 4 M NaCl, in the absence of divalents, and at 4 °C. I-V curves were linear within the range studied, i.e., -40 to +40 mV, with slope conductances of 27-30 pS. Channels opened promptly at the onset of the depolarizing pulses and then closed and re-opened several times before inactivating. Intense flickering was frequently observed at potentials as positive as +40 mV. The probability of opening as a function of voltage saturated at potentials more positive than 0 mV. Mean open times (MOT) measured with a bandwidth of up to 8 KHz ranged between 80 and 300 μs and they increased with depolarization up to around 0 mV, decreasing again as the membrane was made more positive. Pronase-treated patches also showed intense flickering without a change in the MOT. These results suggest that the exit from the open state involves a step with a very small voltage dependence, most likely between the open and the first close states. At positive potentials, open time histograms show two components pointing to the existence of two open states.

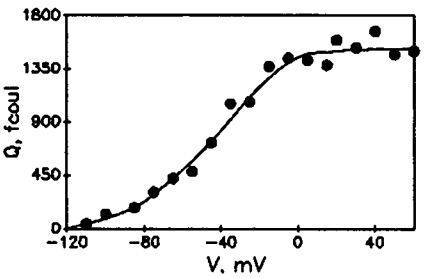
SUPPORTED BY USPHS GRANT GM30376.

M-Pos269

GATING CURRENTS IN ELECTROCYTE Na CHANNELS OF THE ELECTRIC EEL *E. Electricus.*

S. Shenkel and F. Bezanilla. Dept. of Cellular and Molecular Physiol., Yale Univ., New Haven CT, Dept. of Physiology , UCLA, Los Angeles CA and MBL, Woods Hole MA.

Currents associated with the gating of the eel Na channels were measured at ~11° C in cell-attached and dettached 'inside-out' patches from electrocytes of the main electric organ. Ionic currents were eliminated by replacement of Na and K by N-methyl-glucamine and the presence of TTX in the pipette. Linear capacitive and leakage currents were subtracted using a P/4 procedure. Recordings were made with a bandwidth of 20 KHz. Qualitatively, gating currents in eel Na channels appear to be similar to those in other preparations (Fig shows a Q-V of a cell attached patch). A minimum valence of 1.2 ± 0.3 e^- (mean ± SD, 16 measurements from 10 patches) was determined from the foot of the Q-V curves. Inactivation immobilizes gating charge and the time-course and voltage dependence of charge immobilization were similar to those observed for inactivation of ionic currents. The midpoint of the h_∞ curve was around -90 mV.



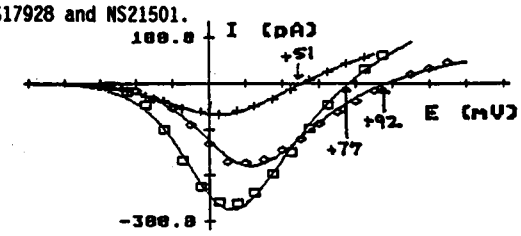
SUPPORTED BY GRANTS GM30376, NS 17928 AND NS21501.

M-Pos270

SELECTIVITY RATIO VARIATION IN ELECTROCYTE Na CHANNELS FROM PATCH-TO-PATCH ON THE SAME CELL

S. Shenkel and F.J. Sigworth. Dept. Cellular & Molecular Physiology, Yale University, New Haven, CT 06510.

'Macroscopic' currents were recorded at 22-24 °C in cell detached 'inside-out' patches from the innervated membrane of electrocytes from the electric eel E. electricus under biionic conditions with equal concentrations of Na outside (pipette) and K inside. In 22 patches from 12 cells, reversal potentials measured in 200 mM solution ranged from +51 to +92 mV corresponding to P_{Na}/P_K of 7.7 to 39.6 (Fig: 3 patches, 3 cells). Reversal potentials differed by as much as 37 mV among patches from the same cell. The range of variability was slightly lower in 400 mM solution with P_{Na}/P_K of 20.0 to 46.5 in 6 patches from 3 cells. Results from several control experiments involving symmetric and asymmetric Na gradients and steady-state inactivation curves have ruled out obvious artifactual sources of error. Such rev. pot. variation may result from simultaneous expression in the same cell of electrocyte Na channel subtypes with different selectivity properties and/or post-translational channel modification. Supported by NIH grants NS17928 and NS21501.



M-Pos271

FIELD-DRIVEN CHANNEL MESSENGER THAT TRIGGERS SODIUM CHANNEL ACTIVATION

Y. Rosenthal and Y. Palti, Rappaport Inst. and Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Ion channel gating involves sequential conformational changes of the channel macromolecule. These include a number of closed states, which are considered responsible for the delay between the beginning of depolarization and current onset. We investigated this delay, as a function of conditioning and test pulse potentials, V, and durations, t, in neuroblastoma cells. The delays ranged between 0 and 500 μs, with τ's in the range of 100-300 μs, depending on V and t. The delays were a linear function of the product: (V*t). Moreover, the delays approached zero when the product reached a specific value. The results are consistent with the existence of a pre-gating process involving the movement of a charged or polar membrane or channel element (FDM) by the electric field. The actual activation begins only after the FDM reaches a specific location, the gate triggering site (GTS), most likely on the channel molecule.

M-Pos272

THE QUANTAL GATING CHARGE OF Na CHANNEL INACTIVATION OBTAINED FROM GATING CURRENTS AND IONIC CURRENT RATE ANALYSIS.

N.G.Greeff and I.C.Forster. Physiologisches Institut, Universität Zürich CH-8057 and Station Biologique, Roscoff, F-29211, France.

We have reported a slow gating current I_g in low-noise recordings in the squid giant axon (Forster and Greeff,J.Physiol.1989,abstr.7/89) and developed an analytical technique allowing to check if it is due to open to inactivated (O-I) transition alone and thus seen as $I_{g,h}$ resulting from a voltage dependent inactivation gate with quantal charge q_h. From an isochronic plot of the experimentally obtained I_g vs $\dot{g}_{Na}$ the ratio $I_g/\dot{g}_{Na}$ approaches a common limiting slope in the later phase of inactivation and for $V_p > -10mV$. We concluded that only in this region the slope (1.54e^-/pS at 5°C) corresponds to q_h/δ and knowing δ the figure of 1.25 e^- for q_h was obtained. Below -10mV the slope is larger indicating additional I_g from C-O transitions.

We now report the implications for the rate analysis of Na current. From the above constraints the apparent τ_h is given by the O-I transition alone only for $V_p > -10mV$. Using conventional rate analysis from $\ln(\tau_h)$ vs V_p, $q_h \cdot d = 0.52\ e^-$ (d = fractional distance of energy barrier betwen O and I). From $\tau_{recovery}$ at potentials below -100mV we found $q_h(1-d) = 0.67e$. Then the total q_h is 1.19 e^- corroborating the 1.25 e^- obtained by the totally different isochronic method. In conclusion i) a rather accurate figure for the quantal inactivation gating charge is obtained which ii) can be used to test molecular dynamical models and iii) the divergent figures for q_h from previous rate analyses appear to result from uncertainties about the constraints reported here. (Supported by Swiss NF grant 3.143-0.85).

M-Pos273

CURRENT THROUGH SINGLE TTX-SENSITIVE AND TTX-RESISTANT Na CHANNELS IN FROG DRG CELLS.

Donald T. Campbell, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Vertebrate DRG cells exhibit two subtypes of voltage-gated Na channel: rapidly inactivating channels blocked by TTX at nM concentrations and slowly inactivating channels insensitive to TTX up to 100 μM. Large cells contain only TTX-sensitive channels; small cells may contain both subtypes. Single channel currents were measured from cell-attached patches in frog DRG cells. The pipette contained (in mM) 300 NaCl to maximize Na current amplitudes, 0.3 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES at pH 7.4. Currents were elicited by +30 to +35 mV steps from rest. In 5 patches from relatively large cells studied with 0 TTX in the pipette, single channel currents averaged 1.9 pA. In 4 patches from small cells studied with 1 μM TTX in the pipette, single channel currents averaged 0.9 pA. Thus, in resistant cells, where the TTX-resistant current averages ~1/3 of the total Na current, an average of~1/2 the Na channels are TTX-resistant. Supported by USPHS.

M-Pos274

SODIUM CURRENTS IN PANCREATIC ISLET B CELLS. D. Pressel and S. Misler, Washington Univ., St. Louis, MO.

B cells produce action potential (AP) trains in response to stimulatory concentrations of glucose. The role of Na^+ currents in AP generation is unclear. In current clamp mode using the "nystatin perforated patch" technique, we have recorded Na^+ APs in both canine and human B cells in response to depolarization to Vm=-50 to -40 mV. In companion conventional "whole cell" voltage clamp experiments on canine B cells, the underlying Na^+ current displays steep voltage dependent activation and inactivation over the range -50 to -40 mV. The Na^+ current is blocked by TTX with a K_I=3.2 nM and has a reversal potential which changes with $[Na^+]_o$ as predicted by the Nernst equation. These Na^+ currents may permit B cells to initiate and maintain electrical activity at levels of membrane potential insufficent to open high-threshold, voltage-sensitive Ca^{2+} channels. Support: NIH DK37380 and HL07275.

M-Pos275

GLYCOSYLATION INHIBITORS AFFECT SODIUM CHANNEL SORTING. W.F. Gilly & M.T. Lucero, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950. Na channels are present at high density in squid giant axon but are absent from its somata in the giant fiber lobe (GFL) of the stellate ganglion. When GFL cells are dispersed onto culture dishes, the axon stump can remain viable for many days in vitro. Over this time swellings (bulbs) form at the axonal termination. Na channel distribution was studied in vitro using whole cell voltage clamp on both bulbs and somata after cutting the axons. After 6 days, Na conductance (GNa) in bulbs was 10.2 ± 0.4 nS/pF vs 0.6 ± 0.1 in somata. Tunicamycin, a glycosylation inhibitor, reduced bulbar GNa to 2.3 ± 0.9 nS/pF, but it had no effect on the axotomized somata or on 'axonless' cells which had failed to retain an axon. K conductance was unaltered by tunicamycin treatment. Thus, glycosylation of Na channels may be necessary to achieve the high channel density appropriate for axonal membrane.

M-Pos276

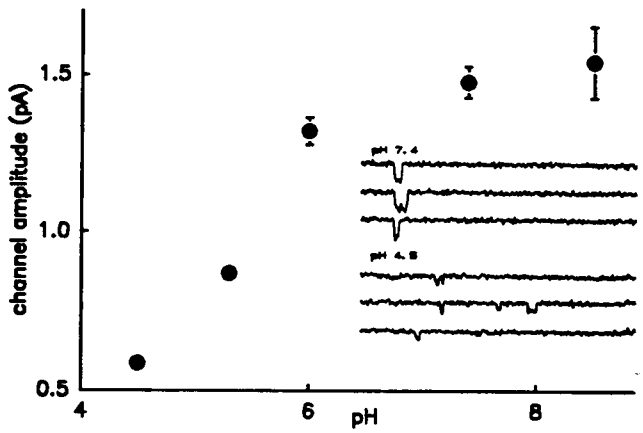
CHIRIQUITOXIN AND IONIC CURRENTS IN FROG SKELETAL MUSCLE FIBERS. L. Yang and C. Y. Kao. Department of Pharmacology, SUNY Downstate Med Ctr, Brooklyn, NY 11203.

Yotsu and Yasumoto have isolated some new chiriquitoxin (ChTX) from the skin of the Costa Rican frog *Atelopus chiriquensis*, and have determined its structure. ChTX differs from TTX in having $-CHOHCHNH_2COOH$ on C-11, in place of $-CH_2OH$. On voltage-clamped (vaseline-gap method) skeletal muscle fibers of frogs, the ED_{50} for reducing I_{Na} is 3.80 nM for TTX and 3.76 nM for ChTX at pH 7.25. In contrast, the ED_{50}'s of two other analogs modified in the same region, 6-epi TTX and 11-deoxy TTX, are 134 nM and 437 nM. The high potency of ChTX must be ascribed to the new substituent groups. The pH dependence of the potency of ChTX differs from that of TTX: at pH 8.25, ED_{50} is 4.32 nM for TTX and 2.14 nM for ChTX; at pH 6.50, it is 3.75 nM for TTX and 5.62 nM for ChTX. Also, ChTX slows the activation of the fast I_K, and reduces the steady-state (20 ms) I_K. Although the I_K effects are variable and complex, ChTX is clearly a useful agent for characterizing the TTX binding site on the Na^+-channel, and possible similar site(s) on some K^+-channels. (Supported by U. S. Army contract DAMD17-87-C-7094 and NIH grant NS14551).

M-Pos277

EFFECTS OF EXTERNAL pH ON CARDIAC SODIUM CHANNELS. J.F. Zhang and S.A. Siegelbaum. Dept. Pharmacol. Columbia U. NY NY 10032

Low external pH reduces sodium current in nerve and muscle, although the effects on single sodium channel currents have not been studied in detail. Using single channel recording from cell-attached patches on guinea pig ventricular cells, we find a dose-dependent reduction in single sodium channel amplitude upon lowering the pH in the patch pipette (Fig.). This is accompanied by a decrease in channel open time (at -40 mV, mean open time=0.90 ms at pH 7.4 and 0.26 ms at pH 4.5) and slowing of channel opening that may reflect a shift in voltage-dependent gating to more negative potentials, consistent with a reduction in negative surface charge.



M-Pos278

BLOCKING EFFECTS OF 711389-S ON THE SODIUM CURRENT IN ISOLATED GUINEA-PIG VENTRICULAR MYOCYTES

I. Hisatome, S. Matsuoka, J. Miyamoto, H. Kotake, H. Mashiba, and R. Sato*, Tottori University, Yonago and Kinki University*, Osaka, Japan.

We studied the effects of 711389-S on I_{Na} using the whole cell voltage clamp. Experiments were conducted at 17°C utilizing 10 mM $[Na]_o$ and $[Na]_i$. 711389-S blocked I_{Na} under steady-state condition (Kd_{app} = 5 uM at HP = -90 mV: Kd_{rest} = 30 uM: Kd_i = 2.4 uM) and shifted Na^+ availability curve to hyperpolarizing direction without changes in slope factor. 711389-S blocked I_{Na} in use-dependent fashion. Brief single or train of brief conditioning pulse (2-10 msec) could produce use-dependent block as well as long single or train of long conditioning pulse (30-300 msec). These results suggest that 711389-S had higher affinity to inactivated state than rested state under steady-state condition and had higher affinity to activated state during pulse as well as inactivated state, making channels unavailable for conduction upon activation.

M-Pos279

Block of different cloned Na^+ channels by internal Mg^{2+} and external Ca^{2+}

Michael Pusch (Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen)

Rat brain type II Na^+ channels and two mutants of type II, designed by Stühmer *et al.* (Nature **339**: 597-603, 1989), were expressed in *Xenopus* oocytes for patch recording from inside-out patches. The long open times of the mutant "cZ-2" allowed a characterization of the block of outward currents by internal Mg^{2+} at the single-channel level. It was found that Mg^{2+} decreases the channel amplitude with half block at V=0 in 1.8 mM Mg^{2+} and with an e-fold increase of the block per 39 mV. Additionally Mg^{2+} increases the noise level of the channel current indicating that Mg^{2+} acts as a fast blocker.

The mutant "K226Q" which lacks one positive charge in the S4-segment of repeat I was compared with wildtype channels for its sensitivity to block by internal Mg^{2+} and external Ca^{2+}. A comparison of macroscopic tail-currents did not reveal different sensitivities to internal Mg^{2+}. However, the concentration- and voltage-dependence of the block by external Ca^{2+} was different in K226Q channels compared to the wildtype. These findings are interpreted in terms of current models of the transmembrane topology of the Na^+ channel.

M-Pos280

PROPERTIES OF A FAST DISPLACEMENT CHARGE RECORDED FROM THE SQUID GIANT AXON.

I.C. Forster and N.G. Greeff, Physiologisches Institut, Universität. Zürich, CH-8057, Switzerland and Station Biologique, Roscoff,F-29211, France.

The fast displacement charge movement previously reported by us in squid giant axons (Biophys. J., 55,316a) has been studied further with improved time resolution (0.5 μs/point) and precautions taken to ensure smooth settling of the true membrane potential. Using a voltage step protocol having a variable-width depolarizing step to +20 mV, we have recorded the displacement charge which flows following an off-step to -100 mV. Analysis of these data show that the amount of fast charge returned (1-2 nC/cm^2) and its relaxation time constant (10-20 μs) are independent of the depolarizing pulse width and are in addition unaffected by the presence of an inactivating pre-pulse to 0 mV for 20 ms. The Q-V and τ-V curves for this component exhibit the characteristics normally associated with a two state process. These results allow us to exclude distortion of the main gating charge movement by hidden membrane overcompensation as a source for this fast component and furthermore instrumentation asymmetries account for < 20% of the charge involved. This substantiates our conclusions that it is a displacement charge acting independently and in parallel with the main sodium channel gating charge.

(Supported by Swiss NF grant 3.143-.0.85).

M-Pos281

NONLINEAR RELATIONSHIP BETWEEN $V^{\cdot}_{max}$ AND h_∞ DURING REPETITIVE ELECTRICAL ACTIVITY OF SKELETAL MUSCLE.

P.P. Nánási, M. Dankó, A. Varró & D.A. Lathrop. Dept. of Physiology, Univ. of Debrecen, Debrecen, Hungary. Dept. of Pediatrics, Univ. of Cincinnati, Cincinnati, OH 45229. (*Intro. by* Dr. Taitzer Wang).

The relationship between the maximum upstroke velocity ($V^{\cdot}_{max}$) of propagating action potentials during repetitive electrical activity and steady-state Na^+ channel inactivation (h_∞) was examined in isolated frog skeletal muscle using voltage clamp and standard microelectrode techniques. A volley of repetitive discharges was evoked by a single depolarizing current pulse applied in the presence of 1 μM cevadine while $[K^+]_O$ was varied between 0.5 and 5 mM. At each value of $[K^+]_O$, $V^{\cdot}_{max}$ of the initial stimulated action potential upstroke and of the 4th repetitive spike in the volley was plotted as a function of it's take-off potential and compared to the h_∞ curve obtained from voltage clamped muscle fibers. These experiments revealed nonlinearities between h_∞ and $V^{\cdot}_{max}$. The results of this study suggest that the observed nonlinearity between $V^{\cdot}_{max}$ and h_∞ reflects the behavior of normal frog skeletal muscle Na^+ channels rather than that of cevadine-modified channels.

[Supported by a grant from the Hungarian Ministry of Health (Grant No. 7/053 and OTKA 2/666/88)]

M-Pos282

ACTIVITY OF Na CHANNELS IN RAT LACTOTROPES AND ITS RELEVANCE FOR PROLACTIN SECRETION.
G. Cota, J. Horta, A. Marin and M. Hiriart. Dept. of Physiol., CINVESTAV-IPN, and Dept. of Neurosciences, IFC-UNAM, Mexico, D.F.

Prolactin (PRL) secretion from individual lactotropes in cell cultures of the male rat adenohypophysis was studied with the reverse hemolytic plaque assay. In this assay, the extent of hemolysis (plaque area) around a cell is a measure of the amount of its PRL secretion. Adding 2 μM TTX to the culture medium decreased by 70-80% the total amount of PRL released from the pituitary cells during a 1-hour period at 37°C. In addition, TTX shifted the frequency distribution of plaque areas from bimodal (small and large modes) to unimodal (small mode): TTX may preferentially suppress a subpopulation of lactotropes secreting large amounts of PRL per unit time. Whole-cell patch clamp experiments confirmed that most of the lactotropes expressed functional, TTX-sensitive Na channels. The maximum amplitude of I_{Na} (normalized by cell capacitance) was 6-7 times bigger in large-plaque lactotropes than in small-plaque cells. The fraction of Na channels available to open at the HP (-80 mV) was close to 1.0 in both supopulations of lactotropes.

M-Pos283

VOLTAGE SENSITIVE ION CHANNELS COULD BE GATED BY ELECTRON TRANSFER FROM TYROSINE TO TRYPTOPHAN.
Chyuan-Yih Lee, Chemical Dynamics Co., 9560 Pennsylvania Avenue, Upper Marlboro, MD 20772.

Tyrosine(Y)-tryptophan(W) is the only amino acid pair capable of having intramolecular long range electron transfer. The sequenced voltage-gated channel proteins **ALL** contain the Y-W pair separated by four other amino acids. The YXXXXW segment is located before the S3 segment of both Na channel (domains II, III and IV) and L-type Ca channel (domains I and IV), and before H1 segment of K channels. Electron transfer from Y to W becomes more efficient (thus higher probability for channel's opening) when there is more electron deficiency (oxidized) on the indole NH of tryptophan. This may explain the action of BTX and other alkaloid neurotoxins, whose **"oxygen triad"** has been demonstrated to be essential. The oxidizing F_3 group of BAY K 8644 may also account for its agonist action on the L-type Ca channel. Involvement of the YXXXXW segment can be tested by antibody binding, functional expression with site specific mutagenesis, or comparison of tryptophan fluorescence with that modified by drugs.

M-Pos284

VERATRIDINE AND BTX-MODIFIED Na+ CHANNELS FROM LOBSTER PERIPHERAL NERVES IN PLANAR LIPID BILAYERS.
C. Castillo Whittembury*, R. Villegas* & E.Recio-Pinto**. **Depts. Anest. & Phys. Cornell U. Med. Coll., NY, NY10021. *IDEA Caracas 1015-A, Venezuela.

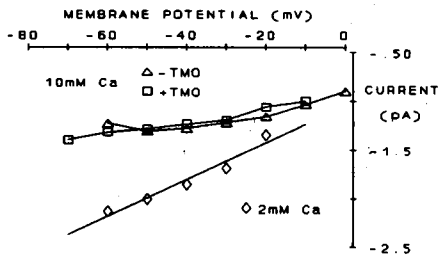
Na+ channel activity was studied in 0.5M NaCl[1]. The I-V curves for veratridine-modified channels with the predominant conductance were linear and symmetrical. The slope conductance (10pS) was similar to that of channels from muscle[2] and eel electroplax[1,3] but higher than that of mammalian brain channels[3]. The I-V curves for BTX-modified channels were linear and symmetrical. The slope conductance (16pS) was lower than those of BTX-modified channels (24-29pS) from central nervous[4] and muscle derived[1,5] tissues. The channel activation was voltage dependent and similar for veratridine and BTX-modified channels. This behavior was comparable to that of veratridine-modified eel channels[3] but not[1,4,5] to that of other BTX-modified channels. The PNa/PK ratio (2.2) measured under mixed ionic conditions was similar for BTX and veratridine-modified channels.

[1]Recio-Pinto et al.,1987 JGP 90: 375; [2]Garber & Miller, 1987 JGP 89:459; [3]Duch et al., 1989 JGP press; [4]Hartshorne et al., 1985 PNAS 82: 240, Green et al.,1987 JGP 89: 873, Behrens et al., 1989 JGP 93:23; [5]Moczydlowski et al.,1984 JGP 84:665.

M-Pos285

TRIMETHYLOXONIUM (TMO) FAILS TO PREVENT Ca^{2+} BLOCK OF CARDIAC Na^+ CHANNELS.
S.C. Dudley, Jr. and C.M. Baumgarten
Medical College of Virginia, Richmond, VA 23298

In nerve, skeletal muscle and heart, Ca^{2+} blocks open Na^+ channels at a site within the membrane field. Carboxyl-specific O-methylation of neural and skeletal muscle Na^+ channels by TMO prevents Ca^{2+} block and reduces binding of TTX and STX. We tested whether TMO modification would prevent Ca^{2+} block in rabbit ventricular myocytes. Myocytes were incubated on ice for 10 min in (mM): 50 TMO, 90 KCl and 100 HEPES (initial pH=8.08, final pH=7.31±0.03). Unitary Na^+ currents were measured at 10°C in cell-attached patches. Control conductance (γ) was 18.8 pS with 280 mM Na^+ and 2 mM Ca^{2+} in the pipette (n=3) and was reduced to 6.9 pS with 10 mM Ca^{2+} (n=7). In contrast to TTX-sensitive Na^+ channels, Ca^{2+} block of ventricular Na^+ channels was **not** prevented by TMO; in 10 mM Ca^{2+}, γ was 6.4 pS (n=5). This finding and the relatively high sensitivity of cardiac Na^+ channels to block by Cd^{2+} and Zn^{2+} suggest that the mouth of the cardiac Na^+ channel differs from that in nerve and skeletal muscle.



M-Pos286

EFFECTS OF LIDOCAINE AND CHLORPROMAZINE ON THE TETRODOTOXIN RESISTANT CURRENT OF MAMMALIAN DORSAL ROOT GANGLION NEURONS. M.L. Roy and T. Narahashi (Intro. by N.T. Slater). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Acutely dissociated dorsal root ganglion neurons isolated from 5-10 day old rats have been found to generate both tetrodotoxin-resistant (TTX-R) and tetrodotoxin-sensitive (TTX-S) sodium currents in varying proportions. Whole cell patch clamp techniques were used to record sodium currents with or without 10 μM TTX at 13-18°C to elucidate the mechanism of action of lidocaine (LDC) and chlorpromazine (CPZ) on both types of currents. The majority of sodium currents showed a relatively slow time course and was composed largely of the TTX-R current. In some cases, however, a much faster sodium current was recorded and composed of the TTX-S current only. LDC (100 μM) reduced TTX-R current by more than 50%, whereas CPZ (10 μM) inhibited the current to a negligible extent. TTX-S current appeared to be sensitive to both LDC and CPZ. The differential effects of CPZ and LDC on the two types of sodium currents may provide insight into their therapeutic and side effects on the CNS, respectively. Supported by NIH grant NS14143.

M-Pos288

KINETICS OF THE INWARD CURRENT OF HUMAN RETINOBLASTOMA BEFORE AND AFTER IN VITRO INDUCED DIFFERENTIATION. M. Gomez, G. Waloga and E. Nasi, Dept. of Physiology, Boston University School of Medicine.

Y-79, a cell line from human retinoblastoma, can be induced to differentiate in vitro towards various retinal cell types. We have previously shown that in the tumoral state, Y-79 cells have a voltage-dependent inward current (I_i) carried by both Na^+ and Ca^{2+}. After neuronal differentiation by plating on a laminin and poly-D-lysine substrate, I_i is carried by Na^+ exclusively, and can be completely blocked by TTX. Here we show that differentiation also results in profound changes in the activation and the kinetics of I_i. In the undifferentiated cells, the activation threshold is -15 mV and the maximum current amplitude is attained at 10-15 mV, with a time-to-peak of about 10 ms. The current inactivates with a single time constant of 15-25 ms. Differentiation shifts the threshold to more negative values by 15 mV, and the peak amplitude occurs around -5 mV. The kinetics of both activation and inactivation become markedly faster: the conductance opens fully within 1 ms at 0 mV, and the current then declines with τ = .8-1.2 ms. The study indicates that Y-79 is a valuable model system to investigate functional aspects of cell differentiation in the human retina. Supported by NIH grants EY04777 and EY07559.

M-Pos287

INTERNAL APPLICATION OF SP19 ANTIBODY IN SQUID AXONS. J. TANGUY AND J. Z. YEH. DEPT OF PHARMACOL, NORTHWESTERN UNIV., CHICAGO, IL.

From amino acid sequences and site-directed antibody studies, the SP19 peptide corresponding to the residues 1491-1508 of the α-subunit of the rat brain Na channels R_{II} was found to be highly conserved in a wide range of Na channels in different species. The SP19 peptide contains the target sites for proteases and N-bromoacetamide, both of which remove the fast Na inactivation. The antibody against this peptide (Ab_{SP19}) slows the fast inactivation when applied through the patch pipette to rat muscle cells (Vassilev et al., 1988). Both observations suggest a direct role for the SP19 in the fast inactivation. Taking advantage of the internal perfusion technique, we directly applied Ab_{SP19} in voltage-clamped squid axons. When internally perfused, up to 2 hr at 10-12 °C, Ab_{SP19} did not show any appreciable effect on the voltage-dependency of the time constant of the decay of Na current (τ_h) and of the steady-state inactivation (h_∞). The activation process was not significantly altered. After the axon had been exposed to Ab_{SP19}, N-Bromoacetamide and pronase were as effective in removing the fast Na current inactivation as in the control. Thus, Ab_{SP19} did not affect the fast inactivation process in squid axons, suggesting that the fast inactivation in squid Na channel and in mammalian Na channel might be structurally different. (Supported by NIH grant GM 24866).

M-Pos289

DIFFERENTIAL GATING OF TTX-SENSITIVE AND -RESISTANT Na^+ CURRENTS IN BULLFROG SENSORY NEURONS. Xiaotao Guo and Gary Strichartz, Anesthesia Research Labs, Brigham and Women's Hospital, Dept. of Biol. Chem. & Molec. Pharmacol., Boston MA, 02115

I(Na) in R. catesbeiana sensory ganglion cells, under whole-cell clamp, was separated into TTX-sensitive (S) and TTX-resistant (R, in 0.1 or 1 μM TTX) classes. Ringer's contained (mM): 100 NaCl, 14 TEACl, 1.8 $CaCl_2$, 2.5 KCl, 10 MOPS/NaOH, pH 7.2; internal sol'n=10 NaCl, 105 CsCl, 10 MOPS/NaOH, pH 7.2 (T=23-24.5°C). G(Na) activation differed between the two classes [V0.5=-30.7±6.2 mV (S,n=4), -18.9±2.5 (R,4); k=7.2±0.9 mV/e (S,4), 6.6 ± 0.4 mV/e (R,4)], as did inactivation [h-inf. (50 ms) V0.5 = -60.6±1.8 mV (S,4), -40.3±2.7 mV (R,5); k=6.8±0.8 mV/e (S,4), 11.7±0.8 mV/e (R,5)]. Fast inactivation developed differentially, yet biphasically for both S & R [(at 0 mV) S:τ1=0.37±0.062 ms (89%),τ2=2.70±0.73 ms (11%) n=5, vs R: τ1=1.32±0.20 ms (65%), τ2=7.65±2.07 ms (35%) n=5], and recovered exponentially, and differentially [(at-90 mV) S:τ=2.70±0.38 ms (n=5) vs R: 6.96±0.66 ms (n=5)]. Such differences account in part for an intrinsic, differential use-dependent depression of I(Na).

M-Pos290

ISOLATION OF A HUMAN SKELETAL MUSCLE Na^+ CHANNEL cDNA CLONE. A.L. George, R.G. Kallen, and R.L. Barchi (Intro. by S.W. Englander). Mahoney Institute of Neurological Sciences, University of Pennsylvania, Philadelphia PA 19104

A fetal human skeletal muscle cDNA library containing 10^6 independent recombinants has been screened under low stringency conditions using antisense probes generated from rat skeletal muscle subtype I sodium channel (SkM1) cDNA clones. Using either a partial length probe of SkM1 including domains II - IV and the C-terminus, or a full-length probe of the entire coding region, approximately 20 partial length (0.6 - 4.0 kb) cDNA clones were isolated including one containing a 3.6 kb fragment (C6b). The complete nucleotide sequence of cDNA clone C6b been determined and compared with the two known rat skeletal muscle Na^+ channel sequences (SkM1, SkM2). C6b encodes 435 amino acids corresponding to residues 1395 - 1840 of rat SkM1 and includes a portion of domain IV and the entire C-terminus. In addition, C6b has a 2.1 kb 3'-untranslated region which includes a consensus polyadenylation signal sequence (AATAAA) and a poly - (dA) tail. C6b is closely related to rat SkM1 with only 6 substitutions of 200 amino acid within domain IV and 91% overall amino acid sequence identity. Comparison of C6b with SkM2, a second Na^+ channel isoform cloned from rat skeletal muscle, reveals only 78% amino acid sequence identity and 34 amino acid substitutions within domain IV. C6b appears to encode a portion of a human skeletal muscle Na^+ channel homologous to rat SkM1, the major TTX-senstive channel of innervated adult muscle.

M-Pos292

SLOW INACTIVATION OF CARDIAC Na CHANNELS IN EXCISED MEMBRANE PATCHES.

Carmen Valenzuela & Paul B. Bennett, Department of Pharmacology, Vanderbilt University, Nashville, TN

Single Na channels were investigated in membrane patches excised from enzymatically dispersed guinea pig ventricular myocytes. Channel gating was studied after removal of inactivation by the specific endopeptidase, α-chymotrypsin (0.3 mg/ml). Brief exposure times (< 5min) appeared to minimize effects other than the removal of fast inactivation as evidenced by unaltered single channel conductances and latencies to first openings. Open times were predictably lengthened and were voltage dependent. The results were modeled by a 4 subunit activation model (Bennett, Balser & Hondeghem, 1989). Slow inactivation was investigated using 1 minute pre-pulse depolarizations after enzymatic removal of fast inactivation. When fast inactivation was intact, channels completely inactivated within 20 msec at 0 mV, and recovered within 500 msec at -120 mV. Long prepulses after removal of fast inactivation increased blank sweeps and lowered opening probability in sweeps with openings, suggesting the slow inactivation process was not destroyed by the enzyme. Supported by NIH HL40608 and The American Heart Association.

M-Pos291

ACTIVATION UNBLOCK OF CARDIAC Na^+ CHANNELS WITHOUT CHANNEL OPENING.

Takafumi Anno, Paul B Bennett, Luc M Hondeghem, & Dirk J Snyders. Vanderbilt University, Nashville,TN

Use-dependent unblock (UdUB) has been shown to be associated with Na^+ channel opening. It is possible that activation has many substates, only one of which may manifest channel opening. We tested whether activation without channel opening can also induce UdUB. Cardiac Na^+ currents (I_{Na}) were recorded from isolated guinea-pig myocytes in whole cell voltage clamp mode at 17 °C. In the presence of flecainide (6μM), nearly 95% block was established by a train of short pulses. Following maximum block induction the membrane was hyperpolarized (-180 mV) to maximize the availability for UdUB. Next a subthreshold depolarization train from -180mV (SDTr: 50 msec pulse, 10 Hz, N=18) was imposed and followed by the test pulse (-10 mV after 2 sec at -120 mV). A SDTr with pulses more negative than -85 mV, did not induce unblock. Between -80 to -60 mV however, the SDTr produced a significant unblock in comparison with a SDTr to -100mV (ΔI_{Na}= 0.32±0.11 and 1.6±0.4 nA at -80 and -60 mV respectively, n=7, $p<0.05$). Over this voltage range, no I_{Na} was recorded in control nor drug conditions. We conclude that sodium channel blockers can unbind from the channel upon subthreshold depolarizations, which suggests the existence of activated states without channel opening.

M-Pos293

STEREOSPECIFIC BLOCK OF CARDIAC Na^+ CHANNELS BY BUPIVACAINE.

C Valenzuela, PB Bennett & LM Hondeghem. Vanderbilt University. Nashville TN 37232.

Na^+ currents were recorded in isolated guinea pig cardiac myocytes using the whole-cell configuration of the patch-clamp technique. The effects of the two optical isomers of bupivacaine, R(+) and S(-), were studied at 10 μM. A single long depolarization yielded extensive block, while trains of short depolarizations were less effective in reducing the Na^+ current. Thus, both isomers preferentially blocked inactivated Na^+ channels and exhibited relatively little block during activation. In the absence of activations, the kinetics of development and recovery from block were similar. However, the R(+) isomer induced substantially more steady state block than the S(-) isomer. In terms of the modulated receptor hypothesis, this could only occur if activation unblocking were larger for the S(-) than for the R(+) isomer. Following block of more than 95% of the channels, a single activation from -180 mV resulted in 64±0.3% of the control Na^+ current for the S(-) isomer, but only in 32±18% for the R(+) isomer. The R(+) isomer induced a larger voltage shift of channel gating than the S(-) isomer. We conclude that bupivacaine interactions with cardiac Na^+ channels are stereospecific and the different potency of the two isomers result from differences in the degree of activation unblocking and the magnitude of the voltage shift in gating kinetics of drug associated channels.

M-Pos294

MUSCLE SODIUM CHANNEL TERTIARY STRUCTURE PROBED WITH PROTEOLYSIS IN VITRO. S. Zwerling, S. Cohen, and R. Barchi, Mahoney Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The tertiary structure of the skeletal muscle sodium channel was probed in vitro by determining regions of sensitivity to the proteases chymotrypsin, trypsin, and V-8 protease. Channel fragments were identified with antibodies to defined segments distributed along its primary structure. Time-dependent proteolysis was followed with channel protein in either membrane fragments or detergent-phospholipid micelles, as well as with denatured channel. While all enzymes rapidly and completely degraded the denatured channel, proteolysis in membranes and micelles occurred in discrete and reproducible steps that were the same in either system. Although the MW of intermediates varied slightly with the protease used, their sequence of appearance was the same for all enzymes indicating that the observed pattern was determined by the accessibility of segments within the channel's tertiary structure to these soluble enzymes rather than by the substrate preference of a given enzyme alone. No major change in channel organization appears to occur after solubilization in nonionic detergents. Consistent with earlier studies of endogenous proteolysis, highly accessible sites include loops linking the internal repeat domains as well as the amino and carboxyl termini, while individual repeat domains are resistant to proteolysis unless the protein is denatured. Unlike our previous observations on endogenous proteolysis, the 3-4 interdomain is easily cleaved by exogenous proteases.

M-Pos295

ION CONDUCTION AND KINETIC PROPERTIES OF BTX-MODIFIED SODIUM CHANNELS FROM FROG MUSCLE MEMBRANES. David Naranjo, Osvaldo Alvarez, and Ramón Latorre. Departamento de Biología, Facultad de Ciencias, Universidad de Chile and Centro de Estudios Científicos de Santiago, Santiago, Chile.

Membrane preparations from frog skeletal muscle and enriched in transverse tubules were obtained in the presence (PI-TT) or in the absence of protease inhibitors (AI-TT). Sodium channels were incorporated into planar bilayers in the presence of 200 nM batrachotoxin (BTX). When PI-TT preparations were used, sodium channels exhibit a 16 pS conductance in 200 mM NaCl. Infrequently, we also observed a conductance substate of 11 pS. When AI-TT preparations were used the most probable conductance state is that of 11 pS. The current vs $[Na^+]$ curves for both conductance states have the same K_D but different I_{max}. In biionic conditions, both conductance states show $P_{Li}/P_{Na} \approx 0.8$. Ionic conduction through the channel was modelled using energy barriers. This approach indicates that the differences between the two conductance states resides in the height of the barriers and not in well deep. Kinetic analysis show that both conductances states have at least two open states and three close states. Correlation analysis indicates that long closed durations are flanked by short openings and short closed durations are flanked by long openings. These results are not consistent with linear kinetic models for the gating of BTX-modified sodium channels. (Supported by NIH and Fondecyt 451/88).

M-Pos296

MUSCARINE(M)-INDUCED RELEASE OF INTRACELLULAR Ca^{+2} RESULTS IN COMPLEX EFFECTS ON 2 Ca^{+2}-DEPENDENT K^+-CURRENTS IN SINGLE RAT CHROMAFFIN CELLS A. Neely & C.J. Lingle*, Dept. Anesthesiology, Washington Univ. Sch. of Medicine., St. Louis, MO 63110

Whole-cell current and intracellular Ca^{+2} were separately measured to study the effect of muscarinic receptor (mAChR) activation on single rat chromaffin cells.

1. Short (1-5 sec) or prolonged (>30 sec) puffs of M (2-15 μM) transiently increase $[Ca]_i$. The $[Ca]_i$ increase persists following short exposure to Co^{+2} or Ca^{+2}-free saline.

2. Voltage steps activate two components of Ca^{+2}-dependent K^+-current: a voltage-independent, apamin- and curare-sensitive current (SK) and a voltage-dependent, TEA-sensitive current (BK). Maximal g_{BK} and g_{SK} are similar.

3. Puffs of M activate both SK and BK in a manner consistent with the transient elevation of $[Ca]_i$. However in some cells a marked transient suppression of BK can occur coincident with or just following the elevation of $[Ca]_i$. BK suppression is washout-sensitive disappearing at a time when activation of SK and BK current is well-maintained. (Support: Florida Affiliate of AHA and NIH DK-37109.)

M-Pos297

N-ETHYLMALEIMIDE UNCOUPLES MUSCARINIC RECEPTORS FROM ACETYLCHOLINE-SENSITIVE POTASSIUM CHANNELS IN BULLFROG ATRIUM. T. Nakajima, H. Irisawa and W. Giles. University of Calgary, Calgary, Canada.

The effect of N-ethylmaleimide (NEM), a sulphydryl alkylating agent, on the acetylcholine-activated K^+ current, $I_{K(ACh)}$, has been studied in bullfrog atrial cells using a whole-cell voltage clamp technique. Addition of NEM (5×10^{-4} M) produced a time-dependent complete block of $I_{K(ACh)}$. Intracellular dialysis of guanosine-5'-O-(3-thiotriphosphate) (GTPγS, 5-10 x 10^{-4} M), a non-hydrolysable GTP analogue, gradually activated $I_{K(ACh)}$ even in the absence of acetylcholine, due to a GTPγS-induced dissociation of G-proteins into subunits which can directly activate $I_{K(ACh)}$. After the GTPγS effect developed, NEM failed to inhibit it, indicating that NEM likely does not act on the dissociated G-protein(s) subunits and/or on the K^+ channels. When NEM was applied prior to GTPγS, it markedly reduced this GTP-dependent K^+ current, perhaps by preventing the formation of G-protein subunits. In NEM-treated cells, the stimulatory effect of isoproterenol on I_{Ca} remained, but the muscarinic inhibitory action of ACh on I_{Ca} was completely abolished.

M-Pos298

MULTIPLE GENES CONTRIBUTE TO K^+ CHANNEL DIVERSITY IN THE MOUSE KG Chandy, CB Williams, RH Spencer, B Aguilar, S Ghanshani, G Chandy, B Tempel$^+$, GA Gutman (Intro. by ME Barish). UC Irvine & UW Seattle$^+$.

To understand the molecular mechanisms responsible for K^+ channel diversity in mammalian cells we isolated 30 genomic clones from mice that hybridize with two K^+ channel cDNAs, MBK1 and K41. Ten clones have been characterized, revealing at least two families of K^+ channel genes. One family comprises 3 related voltage-gated K^+ channels (MK1, MK2, MK3) which are encoded at distinct genomic loci. The coding regions of MK1, MK2 and MK3 exist as single, uninterrupted exons in the mouse genome, precluding the generation of multiple forms of the protein by alternative RNA splicing. The deduced amino acid sequences show remarkable conservation except in the 3 extracellular loops. A fourth gene, MK4, differs significantly from the MK1 family, both in nucleotide sequence and in the presence of at least 1 intron in the region encoding the S1 membrane spanning segment. The region extending from S1 to the 3' end is contained in a single exon. All four genes differ dramatically from the *Shaker* K^+ channel locus in *Drosophila*, which contains 21 exons spanning 130 kb. Thus, the expression of multiple genes contributes to the generation of K^+ channel diversity in mice.

M-Pos299

UNITARY PROPERTIES OF A DELAYED RECTIFIER K-CHANNEL EXPRESSED IN MAMMALIAN CELLS AND OOCYTES. *D.E. Logothetis, E.R. Liman, G.Koren, B. Nadal-Ginard and P. Hess.* Harvard Medical School, Boston, MA 02115.

We have expressed a delayed rectifier K channel (RCK1) from brain and muscle in frog oocytes and SOl-8 cells (Koren et al., Neuron 3, in press). The unitary conductance was 14 pS. Channel activity consisted of bursts of openings during which rapid, incompletely resolved flickerings were observed. Analysis of the open channel noise revealed a single Lorentzian component. The observed extra noise in the open channel could be attributed to rapid opening and closing transitions. The rate of tail current deactivation saturated at negative voltages, suggesting that the closing rate is voltage independent. Since opening and closing steps did not appear to be strongly voltage dependent, the voltage dependence of channel activation must reside in the transitions between closed states preceding the final opening transition. Channel inactivation occurred slowly and did not overlap with activation. Inactivation showed a biexponential time course with time constants of 7 and 40 sec. The rate of inactivation was voltage independent for voltages > -30 mV and appears to be coupled to channel activation.

M-Pos300

BLOCKING BY LANTHANIDES OF A HIGH CONDUCTANCE Ca-ACTIVATED K CHANNEL.

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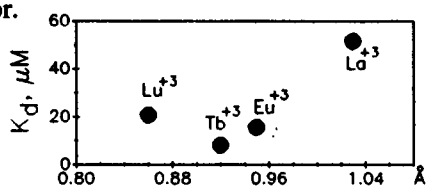
The lanthanides are trivalent cations of graded size and "noble gas" electron shells, whose chemistry should be predominently electrostatic. We used these ions to probe the sites of the Ca-activated K ("K(Ca)") channel of rat T-Tubules in bilayers. In 50 mM KCl, micromolar lanthanides added internally block K current as if bound to a site sensing 0.3 of the applied voltage. The blockade is V-dependent and can be well fitted to a Woodhull model extended to allow for the blocker to pass through the channel (as indicated by the observed decrease in the valence-distance product with applied potential). The K_d's for block (see fig.) fall on a smooth curve with a maximum affinity for the intermediate sized Tb. This topology is expected for an Eisenman Coulomb Pattern. A similar maximum is found for G-Actin (Curmi et al, Eur. J. Biochem. **122**, 239) and a Ca channel (Lansman, BJ **55**, 595a). The ability of a trivalent cation to cross the K(Ca) channel implies that its interior provides efficient screening from the lipid membrane interior.



Supp. by grants FONDECYT 1112-1989 & DTI B-2805, Chile & USPHS GM-24749, USA.

M-Pos302

ACTIONS OF PINACIDIL ON ATP-SENSITIVE K^+ CHANNEL CURRENT IN GUINEA-PIG VENTRICULAR MYOCYTES

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A vasodilator, pinacidil (5-500 μM), increased time-independent outward current. The dose-response curve of pinacidil was non-competitively inhibited by increased intracellular ATP concentration ($[ATP]_i$). In inside-out patches, pinacidil activated the ATP-sensitive K^+ channel current in the presence of $[ATP]_i$. Pinacidil increased the open probability of the channel by prolonging the mean lifetime of bursts, and shortening the slow component of the mean closed time and the long interburst intervals. These effects were opposite to those of increased $[ATP]_i$. Pinacidil did not change the channel conductance, nor its reversal potential. In ATP-free conditions, pinacidil higher than 30 uM inhibited the channel activity at positive voltages but not at negative voltages. These results indicate that there are, at least, two different sites for pinacidil to interact with the ATP-sensitive K^+ channel.

M-Pos301

THE PROPAGATING HELIX MODEL OF VOLTAGE-GATED CHANNELS

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Shot noise experiments of sodium channel gating currents indicate that 2.3 charges cross the membrane for each of 3 voltage-dependent conformational changes. Mutagenesis experiments suggest that most of the charge that moves is localized to 3 positive charges on the S4 segment. A model was developed consistent with these data. When C-termini portions of most or all S4 segments have an α helical conformation they occludes the center of the closed channel and their N-termini have a β conformation. Activation occurs when S4 helices 'propagate' toward their N-termini for six residues, leaving their N-termini in an α helix conformation on the extracellular surface and their C-termini forming part of an 8 stranded β barrel. Negatively charged SS2 segments form the other 4 strands of the β barrel which is the ion-selective portion of the channel. This β barrel is surrounded by 8 α helices formed by SS1 and C-termini half of S6 segments. These helices are surrounded by a cylinder of 16 helices formed by S1, S2, S3, and S5 segments.

M-Pos303

Mg^{2+} AND Ca^{2+} BLOCK AN INTRINSICALLY LARGE Na^+ CONDUCTANCE THROUGH A CARDIAC K^+ CHANNEL.

Raman Mitra, Johan Vereecke+, and Edward Carmeliet+. *Department of Cardiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and the +Laboratory of Physiology, Gasthuisberg, Herestraat B-3000 Leuven, Belgium.*

The effect of internal and external divalent cations on the K^+/Na^+ selectivity of the inwardly rectifying K^+ channel in guinea pig ventricular myocytes was studied. We report that with hyperpolarization (< -100 mV) and in the presence of K^+, external Na^+ does not block this channel and is permeant when divalent cations are absent or low in the external solution and the $[Mg^{2+}]_i$ < 50 uM ($[Ca^{2+}]_i$ < 10 nM). In the presence of divalent cations, Na^+ ions can still pass through the channel during the peak of the inward current. Occupancy of the channel by Na^+, however, enables voltage and time dependent block (inactivation) by external Ca^{2+} or Mg^{2+} and *internal* Mg^{2+} (whereas K^+ can clear these divalent cations from the channel), thereby inducing K^+ selectivity of the channel in the steady state. These results account for the paradoxical "facilitating" and "blocking" effects of Na^+ on K^+ currents through this channel.

M-Pos304

MECHANISM(S) OF G-PROTEIN MODULATION OF K_{Ca} CHANNELS IN UTERINE SMOOTH MUSCLE. J. Ramos-Franco, L. Toro, and E. Stefani. Dept. Mol. Physiol. & Biophys. Baylor College of Medicine. Houston, TX 77030.

Ca^{2+}-activated K^+ channels (264 pS) from pig uteri reconstituted into planar lipid bilayers are G-protein activated. We examined the action of GTPγS + $MgCl_2$ on: a) the open and close states, and b) the voltage dependence of the channel. We found at least two open (short, τ_{O1}=3 ms; and medium, τ_{O2}=26 ms) and three close states (short, τ_{C1}=2 ms; medium, τ_{C2}=100; ms and long, τ_{C3}=2 s) in control conditions. G-protein activation increased τ_{O2} (68 ms) or made more evident a third component (100 ms). For the close states: τ_{C1} remained almost the same (1.9 ms), but its amplitude factor (a.f.) increased 3 times; and τ_{C3} diminished to 870 ms and 16% its a.f.. This pattern is similar when Ca^{2+} is the activator (Magleby and Pallotta, J. Physiol. 344: 584, 1983). GTPγS displaced the voltage dependence of K_{Ca} channels. P_O (fraction open time) vs. voltage relationship was fitted to a Boltzmann distribution with $V_{1/2}$=35 mV and k=10 mV, at pCa 6.2. Addition of 100 μM GTPγS decreased $V_{1/2}$ to 5 mV without modifying k (9 mV). Our results suggest that G proteins influence the gating of these channels by shifting their voltage dependence possibly by increasing the affinity of the Ca^{2+} sensor. Supported by NIH.

M-Pos305

POTASSIUM CURRENTS IN ACUTELY DISSOCIATED UTERINE SMOOTH MUSCLE CELLS. E. Piedras-Rentería*, L. Toro & E. Stefani. Department of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX 77030.

Acutely dissociated smooth muscle cells from adult non-pregnant rat uteri (in oestrus) were studied with the whole cell patch clamp method. The external solution was Ringer-Krebs and the patch pipette contained (in mM): 140 K-aspartate, 1 $MgCl_2$ and 10 HEPES. We characterized three types of K currents that are charybdotoxin (100 nM)-sensitive: a transient current (T) with an activation time constant (τ_a) of 1.6 ± 0.21 ms (n=12) and an inactivation time constant (τ_i) of 8.7 ± 1.49 ms (n=8); a "fast" Ca-dependent current (K_f) with τ_a = 3.0 ± 0.43 ms (n=6); and a "slow" current (K_s) with τ_a = 8.7 ± 0.88 ms (n=6), at V_m= 50 mV. The sequence for charybdotoxin sensitivity is: K_s(100%) > T(76%) > K_f(32%). Supported by NIH and AHA.

*Universidad Autónoma Metropolitana-Iztapalapa, México.

M-Pos306

FUNCTIONAL RECONSTITUTION OF THE SOLUBILIZED CARDIAC SARCOPLASMIC RETICULUM (SR) K^+ CHANNEL.

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The muscle SR K^+ channel has been characterized functionally (Hill *et al*, *Biophys. J.* 55, 35, 1989) but not structurally. We have employed solubilization/reconstitution as an approach aimed towards identifying the protein component(s) of the SR K^+ channel. Canine cardiac SR proteins were solubilized in CHAPS and sedimented through linear gradients of sucrose. Gradient fractions containing the SR K^+ channel were identified by reconstitution into planar lipid bilayer membranes. Incorporated channels displaying similar temperature-dependent conductance, $[K^+]$ dependence and major sub-state O_1, to the native cardiac SR K^+ channel, were enriched in the ~20S region of the gradient. SDS-gel analysis of the gradient fractions indicated specific comigration of K^+ channel activity with several polypeptides ranging from M_r ~70K to 250K. Supported by NIH and MDA.

M-Pos307

POTASSIUM CHANNEL CONTROL OF REPOLARIZATION IN THE HUMAN NEUROBLASTOMA CELL LINE LA-N-5.

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Whole-cell currents from LA-N-5 neuroblastoma cells were studied with patch electrodes containing nystatin (360 uM) to permeabilize the membrane under the pipet tip. The current-voltage relationship (I-V), measured by ramps (dV/dt=80 mV/s) of membrane voltage (V_m), was markedly non-linear with pronounced outward rectification at V_m positive to -15 mV. Below -15 mV the I-V was nearly linear with a 500 pS slope conductance. Increasing external [Ca] from 2 to 25 mM decreased the slope conductance at negative V_m, shifted the activation of outward current at positive V_m by approximately +20 mV, and decreased the maximum current recorded at +60 mV. Increasing external [Mg] similarly decreased the slope conductance and shifted the outward current activation, but had little effect on the current at +60 mV, suggesting that the shift in activation arises from surface charge effects. TEA or internal Cs blocked the outward current. On-cell single channel recordings revealed a 55 pS channel, permeable to K^+, that was sharply voltage dependent (P_o increasing e-fold for 7 mV). The single channel properties suggest that this K channel underlies the large outward current observed in whole-cell recordings and is a major determinant of the time course of the action potential in these cells.

Supported by grants from the CA and FL affiliates of the AHA, the Louis Sklarow Memorial Fund, and the NIH.

M-Pos308

WHOLE CELL K CURRENTS IN INSULIN SECRETING CELLS MEASURED USING THE NYSTATIN PERMEABILIZED PATCH METHOD.

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The nystatin permeabilized patch method permits whole cell current recording with minimal disturbance of the intracellular *milieu*. The hamster insulin secreting cell line HIT was used to investigate whole cell K currents. Using a ramp clamp from -70 mV (V_H) to +120 mV (dV/dt = 13 mV/s) HIT cells display two distinct K current components in zero glucose. One is in evidence close to V_H and as it rectifies with depolarization and is blocked by 100 uM tolbutamide or 10 uM glyburide it may be ascribed to ATP sensitive K channel (K(ATP)) activity. The second is voltage dependent, activity commencing around -45 mV and is blocked by charybdotoxin (CTX) (kindly supplied by Dr. C. Miller, Brandeis University), good evidence that the calcium- and voltage-dependent K channel (K(Ca,V)) underlies this current. With both 100 uM tolbutamide and 50 nM CTX present the outward current is reduced by >95%, indicating that K(ATP) and K(Ca,V) comprise the major K channel species in these cells. Addition of 20 mM glucose provokes reduction in both components of K current, confirming the integrity of metabolic channel regulating mechanisms. Results from glucose dose-response studies and studies of the time dependent glucose-induced reduction of K(ATP) and K(Ca,V) channel currents are consistent with cell-attached patch single channel studies (Eddlestone et al. *J. Membrane Biol.* 109:123-134, 1989), strengthening the hypothesis that K(ATP) channel activity regulates the membrane potential of insulin secreting cells between rest and the level at which the characteristic burst activity occurs while K(Ca,V) channel modulation is associated with burst activity regulation. (Supported by American Diabetes Association grant W-P-880513 and a grant from the MDA).

M-Pos309

BETA CELLS OF THE LEAN MOUSE, THE HOMOZYGOUS COUNTERPART PART OF THE OB/OB MOUSE, DISPLAY TYPICAL GLUCOSE-SENSITIVE K^+-CHANNEL ACTIVITY. G.A.R. Mealing, L.A. Fournier*, N. Bégin-Heick*, J.F. Whitfield and J.L. Schwartz. Biological Sciences, National Research Council, Ottawa, and *Department of Biochemistry, University of Ottawa, Canada.

The glucose-sensitive K^+-channels of cultured β-cells of the C57BL/6J ob/ob mouse appear to be atypical (Schwartz et al., Biophys J, 1986, 49, 162a). However, there is no information on these channels in the β-cells of its homozygous lean counterpart. Single-channel currents were recorded from β-cells from the lean mouse (Jackson Laboratory, Bar Harbour, MA) which had been in culture for 7 to 14 days. This monolayer culture consisted of clusters of about 50 cells each, which stained positively for insulin using immunofluorescence. In the cell-attached configuration, a 50-pS potassium-selective channel was observed about 80% of the time with 2.8 mM glucose in the bath at room temperature. This channel was voltage-independent. It frequently displayed rapid bursting activity followed by periods of quiescence, lasting up to a minute. In about 80% of the cells tested, this channel was reversibly blocked in the presence of 16.8 mM glucose. Blockage took between 5 and 10 minutes to develop. Reactivation after return to 2.8 mM glucose required up to 15 minutes. In the inside-out configuration this channel was instantaneously blocked by applying 1 mM Na-ATP on the cytoplasmic side of the membrane patch. This ATP-blockage was rapidly reversible.

In conclusion, the glucose-sensitive K^+-channel of the lean mouse behaves like that of the NMRI mouse (Rorsman and Trube, Pfluegers Arch, 1985, 405, 305-309). The β-cells of the homozygous lean mouse appear to be acceptable controls for electrophysiological studies of the obese mouse β-cells.

M-Pos310

EFFECT OF VERAPAMIL ON THE Ca^{2+}-ACTIVATED K^+-CHANNELS OF AORTIC MYOCYTES. S. F. Fan* and C. Y. Kao#. #Departmenmt of Pharmacology, SUNY Downstate Medical Center, Brooklyn, NY 11203, and *Department of Anatomical Sciences, Health Sciences Center, SUNY at Stony Brook, NY 11794.

In freshly dispersed aortic myocytes of guinea pigs and rats, the predominant K^+-channel activities are those with unitary conductance of ~250 pS under symmetrical [K^+] conditions. Their open probability (p) is sensitive to both $[Ca^{2+}]_i$ and membrane potential. The unitary conductance decreases in the order of $K^+ > NH_4^+ > Rb^+$. Verapamil increases their p appreciably; e.g., at 45 mV, p rises from 0.98% to 2.32% in 30 μM verapamil. This effect differs sharply from those in guinea pig taenia coli myocytes, and in chick B-lymphocytes, where verapamil decreases p. The increased p of K^+-channels in aortic myocytes would lead to a hyperpolarization and relaxation of the myocyte, contributing to a reduction of vascular tone. Such actions on single K^+-channels could account in part for the therapeutic antihypertensive effect of verapamil in addition to its Ca^{2+}-channel blockade. (Supported by NIH grants HD00378 and DK39731).

M-Pos311

COMPARATIVE STUDY OF THE ATP- AND ADP-SENSITIVITY OF THE GLUCOSE-SENSITIVE K^+-CHANNEL IN BETA CELLS OF LEAN AND OBESE MICE. L.A. Fournier, G.A.R. Mealing*, J.F. Whitfield*, N. Bégin-Heick and J.L. Schwartz* (Intro. by S.L. Jacobson). Department of Biochemistry, University of Ottawa, and *Biological Sciences, National Research Council, Ottawa, Canada.

The effects of ADP and ATP on the activity of the glucose-sensitive K^+-channel of cultured β-cells of the lean (ln) and obese (ob) mouse were investigated using single-channel recording. The probability of channel opening in the presence of ATP (15, 30 uM, or 1 mM) or ADP (100 uM) on the cytoplasmic side of an inside-out patch was compared to that in the absence of these nucleotides. The ob channel was less responsive to ATP inhibition and its response range was wider than its ln counterpart. At 15 uM ATP, the reduction in the opening probability ranged from 78.8 to 98.8% in the ln mouse, and from 30.8 to 99.7% in the ob mouse. With 30 uM ATP, it ranged from 96.3 to 99.9% in the ln mouse and from 66.0 to 99.7% in the ob mouse. 1 mM ATP completely blocked both the ln and ob channels. ADP inhibited the ln channel, and inhibited but occasionally activated the ob channel. The range of response to ADP was wider in the ob mouse, 50% reduction to 40% activation, than in the ln mouse, 25.9 to 88.2% reduction. In view of the role of the ATP-sensitive K^+-channel in the coupling of glucose to insulin secretory activity, the differences described above may in part explain the abnormal insulin secretory activity previously reported for the ob mouse.

M-Pos312

SUBCONDUCTANCES IN CALCIUM-ACTIVATED POTASSIUM CHANNELS FROM AIRWAY SMOOTH MUSCLE.

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The single channel patch clamp technique was used to analyze the 260 pS calcium-activated potassium channel in canine airway smooth muscle. First described by McCann and Welsh (J. Physiol. 372:113, 1986), this channel shows high calcium sensitivity (P_{open}>0.8 in 0.1 μM Ca^{++} and P_{open}<0.1 in 1 nM Ca^{++} at -60 mV). We find that the channel spends a considerable amount of time in 30% and 50% subconductance states. The kinetic characteristics of these subconductances has been examined at different voltages and calcium concentrations. The effect of monovalent cation concentration has also been examined. A kinetic model accounting for subconductances will be considered.

This work has been supported by the Alberta Heritage Foundation for Medical Research.

M-Pos313

CROMAKALIM AND RP49356 MODULATION OF THE ATP-SENSITIVITY OF CARDIAC K_{ATP} CHANNELS

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K_{ATP} channels open when $[ATP]_i$ falls, and underlie cardiac action potential shortening in metabolic blockade (Lederer et al., 1989, J. Physiol., 413, 329-349). Potassium channel opening drugs, such as cromakalim (CR) and RP49356 (RP) also shorten the cardiac action potential by opening K_{ATP} channels (Escande et al., 1988, Biochem. Biophys. Res. Comm., 154, 620-625). We have investigated the interaction of these drugs and ATP in the regulation of cardiac K_{ATP} channels using isolated inside-out patches from adult rat and guinea-pig ventricular myocytes. In guinea-pig K_{ATP} channels (at 37 °C), 40 μM CR caused an apparently competitive shift in the $k_{1/2}$ ([ATP] required for half-maximal inhibition of channel activity) from 79 μM to 152 μM, without shifting the maximum or minimum channel activity at high, or low, [ATP]. RP caused a similar shift in rat heart K_{ATP} channel ATP-sensitivity (at 20 °C). These results imply that the in vivo effects of RP, or CR, on K_{ATP} activity will depend critically on $[ATP]_i$.

M-Pos314

CA-DEPENDENT K CHANNEL ACTIVITY IN RAT THYMIC LYMPHOCYTES: EFFECT OF MITOGENIC STIMULATION.

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We are studying the role of Ca-dependent K channels in the membrane potential response of rat thymocytes to mitogenic stimulation by the lectin Concanavalin-A (Con-A). Membrane potential was measured in suspensions of thymocytes using bis-oxonol. Con-A evoked a hyperpolarization which was blocked by cytoplasmic loading with the Ca^{2+} chelator BAPTA or charybdotoxin (50nM). K channel activity was monitored using cell-attached patch recordings. The pipette contained a high KCl (0Ca) saline and the bath contained normal NaCl saline. Under resting conditions, few channels were active; addition of Con-A outside the pipette rapidly activated inward currents at the resting potential. The current-voltage relationship displayed inward rectification (slope at 0mV applied was 25pS) with a reversal potential of approximately -70mV. Channel activity was not strongly dependent on pipette potential within the range +40 to -140mV. This channel has properties indistinguishable from those activated by ionomycin and previously identified as calcium-activated K channels (Mahaut-Smith & Schlichter, J.Physiol. 415, 69-83). We conclude that Con-A, which is known to increase $[Ca^{2+}]_i$, hyperpolarizes thymocytes through activation of IK(Ca) channels.

M-Pos315

CLONING AND EXPRESSION OF A GENOMIC DNA CLONE ENCODING A RAT BRAIN POTASSIUM CHANNEL

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P1, a cDNA clone encoding part of a rat brain K^+ channel, was isolated using a *Shaker* cDNA probe and then used to screen a rat genomic DNA library. A 9 kb genomic clone, B6, was isolated and contains the entire protein coding sequence uninterrupted by introns. The deduced protein contains 535 amino acids, is structurally homologous to the *Shaker* type K^+ channels (it has 6-7 hydrophobic regions including an S4 type domain), and is 69% and 72% identical to K_V1 and K_V2, two rat brain K^+ channels previously isolated in this laboratory (Swanson *et al.*, 1989). A 9.5 kb mRNA encodes this channel and is found in brain, lung, and spleen but not in heart, skeletal muscle, liver, or kidney. No differences in the abundance of this mRNA in brain were evident over the course of development of the rat from neonate to adult. RNA transcribed from the protein coding region of clone B6 and injected into *Xenopus* oocytes induced the expression of K^+ selective outward currents with transient I_A type kinetics. Currents activated in response to depolarizations to voltages > -40 mV. Time to peak current at 40 mV was 22 ms and current was largely inactivated at the end of 200 ms pulses.

M-Pos316

KINETICS OF CLONED K^+ CURRENTS CHANGE WITH LEVEL OF EXPRESSION IN *XENOPUS* OOCYTES. Jeffrey S. Smith, Robert Stein, and Richard Swanson Dept. of Cardiovascular and Molecular Pharmacology, Merck Sharp and Dohme Research Labs, West Point, PA 19486

cDNAs encoding two K^+ channels, K_V1 and K_V2, were cloned from rat brain mRNA based on their homology to a Shaker cDNA probe (Swanson et al.,1989). RNA transcribed from each clone induced the expression of K^+ selective currents when injected into *Xenopus* oocytes. Whole cell currents of 2 μA or less displayed delayed rectifier type kinetics i.e., slow activation (time to peak current was 40 ms at 40 mV) and little or no inactivation during 200 ms pulses. Currents 6 μA and larger had transient I_A type kinetics showing faster activation (time to peak = 15 ms at 40 mV) and almost complete inactivation during 200 ms pulses. Currents of intermediate magnitudes had kinetic properties between those of the smaller delayed rectifier and large I_A type currents. Individual ooytes, which could be voltage clamped repeatedly over a period of several days, demonstrated delayed rectifier currents which were seen to grow in magnitude and develop faster I_A type activation and inactivation kinetics. The mechanism of this transition is not known. Experiments are underway to characterize the phenomenon at the single channel level using the giga seal patch clamp method.

M-Pos317

EXPRESSION AND CHARACTERIZATION OF K^+ CHANNELS ENCODED BY NEW MEMBERS OF THE *DROSOPHILA SHAKER* MULTIGENE FAMILY. M. Covarrubias[1], A. Wei[1], A. Butler[1], K. Baker[1], M. Pak[1], and L. Salkoff[1,2]. Dept. of Anatomy and Neurobiology[1] and Dept. of Genetics[2], Washington University School of Medicine, St. Louis, Mo 63110.

The hypothesis that K^+ channel diversity results from an extended gene family coding for homologous proteins was introduced by the finding that the *Drosophila Shaker* gene has three sister genes (Butler, et al., Science 243:943-947, 1989): *Shal*, *Shab* and *Shaw*. Each *Drosophila Shaker* homolog has a mammalian homolog. We have now expressed the products of all Drosophila genes in *Xenopus* oocytes and show that they code for voltage-gated K^+ currents with different kinetic properties. According to their speed of activation and inactiviation they can be ordered as follows (fastest to slowest): *Shaker*, *Shal*, *Shab* and *Shaw*. These currents also cover a range of voltage sensitivities that is manifested in the current-voltage relationships and steady-state inactivation curves. We hypothesize that some of the biophysical differences may be a reflection of structural differences found in the presumed S4 voltage sensor of the proteins. (Supported by NIH R01 NS24785-01 and a MDA Research Grant).

M-Pos318

PERFORATED-PATCH RECORDING FROM FRESHLY ISOLATED RAT OSTEOCLASTS. M.E.M. Kelly, S.J. Dixon and S.M. Sims. (Intro: M. Sherebrin). Depts. Physiology & Oral Biology, Univ. Western Ontario, London, Canada

We have previously described inwardly rectifying K^+ current (I_K) in isolated rat osteoclasts, using whole-cell recording with patch-pipettes (WCR). Here we describe experiments designed to test for other currents. The perforated-patch technique (Horn & Marty, 1988. J. Gen. Physiol. 92:145) was used in an attempt to preserve any labile currents, by minimizing exchange of cytoplasm with electrode solution. With this technique, osteoclasts exhibited stable resting membrane potentials of -70 mV. As before, inward rectifying I_K was present in all cells studied. In order to test directly for voltage-activated I_{Ca}, we used Cs^+ in the recording pipette and Ba^{2+} in the bath, to block I_K. With both perforated-patch recording and conventional WCR, no voltage-activated inward currents were detected, even in the presence of the Ca^{2+} channel agonist BAY K 8644. When we used WCR and buffered Ca^{2+} in the internal solution to ≈100 nM, ≈25% of cells studied exhibited an outward current at potentials positive to 0 mV. We conclude that inwardly rectifying I_K is the dominant current of rat osteoclasts when recorded with the perforated-patch technique. An additional outward current was evident in some cells.
(Supported by The Arthritis Society and MRC).

M-Pos319

LARGE AND SMALL CONDUCTANCE K^+ CHANNELS IN THE PLASMA MEMBRANE OF RABBIT PORTAL VEIN CELLS. Normand Leblanc, and Joseph R. Hume, University of Nevada School of Medicine, Reno, NV, 89557.

The cell-attached configuration of the patch clamp technique was used to record K^+ channel activity from the surface membrane of single vascular smooth muscle cells enzymatically dispersed from the rabbit portal vein. The cells were depolarized near 0 mV with 150 mM K^+ in the bath. With 150 mM K^+ in the pipette solution, two different unitary conductances activated by depolarization were consistenly identified: large (212 pS; K_{Ca}) and small (70 pS; K_{DR}) conductance channels. These channels were found to be mainly selective for K^+. With 5.4 mM K^+ in the pipette solution, the slope conductance of K_{DR} decreased to 36 pS. The open probablility and mean open time of both channels increased with membrane depolarization. Ensemble averages of repetitive depolarizations that activated K_{DR} or K_{Ca} revealed time-dependent activation kinetics that correlated well with the time course of whole-cell K^+ currents recorded in this preparation (Hume and Leblanc, J. Physiol., 413, 1989). K_{Ca} shares some properties of the large Ca-activated K^+ channel identified in many smooth muscle cells, and probably underlies the slow Ca-dependent transient outward current previously described. K_{DR} might correspond to the Ca-independent delayed rectifier K^+ current, which activates at similar potentials.

M-Pos320

LARGE AND SMALL CONDUCTANCE CA-ACTIVATED POTASSIUM CHANNELS IN ADULT AND TISSUE-CULTURED RAT CEREBRAL CORTEX NEURONS.

Andrew L. Blatz, PhD. Dept. of Physiology, UT Southwestern Medical Center, Dallas, TX, 75235.

Single channel currents through Ca-activated K channels in cerebral cortex neurons were recorded with the patch-clamp technique using cell-attached and excised patch configurations. Tissue-cultured cerebral cortex neurons were obtained from fetal rat brains and grown in a defined (N2) medium. Adult neurons were dissociated from rat cortex using trypsin and mechanical disruption. Tissue-cultured and adult cortex neurons were identified as cells that fired action potentials or stained positive for neuron-specific enolase and did not stain positive for glial fibrillary acidic protein.

Two types of Ca-activated K channel currents were present in adult and tissue-cultured neurons; a large-conductance (250-300 pS), voltage-sensitve, charybdotoxin-sensitive channel (BK-channel) and a smaller-conductance (10-15 pS), apamin-sensitive channel (SK-channel). BK channels were observed in cell-attached configurations and both channel types were present in excised, inside-out patches.

Supported by NIH grant GM39731.

M-Pos321

POTASSIUM CHANNELS IN TASTE RECEPTOR CELLS OF THE CATFISH.

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The catfish taste system is highly sensitive to amino acids. Independent receptor sites for short-chain neutral amino acids (L-alanine receptor) and the basic amino acid, L-arginine (L-arginine receptor) have been established by radioligand binding and neural cross-adaptation studies. Using the patch-clamp technique, we have observed two types of K^+ channels in receptor cells dissociated from catfish taste buds. One type had a unitary conductance of 63 pS and its open time was markedly reduced by membrane hyperpolarization. The other type of K^+ channel had a unitary conductance of 88 pS and displayed inward rectification, being open nearly all of the time at potentials negative to the resting potential and closed at positive membrane potentials. Neither L-alanine nor L-arginine altered the properties of either type of K^+ channel, suggesting that these channels are involved in the control of resting membrane potential. Supported by NSF BNS-8609555.

M-Pos322

BLOCK OF K^+ CHANNELS BY 4-AP DEPENDS ON OPENING OF CHANNELS and pH.

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The mechanism by which 4-AP blocks the delayed rectifier type K^+ channels at LPS activated murine B lymphocytes was investigated using the whole-cell patch-clamp. We found that 4-AP (0-1mM) only blocks open K^+ channels; furthermore the drug remains in the channel once it is closed. 4-AP was added (or washed) for 3-4 min before applying depolarizing pulses to activate the channel (1 s, 100 mV from a HP of -80 mV). During the first pulse after drug application, the current inactivated faster but its peak was unchanged, as compared to the control. The apparent rate of block (Tcontl*Tinac/(Tcontl-Tinac)) was 506±41 ms and 230±51 ms at .1 and 1 mM 4-AP, respectively. During subsequent pulses, the peak current was decreased (Kd=120 uM), but the inactivation rate was slower than in the control, a process that could be explained by a slow unblocking process. During the first pulse after washing out the drug, the channels were initially blocked, and unblocked with at least two rates; a fast one (T=30 ms), and a slower one not resolved. These data suggest two blocked states may exist. 4-AP is a weak base (pK=9), and thus exists in ionized or non ionized form. We found that the Kd of channel block depends upon both internal and external pH; pKd=120(±20) - 1.3(±.055)*(pHext-pHint). This relation suggests that 4-AP acts from inside the cell in its ionized form to block the channels.

M-Pos323

^{125}I-CHARYBDOTOXIN BINDS WITH HIGH AFFINITY TO THE VOLTAGE-GATED K^+ CHANNEL OF INTACT HUMAN T LYMPHOCYTES.

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^{125}I-Charybdotoxin (ChTX) binds to quiescent human T lymphocytes and also blocks the voltage-gated K^+ channel. Steady-state binding is linearly dependent on cell concentration, highly ionic strength dependent, and independent of temperature (4-25°C) and monovalent cation. At isotonic low ionic strength, the inhibition constant for native ChTX blockade of ^{125}I-ChTX binding is 13 pM, a value similar to that obtained for the inhibition constant for channel blockade. Scatchard analysis of the binding of ^{125}I-ChTX gives a K_d ~ 67 pM and V_{max} ~400 sites/cell, in excellent agreement with the channel density obtained electrophysiologically. Rate constants for association and dissociation for ^{125}I-ChTX are $8x10^6$ $sec^{-1}M^{-1}$ and $5x10^{-4}sec^{-1}$, giving a calculated $K_d(k_{-1}/k_{+1})$ of 60pM. Pharmacological characterization has also been done. Supp.by GM 41467.

M-Pos324

BLOCKADE OF CARDIAC SARCOPLASMIC RETICULUM POTASSIUM CHANNEL BY CALCIUM AND BARIUM.

Qi-Yi Liu and Harold C. Strauss. Depts. of Medicine and Pharmacology. Duke Univ. Med. Center, Durham, NC 27710

The canine cardiac SR K^+ channel has been studied in planar lipid bilayers. Ca^{2+} or Ba^{2+} added to either the *cis* or *trans* chamber blocked the K^+ current. Blockade was nearly equally effective with Ca^{2+} or Ba^{2+} added to either chamber, although *cis* addition was more effective for *cis*-to-*trans* current and vice versa for *trans* addition. In 50 mM KAcetate, K_D for K^+ current inhibition (Ba^{2+} or Ca^{2+}, *cis* chamber) was ~5 mM for *cis*-to-*trans* current and ~30 mM for *trans*-to-*cis* current. Blockade was slightly more effective when the blocking ion was present in both chambers. Competitive binding of Ca^{2+} or Ba^{2+} decreased the conductance of both the fully open and sub-staces in a parallel manner. Increasing $[K^+]$ decreased blockade. These data are consistent with divalent ion binding to negative charges associated with the channel. Supported by NIH HL-19216.

M-Pos325

QUININE BLOCKS THE K-Ca CHANNEL IN RAT B-CELLS.

E.Mancilla & E.Rojas (Introduced by P.Carroll). LCBG, NIH, Bethesda, MD 20892

The K-Ca, one of the three K channels described in B-cells, is a high conductance, voltage and Ca dependent K-channel. Quinine is known to block K-Ca channels in other systems, and its effect on membrane potential in B-cells and insulin secretion has been well studied. We tested quinine on the K-Ca channel in rat B-cells in culture using the outside-out configuration of the patch-clamp technique. It produced a reversible fast flickering with an apparent decrease in channel amplitude (Kd= 155 uM). There was an exponential distribution of the mean open and closed times within bursts, corresponding to a simple sequential model for its blocking mechanism. Since quinine also blocks K-ATP channels in the B-cell, its physiological effects may not be specific to one channel.

M-Pos326

EXTERNAL TEA^+ BLOCK OF Ca^{2+}-ACTIVATED K^+ CHANNELS OF ARTERIAL SMOOTH MUSCLE.

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Smooth muscle cells were isolated enzymatically from rat or rabbit mesenteric artery. Unitary currents through large-conductance Ca^{2+}-activated K^+ channels were recorded from inside-out patches. At 0 mV, with physiological K_O and 120 mM K_i, currents were 5.4 ± 0.3 pA (rabbit, n=6) and 5.7 ± 0.1 pA (rat, n=5). TEA Cl included in the pipette solution at 50-500 μM produced a flickery block, increasing open channel noise and reducing mean amplitude. The reduction was consistent with 1:1 binding with a dissociation constant in the range of 150-250 μM, and with the block showing little voltage dependence. Fitting of beta functions to amplitude distributions suggested rate constants around 150 mM^{-1} ms^{-1} for block and 30 ms^{-1} for unblock. Thus TEA is a relatively effective blocker of BK_{Ca} channels of arterial smooth muscle.

M-Pos327

BLOCK OF HIGH CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNELS BY IBERIATOXIN.

O. McManus, M.L. Garcia, G.J. Kaczorowski, G. Katz, and J.P. Reuben, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.

Iberiatoxin (IbTX) isolated from the venom of the scorpion Buthus tamulus is a potent (low nM), and selective reversible blocker of the large conductance calcium-activated potassium channel in vascular smooth muscle. The toxin blocked this channel in both excised outside-out patches from bovine aortic smooth muscle cells and in lipid bilayers in which the channel is incorporated. In the bilayer, 2 to 5 nM produced very long (tens of minutes) blocked and unblocked periods of single channel activity. In excised outside-out patches containing several channels, the onset of block by the toxin was accompanied by a decrease in the mean open time which reversed within 5 minutes of washout. This modification of channel activity in the presence of IbTX was sustained and not interspersed with periods of normal or near normal activity (dissociation of toxin) as occurs with Charybdotoxin (ChTX). This latter point is critical for modeling IbTX action. These results suggest that block of this channel by IbTX differs from block by ChTX in that all of the results cannot be explained by IbTX binding to a single high affinity site on the channel.

M-Pos328

Na^+ ACTIVATED K^+ CHANNEL IN CARDIAC MYOCYTES: RECTIFICATION, OPEN PROBABILITY AND BLOCK. E. Carmeliet, Lab. Physiol. Univ. Leuven, 3000 Leuven, Belgium.

The Na^+ activated K^+ channel, $i_{K(Na)}$, was studied in isolated patches from guinea-pig ventricular myocytes. A linear single channel current-voltage relation was obtained when $K_i^+ = K_e^+$, inward rectification for $K_i^+ < K_e^+$ and outward rectification for $K_i^+ > K_e^+$. The open probability was sensitive to Na_i^+, but variable. Once activated by high Na_i^+, the channel remained active for many minutes even in the absence of Na_i^+. Single channel conductance was not changed by Ca_i^{2+} (1 mM), slightly dependent on Mg_i (0-2 mM), but decreased in a voltage-dependent way by Cs_e^+; it was insensitive to sulfonylurea, but inhibited by 10^{-7}-10^{-6} M R56865, a drug active against digitalis-induced shortening of the action potential. This drug did not modify Na^+, Ca^{2+} or other K^+ currents. Based on rectification characteristics, open probability and block, it is concluded that the $i_{K(Na)}$ channel plays an important role in the shortening of the action potential caused by digitalis.

M-Pos329

INDUCTION AND BLOCK OF Na_i^+ ACTIVATED K^+ CURRENT IN GUINEA-PIG VENTRICULAR MYOCYTES. H-N Luk, Lab. Physiol. Univ. Leuven, 3000 Leuven, Belgium (Intro. by E. Carmeliet)

Inhibition of the Na^+, K^+ pump in cardiac cells is accompanied, after an initial lengthening by a later shortening of the action potential. The hypothesis whether this shortening is due to a Na_i^+ activated K^+ current, $i_{K(Na)}$, was tested by voltage-clamping (2 suction electrodes) and dialysing cells with Na_i^+ of 10,20,45 and 90 mM, with or without pump inhibition. In control an important outward current was induced by 90 mM Na_i^+. However, superfusion with K^+ free solution or a solution with ouabain caused large outward currents at a Na_i^+ of 10 mM. The current showed outward rectification and was inhibited by R56865, known to block the $i_{K(Na)}$ channel in patches (Carmeliet, this volume), or by lowering Na_e^+. It is concluded that $i_{K(Na)}$ can be induced at low Na_i if the Na^+ pump is blocked. This suggests the existence of Na^+ gradients in dialysed cells, which depend on the activity of the Na^+ pump.

M-Pos330

SYNTHESIS AND BIOLOGICAL PROPERTIES OF THE Ca^{2+}-ACTIVATED K^+ CHANNEL INHIBITORS: CHARYBDOTOXIN, IBERIOTOXIN AND CHIMERIC ANALOGS. M.L. Garcia, E.E. Sugg, J.P. Reuben, A.A. Patchett, and G.J. Kaczorowski, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.

Charybdotoxin (ChTX) and Iberiotoxin (IbTX) are potent peptidyl inhibitors of the high conductance Ca^{2+}-activated K^+ channel ($P_{K,Ca}$). Despite displaying 70% homology, these peptides bind at distinct sites on $P_{K,Ca}$ and block channel activity by different mechanisms. Moreover, IbTX is selective as an inhibitor of $P_{K,Ca}$. Complete syntheses of ChTX and IbTX have now been achieved using FMOC-SPPS. The synthetic toxins are identical to their respective native congeners based on indistinguishable behavior in three different experimental paradigms: HPLC; modulation of [^{125}I]ChTX binding to bovine aortic sarcolemmal membrane vesicles; block of $P_{K,Ca}$ in single channel recordings from bovine aortic smooth muscle. The three disulfide bonds of ChTX have been assigned by selective enzymatic digestion: Cys^7-Cys^{28}, Cys^{13}-Cys^{33} and Cys^{17}-Cys^{35}. These results suggest that ChTX is a highly folded structure with its basic amino acid side chains arranged in clusters. The solid phase synthesis of the hybrid molecules $ChTX_{1-19}$ $IbTX_{20-37}$ and $IbTX_{1-19}ChTX_{20-37}$ has also been accomplished. Based on binding and electrophysiological criteria, the hybrid structures demonstrate that the C-terminal 17-37 residues define either ChTX or IbTX receptor selectivity.

M-Pos331

MAXIMUM LIKELIHOOD ESTIMATION OF RATE CONSTANTS FOR CHANNELS ACTIVATED BY ACETYLCHOLINE AND/OR CARBACHOL. **Anthony Auerbach**, Dept. Biophysics, SUNY, Buffalo, NY 14214.

Acetylcholine receptors usually bind two agonist molecules before opening; how do channels behave when the agonists are different? Single cholinergic channels in *Xenopus* myocytes were studied in cell-attached patches (18 °C, V_m = -110 mV, t_{dead} = 50 μs). Patch pipettes contained ACh (2-50 μM), and/or Carb (10-800 μM). Rate constants were estimated for intervals within apparently homogeneous bursts using a maximum likelihood method (Horn and Lange) with a first-order correction for missed events (Roux and Sauve). Initially, a 5-state linear scheme was assumed: C_1 -- C_2 -- C_3 -- O_5 -- C_4, where C_1, C_2 and C_3 are vacant, mono-, and doubly-liganded channels, and C_4 are open-blocked channels. For simplicity binding sites were assumed to be identical and independent (i.e., $k_{12} = 2k_{23}$ and $2k_{21} = k_{32}$). Optimization of the likelihood function was across many patches with k_{12}, k_{23}, and k_{54} scaled by the agonist concentration. The estimated rates were checked by comparing experimentally obtained distributions with computed and simulated distributions. Preliminary estimates for agonist binding and gating for g_{40} ('extrajunctional'-type) channels from the ACh data set (14 files, 45106 intervals) and the Carb data set (13 files, 37467 intervals) are given below ($s^{-1}M^{-1}$ or s^{-1}). These values must be interpreted with caution because the correction for missed events adds an instability to the optimization as there is no contraint on the extent of open channel noise. More complex models are under investigation to determine the opening/closing rates of channels occupied by heterotypic agonists. (NS23513; thanks to R Horn and X Sun)

	ACh	Carb
k_{23}	8.7×10^7	2.3×10^7
k_{21}	2080	4152
k_{35}	5312	3558
k_{53}	270	400

M-Pos332

MOUSE AND *TORPEDO* ACETYLCHOLINE RECEPTORS EXHIBIT DIFFERENTIAL SENSITIVITY TO OPEN-CHANNEL BLOCKADE BY FORSKOLIN. M.L. Aylwin and M.M. White, Depts. of Physiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The rate of desensitization of the *Torpedo* ACh receptor is increased by cAMP-dependent protein phosphorylation. Middleton *et al* (PNAS 83:4967) have shown that forskolin, which elevates intracellular cAMP levels, increases the rate of AChR desensitization in rat myotubes and concluded that the effect of low concentrations (<20 μM) of forskolin is due to an increase in cAMP levels, while at higher concentrations forskolin seems to weakly block the channels. We have since shown that forskolin is a potent local anesthetic, with *Torpedo* AChRs being very sensitive to forskolin blockade at concentrations as low as 5 μM. We have expressed mouse and *Torpedo* AChRs in *Xenopus* oocytes and directly measured the K_D for channel blockade for the two receptors. Forskolin has a 7-fold higher affinity for the ray than rodent receptor (K_D=3.3±0.5 μM vs 23.7±4.3 μM). Local anesthetics are thought to interact with serine OH groups in the M2 region of the receptor. The β subunit of the mouse receptor has two fewer serines in the M2 region than the *Torpedo* β subunit, which may partially explain the difference in affinities. Replacement of the β subunit of the mouse receptor with that from *Torpedo* increases its affinity for forskolin to a level comparable to that of the *Torpedo* receptor (K_D=7.9±2.0 μM), as expected/hoped. Supported by NIH grant NS 23885.

M-Pos333

A NOVEL CALCIUM CHANNEL ACTIVATED BY PERTUSSIS TOXIN (PTx) IN BOVINE CHROMAFFIN CELLS. V. Cena & E. Rojas. Neurochemistry Dept, University of Alicante, Spain, and Biophysics Section, LCBG, NIDDK, NIH, Bethesda, MD

Catecholamine and ATP secretion from cultured Bovine chromaffin cells requires extracellular Ca. Activation of G proteins by PTx induces sustained secretion, also dependent upon Ca. Using patch clamp techniques, we found that PTx did not increase inward current (whole cell) through L-type Ca channels, the only voltage-dependent Ca channels observed in these cells. Pre-incubation for 4 hours with 100 ug/ml PTx increased the activity of an 8 pS single channel (cell attached), only scarcely observed without pre-treatment with PTx. This Ca channel, also permeant to Cs, was similar to receptor activated Ca channels recently described in human pancreatic B-cells and rat gonadothroph cells and may explain PTx-induced secretion.

M-Pos334

DESENSITIZATION OF A CHICKEN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSED IN XENOPUS OOCYTES. Adrian Gross, Daniel Bertrand, Marc Ballivet* and Duri Rungger+, Department of Physiology, CMU, *Department of Biochemistry, Sciences II, +Department of Animal Biology, 1211 Geneva 4, Switzerland. (Intro. by Ian C. Forster)

Desensitization of a neuronal nicotinic acetylcholine receptor (nAChR), reconstituted in Xenopus oocytes was investigated with the voltage clamp technique two days after nuclear cDNA injection of two subunits coding for α4 and non-α. At -100 mV, a steady exposure to a low concentration (100 nM) of ACh induced a constant inward current. In the presence of higher agonist concentrations, however, the current decreased due to desensitization. In order to study the kinetics of desensitization we took advantage of a property of this neuronal nAChR: at depolarized voltages (+40 mV) no ACh-induced current and no desensitization could be observed. Thus, in the constant presence of ACh the effect of this agonist became effective only when the membrane potential was brought back to negative voltage values (i.e. -100 mV). We found that the time course of desensitization could be adequately described with a sum of two exponentials. At -100 mV and with 50 uM ACh the time constants were 0.38 +/- 0.08 s and 69 +/- 19 s, respectively (n=5). The rapid phase of desensitization was more sensitive to voltage and agonist concentration than the slow component. Hyperpolarization (between -60 mV and -140 mV) or rise of agonist concentration (between 2 uM and 20 uM ACh) led to a marked decrease of the fast time constant (-72% and -27%), whereas the slow time constant was only slightly affected (-17% and +7%). The substitution of extracellular sodium with equimolar potassium did not significantly alter the kinetics of desensitization. In conclusion, the desensitizing properties of this neuronal nAChR are rather similar to those of the nAChR described at the frog endplate (Cachelin & Colquhoun, J. Physiol. 415, 159-188).

M-Pos335

LOSS OF CHANNEL MODULATION BY TRANSMITTER & PROTEIN KINASE C DURING SYNAPTOGENESIS. P. Drapeau, McGill Univ. Centre for Res. in Neurosci. & Montreal Gen. Hosp., 1650 Cedar, Montreal, Quebec, Canada H3G 1A4.

When serotonergic Retzius neurons of the leech contact pressure sensitive (P) neurons in culture, they selectively reduce a cationic response to 5-HT and reform the inhibitory, Cl-dependent synapse seen in vivo.

The cationic channel was observed in 52% of cell-attached patches from single P cells. A saturating (30 μM) [5-HT] caused a 4.0 ± 1.1 (n=14) fold increase in P_{open}. Phorbol myristate-acetate (0.5 μM PMA or TPA, but not 4α-PMA), which activates protein kinase C (PKC), increased P_{open} 9.8 ± 2.7 fold (n=7). The PK inhibitor H-7 blocked both 5-HT and PMA activation.

P cells paired with Retzius cells showed a similar incidence of this channel (56% of patches). However, P_{open} was increased <2 fold by 5-HT or PMA. These results suggest that activation by PKC (and not the number) of cationic channels is reduced upon cell contact. The early clearing of the non-synaptic (excitatory) response to 5-HT appears to be a prelude to (inhibitory) synapse formation.

Supported by the MRC, FRSQ, and FCAR.

M-Pos336

SNAKE TONIC MUSCLE FIBER ENDPLATES HAVE ACHRS THAT RESEMBLE EXTRAJUNCTIONAL CHANNELS ON DENERVATED TWITCH FIBERS. **R.L.Ruff, Neurology Dept. & Ctr. for Neurosci., Dept. Vet. Affairs Med. Ctr., & CWRU, Cleveland, OH 44106.**

Tonic fiber endplates of the inferior costocutaneous muscle of the garter snake (thamnophis sp.) have 33pS and 51pS ACHR channels. The 51pS channel was similar in conductance and kinetics to the twitch fiber endplate ACHR. Segments of snake muscle were denervated by cutting several consecutive nerve roots. Endplate regions on twitch fibers were identified by degenerated nerve terminals. The extra-junctional membrane of denervated twitch fibers had ACHR channels that were similar in conductance and kinetic properties to the 33pS channel found at tonic endplates. Thus, adult tonic endplates appear to contain both twitch adult-type and fetal-type (extrajunctional) ACHR. Perhaps the development of tonic muscle fibers is altered in comparison to twitch fibers so that the adult tonic fibers support polyneuronal and multiterminal innervation and continue to express fetal-type ACHR.

Supported by Merit Reviewed Funding from the Dept. Vet. Affairs.

M-Pos337

CONCANAVALIN-A INCREASED A CYCLIC NUCLEOTIDE ACTIVATED CATIONIC CONDUCTANCE IN CULTURED LEECH NEURONES. Sergio Sánchez-Armass and Pierre Drapeau (Intro. by M. C. García) Dept. de Fisiología, Univ. de San Luis Potosí, A.P. 1521-B S.L.P. México and McGill Univ. Centre for Neuroscience and Research Institute, M.G.C. Montreal, Canada H3G 1A4.

During studies about the second messengers that modulate the conductances activated by 5-HT in the leech mechano-sensory P neurones, we observed a cationic current activated by cyclic nucleotides, but not by 5-HT. This conductance (GNC) was greatly enhanced in neurones grown on Concanavalin-A respect to the cells plated on polylysine. The 10 x increase in current was larger than expected from the estimate increase in surface area due to neurite extension. The effect of Con-A seems to be specific since either the resting membrane potential nor the I/V curve for the 5HT-activated Cl conductance were changed. dbcAMP, dbcGMP and forskolin activated GNC. Permeant ions were Na, Tris, N-Me-glucamine and Mg. TEA was impermeant. Surprisingly, tolbutamide (among other effects, it blocks the cAMP-dependent protein kinase) was able to reversible activate GNC. These results suggest that this is one of the least selective channels already described. The larger conductance measured in Con-A is probably due to either the exposition of cryptic channels, or to the redistribution of receptor sites in the neuronal membrane.

M-Pos338

MOLECULAR REGULATION OF DESENSITIZATION TO NEUROTRANSMITTERS IN SYMPATHETIC NEURONS. Mark A. Simmons. Dept. Pharmacol., Marshall Univ., Huntington, WV. The receptor agonists substance P (SP), LHRH and muscarine inhibit a K current, the M current (I_M), in amphibian sympathetic neurons. During continuous agonist application this inhibition desensitizes. Studies on ß-adrenergic receptors (ßAR) have indicated that desensitization of ßARs results from phosphorylation of ßARs catalyzed by a specific ßAR kinase. By whole cell recordings from single dissociated sympathetic neurons from bullfrogs, I have studied desensitization of I_M inhibition. When ATP in the recording pipette was replaced with AMP-PNP, SP still inhibited I_M, but no desensitization was observed, consistent with the notion that phosphorylation is involved in desensitization. On the other hand, desensitization was not affected by heparin or dextran, which are potent inhibitors of the ßAR kinase. When a low dose of muscarine sufficient to inhibit I_M, but not to desensitize, was applied simultaneously with a desensitizing dose of SP, I_M remained depressed. This indicates there may be separate desensitization mechanisms for different agonists. Supported by NINDS.

M-Pos339

ACTIVATION OF NMDA AND QUISQUALATE RECEPTORS BY ENDOGENOUS EXCITATORY AMINO ACIDS. Doris K. Patneau and Mark L. Mayer. Unit of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

The sensitivity of NMDA receptor-mediated responses to glycine and Mg provides a simple method to control which excitatory amino acid receptor subtype, NMDA or quisqualate, is activated by mixed agonists such as glutamate. Dose-response curves for 9 endogenous excitatory amino acids at NMDA receptors have been obtained, utilizing fast perfusion techniques during whole cell recording from cultured mouse hippocampal neurons voltage-clamped at -60 mV. Submaximal responses at quisqualate receptors were evoked with agonist doses 300 x their NMDA receptor Kd.

NMDA receptor-mediated responses (w/ 3 μM glycine and no Mg) showed little or no desensitization. In contrast, QUIS receptor-mediated responses (w/ no glycine and 1 mM Mg) consisted of a rapidly desensitizing, low-affinity peak response, and a much lower amplitude, higher affinity steady-state response. All agonists tested had much higher affinity for the NMDA receptor than the QUIS receptor, with a >5-fold difference in Kd for the NMDA receptor as compared to the steady-state QUIS response and a >100-fold difference for the peak QUIS response.

Glutamate was the highest affinity agonist at both receptor subtypes and quinolinic acid the least. Several sulfur amino acids, including S-sulfo-L-cysteine and L-homocysteic acid, were relatively potent agonists at both receptors. Aspartate was the most selective of the endogenous agonists examined, with high affinity for the NMDA receptor (Kd=16.9 μM) and virtually no activity at the QUIS receptor.

M-Pos340

KINETIC EXPERIMENTS WITH COMPETITIVE NMDA RECEPTOR ANTAGONISTS. Morris Benveniste and Mark L. Mayer. Unit of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

Antagonism of NMDA activated currents was studied using mouse dissociated hippocampal neurons cultured for 10 to 15 days, and whole cell voltage clamp. The kinetics of NMDA receptor antagonism were measured by applying drugs with a multibarrel flow pipe, allowing rapid solution changes around the cell in less than 10 msec. Two competitive NMDA antagonists, CPP and D-AP5, have equilibrium Kd's which vary only 2.7-fold (CPP=0.46 μM and D-AP5=1.23 μM). However, the dissociation rate of CPP is almost 15-fold slower than that of D-AP5. The association rates for CPP (0.25×10^6 M^{-1} sec^{-1}) and D-AP5 (0.38×10^6 M^{-1} sec^{-1}) were similar, but these measurements may be slowed by the dissociation of NMDA, since following rapid removal of agonist the current decays with a mean time constant of 27 msec. Structurally, these two antagonists have similar functional groups: an ω-phosphonate moiety, and α-carboxylic acid and α-amine moieties; however, they differ in α−ω chain length (CPP=7, D-AP5=5). CPP, which contains a piperazine ring, is also much more constrained than D-AP5 which has a saturated hydrocarbon backbone. Kinetic evaluations of an unconstrained analogue of CPP (D-AP7), and a CPP analog containing only 5 atoms between the α carboxyl and ω-phosphonate, will also be presented.

M.B. is supported by a National Research Council Fellowship.
We thank Dr. J.C. Watkins for the CPP analogue.

M-Pos341

EVIDENCE THAT THE AGONIST-, LIPID-, OR LOCAL ANESTHETIC-INDUCED EQUILIBRIUM DESENSITIZED CONFORMATIONS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR ARE THE SAME.

Marjorie A. Moore and Michael P. McCarthy
New Jersey Center for Advanced Biotechnology and Medicine, and Dept. of Pharmacology
UMDNJ-Robert Wood Johnson Medical School

The pattern of 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine [^{125}I]TID incorporation into all four subunits of the acetylcholine receptor (AChR) is dramatically altered by agonist-induced desensitization. The same pattern of [^{125}I]TID incorporation is observed upon reconstitution of the AChR into lipids which have been shown to desensitize the AChR, on the basis of functional assays, and upon pre-equilibration with the desensitizing local anesthetics dibucaine and lidocaine, but not with the open channel blocker phencyclidine. The state of the AChR reconstituted into different lipids or in the presence of different local anesthetics was independently assayed by measuring the inhibition of α-bungarotoxin binding by carbamylcholine, with or without pre-equilibration with agonist. These results suggest that these quite disparate agents induce an equilibrium desensitized conformation of the AChR which is the same at the structural level, in agreement with earlier studies based strictly on functional measures of AChR conformation.

M-Pos342

NMDA-ACTIVATED CHANNEL CURRENTS IN THIN HIPPOCAMPAL SLICES. George J. Strecker & Meyer B. Jackson. Depts. of Physiology and Biology, UCLA, Los Angeles, CA, 90024. Outside-out patches were taken from CA1 pyramidal cells of intact thin slices of 8 day old rat hippocampus. Open times are well described by one exponential, and closed times of less than 200 ms, by two exponentials. Channel conductance is 53 pS, and a subconductance state of 33 pS is seen rarely. Magnesium (Mg) blocks open channels in a voltage and concentration dependent manner. The rate of block is $k_b = 2.7 \times 10^6 \exp(-mV/18)$ $M^{-1}s^{-1}$. The fast closed time decay constant shows no concentration dependence but does show voltage dependence $= 2860 \exp(mV/44)$ s^{-1}. The dissociation constant for Mg block is $K_d = 1.1 \times 10^{-3} \exp(mV/13)$ M. Apart from the voltage dependence produced by Mg, there appears to be voltage dependence in NMDA channel behavior that is independent of Mg. In nominally Mg-free solutions, the channel closing rate $\propto$ (1/mean open time) is $69\exp(-mV/33)$ s^{-1}. There also appears to be a weak voltage dependence in the duration of short closed times in Mg-free solutions, but this effect is not statistically significant.

M-Pos343

THREE "DESENSITIZATION" RESPONSES TO GLUTAMATE. Cha-Min Tang and Marc Dichter. University of Pennsylvania, Dept. of Neurology, Phila. Pa. 19104.

Desensitization can be an ambiguous term when used in the context of glutamate-activated current because it is used to describe three very different phenomena. Most commonly it is used to describe the rapid time-dependent decay of the peak current. A second phenomenon is the concentration-dependent suppression of the peak current by pre-application with low concentrations of glutamate. The K_d for this "steady state desensitization" is 10 uM. The K_d for activation of the peak current is close to 400 uM. This "steady state desensitization" has no effect on the persistent glutamate current. A third phenomenon is the concentration-dependent suppression of the persistent current as glutamate concentration is increased. Whether these three desensitization properties can be explained by a single mechanism at the microscopic level remains to be explored. Physiologically, the three phenomenon described by desensitization are likely to serve different regulatory roles.

M-Pos344

STRUCTURAL MODIFICATIONS TO A STABLE NICOTINIC CHOLINERGIC AGONIST AND A NEW ANALYSIS TECHNIQUE. A.A. Carter & R.E. Oswald, Dept. Pharmacology, Cornell University, Ithaca, NY 14853.

Systematic structural alterations to an agonist of the nACh receptor can affect its capacity for binding and channel opening. Single channel analysis of such derivatives can provide information on the role of more subtle agonist characteristics. A very stable agonist, 1,1-dimethyl-4-acetylpiperazinium iodide (PIP; Spivak et al. *Eur. J. Pharm.* 120, 127 (1986)), is capable of bearing several and varied substituents such as halogens and carbon chains, allowing a wide range of variability to the agonist's character. PIP has the geometric requirements of a nAChR agonist, and is held by its ring structure in approximately the correct configuration for binding. We have synthesized PIP, several substituted derivatives of PIP, and an open chain derivative which is no longer held in the correct configuration for binding. Single channel analysis of these compounds on BC_3H1 cells has been used to compare PIP to its derivatives and to correlate changes in agonist activity with structural modifications.

We are also using this data to compare a new method of exponential extraction from lifetime histograms with the conventional method of maximum likelihood and likelihood ratio testing. This new method, which employs the numerical method of linear prediction, allows an objective determination of the number of exponentials within the histogram. This may provide an easier, and perhaps more accurate, determination of kinetic models constructed from single channel data.

M-Pos345

INTRACELLULAR MAGNESIUM BLOCKS OUTWARD CURRENT THROUGH NEURONAL NICOTINIC ACh RECEPTOR CHANNELS

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Neuronal nicotinic acetylcholine receptors expressed by the rat pheochromocytoma cell line PC12 were studied by voltage clamp. The current-voltage relationship for the ACh-elicited current in these cells is non-linear and inwardly rectifying. Major features of the whole-cell I-V can be explained by the voltage dependence of channel gating and the non-linearity of the single channel I-V relationship. The concentration of intracellular Mg++ determines the extent of the inward rectification of the single ACh receptor channel current. With no intracellular Mg++ (20 mM EDTA), the single channel I-V is linear (i(+30mV)=1.6 pA). Increasing the Mg++ concentration reduces the outward current (with 3mM Mg++ inside, i(+30mV)=0.13 pA). The reduction of the outward current at +30 mV as a function of intracellular Mg++ concentration can be described by the classical Michaelis-Menton equation with a Kd of 290 μM for Mg++.

(Supported by R01 NS22356 and T32H107275)

M-Pos346

OPEN-CHANNEL BLOCK OF THE NICOTINIC ACETYLCHOLINE RECEPTOR AND THE NMDA RECEPTOR.

Mariano Amador, Jennifer Merchant, Thomas Reuhl and John A. Dani. Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

NMDA receptor channels from hippocampal neurons and nAChR channels from the cell line BC3H-1 or expressed in Xenopus oocytes are blocked by MK-801 and H-7. MK-801 is a well known anticonvulsant that blocks open NMDA channels. MK-801 (1-100 uM) also blocks open nAChRs in a voltage-dependent manner but, unlike in the NMDA receptor, MK-801 does not become trapped within the closed nAChR to a large extent. H-7 is a protein kinase inhibitor, but it also directly affects nAChRs and NMDA receptors. H-7 causes a voltage dependent slow block of open nAChRs, leading to fewer openings and shorter burst duration. ACh-induced macroscopic currents in the presence of H-7 have a smaller peak and a faster, more complete falling phase. H-7 causes a voltage dependent "flickery" block of single NMDA channels, but macroscopic currents show little effect. Great caution must be used when interpreting results with the kinase inhibitor, H-7. The difference in the action of open channel blockers may reflect different structural conformations of the two receptors during gating. Supported by MDA, Whitaker Foundation and NIH NS21229.

M-Pos347

ATROPINE ACTIONS ON NICOTINIC RECEPTORS: SINGLE CHANNEL ANALYSIS. J.C.T. Cuns[1], Y. Aracava[1,2] & E.X. Albuquerque[1,2]. (Intro. by L. Mullins) [1]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ, Brazil & [2]Dept. Pharmacol. Exp. Ther., UMAB, Sch. Med., Baltimore, MD.

Studies on endplate currents have shown that atropine and its analog scopolamine block neuromuscular transmission through noncompetitive mechanisms. Single channel currents activated by ACh (0.4 μM) were recorded under cell-attached patch-clamp conditions from dissociated interosseal muscle fibers of the frog *Leptodactylus ocellatus*. Atropine (1-40 μM) induced a concentration- and voltage-dependent shortening of mean channel open time. Brief, randomly spaced events, with no bursting pattern, were recorded. Single channel conductance was unchanged. The frequency of openings was only slightly decreased, and no desensitization-like pattern was observed. The results indicate an open channel blockade with a rather slow rate of drug dissociation. Recent evidence demonstrating conservation of ion channel proteins suggests that similar blockade may occur at central nervous system nicotinic receptors which could contribute to the pharmacological effects of atropine-like compounds. Support: FINEP/UMAB, CNPq, US Army Med. Res. Devel. Comm. Contract DAMD17-88-C-8119.

M-Pos348

NOREPINEPHRINE (NE) POTENTIATES THE NMDA RESPONSE IN RAT HIPPOCAMPAL NEURONS. Mônica S. Rocha and Ary S. Ramoa (Intro. by Albert Hybl) Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, Brazil.

Previous studies have shown that NE facilitates neuronal responses to glutamate application (*Brain Research* 490:64, 1989). To examine the effects of NE on the glutamate receptor selective to N-methyl-D-aspartate (NMDA), we have recorded ion currents from cultured rat hippocampal neurons using the outside-out patch clamp configuration. Response to NMDA was potentiated by application of NE (0.1-100 μM), as revealed by an increased frequency of single channel openings in 55% of 20 patches studied. NE also induced an increase in channel burst duration. These results are similar to those observed with glycine, an endogenous modulator of the NMDA receptor. Moreover, whenever NE failed to increase the NMDA response, glycine also was ineffective, suggesting a common mechanism of action. Thus, the facilitatory effect of NE on the glutamatergic response may be related to its modulation of NMDA receptor function.

Supported by CNPq, FINEP and Mol. Pharmacol. Training Program to E.X. Albuquerque.

M-Pos349

EFFECTS OF ACRIDINE ANALOGS ON THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR). F.C.A. Silveira[1], M.E. Nelson[1], C.M. Himel[1] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. and Exp. Therap., Univ. Maryland Med. Sch., Baltimore, MD 21201 and [2]Lab. Mol. Pharmacol., IBCCF, IFRJ, RJ, Brazil.

1,2,3,4-Tetrahydro-9-aminoacridine (THA) has been used to treat patients with Alzheimer's disease based on its ability to inhibit cholinesterase. THA and several analogs called acridine araphanes blocked indirectly elicited twitch in frog sartorius muscle. Effects on the AChR were further studied on endplate currents (EPCs). THA (10-50 μM), 1,2-propaneacridine araphane (0.5-2.5 μM) and 1,4-butaneacridine araphane (1.0-4.0 μM) depressed EPC peak amplitude and shortened the decay in a concentration- and voltage-dependent manner. These alterations were indicative of an open channel blocking effect. THA produced a double exponential decay. In rat soleus muscle these compounds (1-16 μM) enhanced desensitization to microionophoretic application of ACh, THA being the least potent. These AChR effects may be important to elucidate the basic mechanisms involved in therapeutic effectiveness of these agents in cholinergic diseases. Supported by US Army Res. & Dev. Comm. Contract DAMD17-88-C-8119.

M-Pos350

1,2-PROPANE-9-AMINO ACRIDINE ARAPHANE (1,2-PAA) BLOCKS NMDA CHANNELS IN RAT HIPPOCAMPAL NEURONS. M. Alkondon, C.M. Himel and E.X. Albuquerque. (Intro. by L. Goldman) Dept. Pharmacol. & Exp. Ther., University of Maryland Med. Sch., Baltimore, MD 21201.

Acridine compounds have been shown to be effective in treatment of cognitive disorders such as Alzheimer's disease. Since NMDA-type glutamate receptors appears to be implicated in the above disorders, we investigated the effects of 1,2-PAA on these receptors. Outside-out patch clamp recordings were made from cultured rat fetal hippocampal neurons. 1,2-PAA (1-10 μM) caused a concentration-dependent reduction in the mean channel open time at a voltage range of -40 to -100 mV. In addition, 1,2-PAA prolonged the burst time. An increase in the number of open events and a decrease in the long component of the closed time were evident at -60 mV and at more depolarized potentials. These results indicate that the unblocking rate of 1,2-PAA may be faster at depolarized potentials and slower at hyperpolarized potentials. The findings with 1,2-pAA on NMDA-activated single channel currents appear to resemble the sequential model proposed for nicotinic acetylcholine receptor/ion channel blockade. Supported by U.S. Army Med. Res. & Devel. Command Contract DAMD17-88-C-8119.

M-Pos351

NORTRIPTYLINE HAS POTENT DESENSITIZING EFFECTS ON NICOTINIC RECEPTORS. T. Tano, Y. Aracava, & E.X. Albuquerque. (Intro. by Terry Rogers) IBCCF, UFRJ, Rio de Janeiro, Brazil and Dept. Pharmacol. & Exp. Ther., Univ. Maryland, Sch. Med. Baltimore, MD.

In voltage-clamp studies using frog muscles, nortriptyline decreased endplate current peak amplitude in a voltage- and time-dependent manner indicative of closed channel blockade (*PNAS* 78:5240, 1981). In this work, nortriptyline's actions on kinetics of ACh-induced single channel currents were investigated. Cell-attached patch-clamp recordings were obtained from the perijunctional regions of interosseal muscle fibers of frog *Rana pipiens*. Nortriptyline together with ACh (0.4 μM) produced a marked decrease in frequency of channel openings. Neither the mean open time nor single channel conductance was affected. These results suggest a desensitization-like effect. Since Ca^{++} ions are known to modulate desensitization, we investigated the effects of nortriptyline (20-30 nM) in the absence of Ca^{++} (replaced by Mg^{++} plus 4 mM EGTA). Under these conditions, the desensitization-type effect of nortriptyline was decreased. Considering the homology among ion channel proteins similar actions may be expected at central nervous system synapses. Supported by FINEP, CNPq & NIH P50-MH44211.

M-Pos352

CHEMICAL KINETIC INVESTIGATIONS OF THE CHANNEL-OPENING PROCESS OF THE ACETYLCHOLINE RECEPTOR

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A new chemical kinetic method to investigate the channel-opening process of the acetylcholine receptor has been developed. An inactive, photolabile precursor of carbamoylcholine (caged carbamoylcholine) was designed and synthesized. It is equilibrated with BC_3H1 cells held in the whole-cell current recording mode. A laser pulse liberates carbamoylcholine (within 200 μs) and the resulting current is recorded and analyzed. Analysis of the rising phase of the current allowed us to determine the rate constants for channel opening and closing and the dissociation constant of the site controlling channel opening.

This work was supported by grants awarded to G.P.H. by the National Institutes of Health and the Cornell Biotechnology Program.

M-Pos353

REPTATION THEORY OF ION CHANNEL GATING. Glenn L. Millhauser, Dept. of Chemistry, University of California, Santa Cruz, CA 95064

Reptation theory is a highly successful approach for describing polymer dynamics in entangled systems. In turn, this molecular process is the basis of viscoelasticity. We apply a modified version of reptation dynamics to develop an actual physical model of ion channel gating. We show that at times longer than microseconds these dynamics predict an α-helix-screw motion for the amphipathic protein segment that partially lines the channel pore. Such motion has been implicated in several molecular mechanics studies of both voltage-gated and transmitter-gated channels. The experimentalprobability density function (pdf) for this process follows $t^{-3/2}$ which has been observed in several experimental systems. Reptation theory correctly predicts that channel gating will occur on the millisecond time scale and we believe that this is the first physical model to indicate this. We examine the consequences of reptation over random barriers and we show that, to first order, the pdf remains unchanged. In the case where the reptating helix is charged we show that the tail of the pdf will be exponential. We provide a list of practical experimental predictions to test the validity of this physical theory.

M-Pos354

GABA- AND GLYCINE ACTIVATED CL CHANNELS CAN PASS CATIONS. R. Numann and W. Nonner, Dept. of Physiology & Biophysics, University of Miami, Miami FL 33101.

Many Cl channels reveal a significant permeability for cations, whereas anion substitution experiments on GABA- and glycine activated Cl channels (Borman et al., J. Physiol. 385, 243) have indicated no cation permeability. Since cation permeabilities in other Cl channels are masked under anion substitution, we have determined cation/anion permeability ratios of single transmitter activated Cl channels exposed to salt gradients.

Inside- or outside-out membrane patches from cultured hippocampal neurons of rat embryo were superfused with 75-1200 mM NaCl or TRISCl (pipette: 150 or 300 mM) . Unitary currents activated by 0.5-10 uM GABA or glycine reversed at potentials smaller in size than $E_{Nernst,Cl}$ (e.g. +21 mV for 300//1200 mM NaCl (ext//in), and -20.5 mV for 300//75 mM, compared to the Nernstian +32 and -31.5 mV) reflecting a Na permeability. GHK permeability ratios varied with the ionic conditions; ranges were: P_{Na}/P_{Cl}, 0.14-0.52, and P_{TRIS}/P_{Cl}, 0.41-0.5. Thus, Cl channels activated by GABA or glycine under salt gradients can pass Na or TRIS cations. Supported by NIH GM30377.

M-Pos355

DESENSITIZATION OF THE SEROTONIN RESPONSE IN *XENOPUS* OOCYTES IS ENCODED BY LOW MOLECULAR WEIGHT mRNA. A. Walter, N. Davidson, J. Hoger, C. Labarca, L. Yu[1] and H. Lester. Division of Biology, 156-29, Pasadena, CA 91125.[1]Dept. of Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46223. Following injection with rat brain mRNA, *Xenopus* oocytes express several types of neurotransmitter receptors. A group of receptor types, including 5-HT_{1c}, respond to agonist binding by activating a G-protein, then phospholipase C, resulting in the production of IP_3 and diacylglycerol. Intracellular Ca is elevated by IP_3 and a Ca-activated Cl conductance is increased. These oocytes respond to 5-HT with a two-phase increase in their Cl conductance. The initial phase of the 5-HT response undergoes a prolonged desensitization with full recovery requiring 1 hr or more. In contrast, for oocytes injected with 1 ng of mRNA transcribed from the mouse 5-HT_{1c} receptor clone and tested with 1 nM 5-HT, the period of desensitization of the initial phase lasts 2 min. If, however, low molecular weight rat brain mRNA (< 2 kb) is co-injected with the 5-HT_{1c} receptor mRNA, oocytes exhibit no recovery from desensitization even 5 min after exposure to 5-HT. Thus, a protein(s) encoded by mRNA (<2 kb) partially reconstitutes the prolonged desensitization. This information may aid in the cloning and the characterization of the desensitizing factors. Support: GM-29836, GM-10991, Markey Trust and AHA.

M-Pos356

EFFECT OF ETHANOL ON CATION FLUX RESPONSE TO NICOTINE IN TORPEDO ACETYLCHOLINE RECEPTOR-RICH VESICLES

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We tested the hypothesis that partial agonists at the *Torpedo* acetylcholine receptor are fully effective at opening the ion channel but the observed flux response is reduced due to simultaneous self-inhibition over the activating concentration range. We measured nicotine elicited efflux of $^{86}Rb^+$ from AChR-rich native *Torpedo* vesicles in the presence of 0.25-2.0M ethanol using integrated flux assays (3msec-10sec).

The nicotine concentration-response curve is bellshaped and can be fitted to a biphasic equation allowing estimation of the apparent dissociation constants for activation (K_a) and inhibition (K_b). In both 10-second and quenched flow assays the presence of molar quantities of ethanol had little effect on K_b while decreasing K_a, thus significantly increasing the affinity of the activation site for nicotine. Ethanol dramatically enhanced maximal flux response until it was equivalent to that observed with a full agonist.

Rapid quenched flow was used to measure efflux kinetics. Nicotine alone elicited a very slow rate of efflux. However the addition of ethanol increased the rate upto 60-fold until it was equal to that of the full agonist carbachol.

Supported by an N.I.A.A.A. grant #07040 to K.W.M.

M-Pos357

EFFECTS OF STRYCHNINE ON RETINAL GANGLION CELL RESPONSES IN THE MUTANT MOUSE, *SPASTIC*

Charlene Patrick and Lawrence Pinto, Northwestern University, Neurobiology and Physiology Dept., Evanston, IL 60208

Glycine is a presumptive inhibitory transmitter in the retina. Tauck *et al* (88, Neurosci 27:193) observed a glycine-induced current, reversibly blocked by strychnine, in solitary ganglion cells from the mouse. Glycine receptors in the mutant mouse *spastic* are decreased (B_{max} reduced 80%) throughout the CNS, but K_D is unchanged (Becker *et al.* 86. J. Neurosci 6:1358). We recorded extracellularly from retinal ganglion cells in *spastic* and normal littermates in an isolated, superfused preparation. Action potentials were collected from on-center cells in response to a small, dim flashing spot centered in the receptive field. There were no gross alterations in receptive field organization. Application of 10nM strychnine resulted in a slight decrease in the amplitude of the light response in control retinas. In contrast, the light response in mutant retinas was nearly eliminated by the same dose of strychnine. This latter result cannot be explained by a simple decrease in the density of glycine receptors in the *spastic* retina. Supported by NIH.

M-Pos358

CHOLINERGIC STIMULATION OF PYLORIC SMOOTH MUSCLE CELLS ELICITS NON-SELECTIVE CURRENT Fivos Vogalis & K.M. Sanders (Intro. by N.G. Publicover). University of Nevada School of Medicine, Reno, NV, 89557. The action of acetylcholine (ACh) on resting conductance and on membrane currents was investigated in smooth muscle cells from the canine pylorus. ACh 10^{-5}M-10^{-4}M depolarized cells and decreased membrane resistance. When cells were voltage clamped at -60mV, ACh elicited an atropine-sensitive increase in holding current (I_{ACh}) averaging -10±2pA at 10^{-5}M (n=7) and -24±6pA at 10^{-4}M (n=9). The mean value of the reversal potential of I_{ACh} (E_{ACh}) was -14±2mV (n=12). Elevation of $[K^+]_o$ to 50mM by substitution for Na^+ shifted E_{ACh} positively (-9±3mV, n=4). I_{ACh} was not dependent on the Cl^- gradient and was abolished by external TEA (40mM) and internal Cs^+ aspartate$^-$. ACh also increase a component of outward current but had no effect on voltage-dependent Ca^{2+} current. In the whole cell voltage clamp configuration Single channel currents activated by ACh reversed at -12±3mV and had a mean slope conductance of 30±4pS (n=3). The non-selective current elicited by ACh may explain the depolarization response of pyloric smooth muscle to cholinergic stimulation. (DK 40569)

M-Pos359

[^{3}H]-MEPRAODIFEN MUSTARD REACTS WITH GLU-262 OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) α-SUBUNIT.

Steen E. Pedersen and Jonathan B. Cohen, Washington University School of Medicine, St. Louis, MO 63110.

Reaction of the noncompetitive antagonist meproadifen mustard (2-(chloroethyl-methylamino)-ethyl-2,2-diphenylpentanoate) with the desensitized AChR results in specific incorporation of label into the α-subunit. After cleavage of the α-subunit by CNBr, a labelled fragment corresponding to α244-278 was purified by gel-filtration HPLC. N-terminal amino acid sequencing revealed release of label in the 19th sequencing cycle, corresponding to Glu-262. This observation was corroborated by purification of a fragment that began at Thr-254 from a proteinase K digest of α-subunit and from which label was released at cycle 9. The amount of label released was consistent with the extent of labelling of the α-subunit, indicating that this residue, at the end of the M2 transmembrane segment, is the predominant site of labelling in the α-subunit. Thus, in the desensitized AChR, Glu-262 forms the part of the binding site that stabilizes the positive charge of the noncompetitive antagonists.

M-Pos360

MUTATIONS OF CYS451 IN THE M4 HELIX OF THE γ SUBUNIT OF ACETYLCHOLINE RECEPTOR DECREASE THE ION CHANNEL ACTIVITY.

Lian Li, Andrew Palma, Pamela Pappone*, and Mark McNamee. Depts. of Biochemistry & Biophysics, and *Animal Physiology, Univ. Calif., Davis, CA95616.

Site-specific mutation of Cys451 in the M4 transmembrane domain of the γ subunit of acetylcholine receptor (AChR) to either Ser or Trp was studied using AChR mRNAs injected into *Xenopus laevis* oocytes. Both single mutants displayed a 50% reduction in the normalized channel activity of 1 μM ACh-induced conductance per fmol of surface α-bungarotoxin binding sites. However, the mutations did not change the agonist binding properties as measured by the Hill coefficient of the acetylcholine dose-response curve and tubocurarine inhibition. Moreover, the mutations did not alter the shape of the current-voltage curve, blockade by noncompetitive antagonists, and the slow phase of AChR desensitization as determined by the current decay constant in voltage clamp experiments. This apparent uncoupling of the channel inhibition effects from the other functional properties, which were unaffected, is consistent with our previous results from the chemical modification (Yee et al., (1986), Biochem. 25: 2110). Preliminary patch clamp analysis showed that the mutations also changed the ion channel properties at the single channel level. The significant reduction in AChR ion channel activity associated with the above point mutations, especially the simple change of the -SH group on Cys451 to the -OH group, suggests that this thiol group on Cys451 in the M4 helix of gamma subunit may play an important role in AChR ion channel function. (Supported by USPHS Grant NS 22941).

M-Pos361

ALTERATION OF THE VOLTAGE DEPENDENCE OF ENDPLATE CURRENT DECAY BY IRREVERSIBLE ACETYLCHOLINESTERASE INHIBITORS.

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We have found that methanesulfonyl fluoride (MSF) decreased the voltage dependence of endplate current (e.p.c.) decay (T=24°C) in frog c.p. muscle (control, n=14; H=.0086 ± .0008 and MSF (10mM) treated, n=20, H=.0067 ± 0.0006; $p < .025$). H (mV^{-1}) is obtained from $\alpha(V_m)=\alpha_0(V_m=0)\exp(-V_mH)$, where $\alpha(V_m)$ is the rate constant of e.p.c. decay at the holding potential (V_m). At T=10°C the e.p.c. decay was prolonged, voltage dependence persisted (H=.0121 ± .0013), and MSF reduced H (.0078 ± .0013 $p < .005$). MSF had no effect on the peak e.p.c. at either temperature. We have previously shown that two organophosphate agents (217A0 and Tetram, 10μM) also greatly reduced H (control, H=.0108 ± .0023 and Tetram, H=.0008 ± .0006). Muscles treated with MSF and washed with Ringer's (1 hr) showed a further decrease in H after the addition of Tetram (.0013 ± .0008). These studies suggest that these agents had a direct effect on the ACh receptor.

M-Pos362

EFFICACY OF AGONISTS AT THE ACETYLCHOLINE RECEPTOR AS DETERMINED BY RAPID PERFUSION.

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Direct determination of agonist efficacy at nicotinic acetylcholine (ACh) receptors is hampered by rapid desensitization while calculations of efficacy from single channel data rely on assumptions about kinetic activation schemes. We previously described how these limitiations can be overcome by rapid perfusion of ACh to outside-out patches (1). Here, we extend these measurements to carbachol (Carb) and suberyldicholine (Sub). Rapid applications of 1 μM-50 mM Carb or 0.3-300 μM Sub were made to patches excised from BC3H-1 cells. Ensemble fluctuation analysis was used to determine the number of channels, single channel current and initial open channel probability. At -50 mV and 21°C, maximum open probability is 0.9 for Carb and 0.7 for Sub (compare with 0.93 for ACh). Kinetics of desensitization were also determined. The decay time constant decreases as a function of agonist concentration and saturates at 30-80 ms for all agonists. The time constant for recovery from desensitization is 200-500ms for ACh and 100-200 ms for Carb.

1. JPD & RS Brett, Biophys J. (submitted).

M-Pos363

PH EFFECTS ON *TORPEDO CALIFORNICA* NICOTINIC ACETYLCHOLINE RECEPTOR.

Andrew Palma, Lian Li, Xiaojiang Chen, Pamela Pappone*, and Mark McNamee. Depts. Biochemistry and Biophysics, and *Animal Physiology, U.C. Davis, Davis, CA 95616.

We studied the effects of extracellular pH on the nicotinic acetylcholine receptor from *Torpedo californica* (TnAChR) using single channel recording, two-electrode voltage clamping, and ion-flux measurements to assess the role of ionizable group(s) within the TnAChR in its function. Electrophysiological recordings were performed on TnAChR expressed in *Xenopus laevis* oocytes, while ion-flux was done on purified TnAChR reconstituted into liposomes. Observed effects were reversible over the investigated pH range of pH 5.5 to 9.0. The single channel conductance, whole cell current response, and ion-flux, all decreased at acid pH, with titration curves resembling that for a single weak acidic group. The channel open time and decay time constant of ACh-activated currents, measured respectively by patch and voltage clamping, gave bell shaped curves indicative of the involvement of more than one ionizable group in these processes. The two curves were similar and showed maxima between pH 7.5 and 8.0. The conductance data are consistent with a carboxylic acid residue as the titratable group, whereas more than one ionizable group influences channel gating properties. Supported by USPHS Grant NS 22941, NIH NS07300, and NIH AR34766.

M-Pos364

CATION PERMEABILITY OF 5-HT_3 RECEPTOR CHANNELS IN CULTURED N18 CELLS.

Jian Yang and Bertil Hille (Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195).

Ionic currents induced by serotonin were studied in N18 neuroblastoma cells using whole-cell voltage-clamp. Evidence that the current is mediated by 5-HT_3 receptors and that the receptors are ligand-gated channels is: The response 1) is blocked by ICS 205-930 or MDL 72222 (<10 nM), but not by 1 μM spiperone or ketanserin; 2) can be activated with a delay of <20 ms); 3) does not require internal ATP or GTP; 4) persists with internal CsF (90 mM), AlF_4^- or GTPγS (100 μM). The relative permeability of the channel to cations is determined from reversal potentials (table). Permeability ratios are very similar to that of the nAChR channel, suggesting a minimum pore size of about 6.5 Å. Supported by NIH grant #NS08174.

x	P_x/P_{Na}
Cs^+	1.18
K^+	1.07
Li^+	1.04
Rb^+	0.97
Ca^{2+}	0.25
Mg^{2+}	0.22
Ba^{2+}	0.17
Ammonium	2.74
Methylamine	1.42
Ethylamine	0.84
Dimethylamine	0.71
Methylethanolamine	0.38
Diethanolamine	0.24
Tris (pH = 7.0)	0.19
Glucosamine (pH=6.3)	<0.09

M-Pos365

FACTORS INFLUENCING VOLTAGE-DEPENDENCE OF N-METHYL-D-ASPARTATE (NMDA) CHANNEL BLOCK BY TILETAMINE.

NOWAK, L.M., & WRIGHT, J.M., Department of Pharmacology, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY. (Intr. by A. B. Ribera).

Ketamine, phencyclidine and the anticonvulsant MK-801 inhibit NMDA responses by interacting with a site on the activated NMDA receptor-channel complex and acting as slow channel blockers. Conflicting reports have appeared concerning the voltage-dependence or independence of the inhibition. In studies reporting voltage-dependence, attempts to localize the site of block with the potential field have placed it between 50% and 100% of the way through the field. We have examined these questions by measuring the effects of the dissociative anesthetic tiletamine on NMDA single channel currents in outside-out patches from mouse cortical neurons bathed in Mg-free saline using Cs-filled pipettes. Increasing tiletamine from 200 nM to 2 μM increased long closed times and decreased mean open time (τo) without introducing fast closures or effecting single channel conductance. The decrease in τ_o was virtually voltage independent; however probability of NMDA channel opening (nPo) decreased in a concentration and voltage-dependent manner between +50 and −100 mV. Thus, voltage dependence was primarily due to the slow exit of tiletamine from its blocking site rather than being in its association rate. Voltage-independent effects could be observed when patches were not permitted to equilibrate 5 to 10 minutes at each membrane potential. Results will be presented in terms of the underlying voltage dependence of NMDA channel nPo and the apparent voltage-dependence of tiletamine dissociation. Supported by NIH grant #NS24467.

M-Pos366

VOLTAGE DEPENDENT CHANGE IN PROBABILITY OF OPENING (nPo) OF N-METHYL-D-ASPARTATE (NMDA) CHANNELS IN MG FREE SOLUTIONS.

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In excised outside out patches, channels activated by 10 μM NMDA exhibit a voltage dependent change in nPo when the patch is depolarized. The amount of increase was highly variable among patches, ranging from 1.4 to 5.6 fold at positive potentials relative to −60mV. The average increase was 3.2 fold at positive potentials (N=11). The increase was due to 2 effects: an 18% increase in mean open time from 5.6 ms at −60 to 6.6 ms at positive potentials, and an increase in frequency of opening. nPo changed very little over the range of −70 to −40 mV. Neither inclusion of 2mM ATP in the pipet, nor reduction of the disulfide bond with 1mM dithiothreitol (DTT) in the bath affected the voltage dependence. However, DTT greatly increased efficacy of NMDA, primarily by increasing rate of opening. After DTT, mean open time was increased slightly at −60mV (6.6ms) and at positive potentials (7.74 ms). However, nPo doubled at +30mV compared with −60mV (n=4) and increased frequency of opening accounted for most of the increase. Supported by NIH Grant #NS24467.

M-Pos367

VOLTAGE SENSITIVITY OF NMDA-RECEPTOR MEDIATED POSTSYNAPTIC CURRENTS

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Excitatory synaptic transmission mediated by glutamate receptors was investigated by applying patch-clamp techniques to granule cells in thin hippocampal slices (Edwards et al., Pflügers Arch. 414, 600-12). Excitatory postsynaptic currents (EPSCs) were evoked by field stimulation of the perforant path. NMDA receptor mediated EPSCs were pharmacologically isolated by bath application of 5μM CNQX, a specific antagonist for glutamate receptors of the quisqualate-kainate type. The time course of decay of NMDA-EPSCs was strongly dependent on membrane voltage, being prolonged by depolarisation in an exponential manner. Both Mg_o^{2+}-dependent and Mg_o^{2+}-independent processes contributed to this voltage sensitivity. Through this mechanism, even moderate depolarisations prolonged the duration of NMDA-EPSCs, which is a critical determinant of neuronal function.