M-Pos89

LINKER INSERTION MUTAGENESIS: A NOVEL APPROACH TO THE STUDY OF GATING MECHANISMS IN VOLTAGE-DEPENDENT SODIUM CHANNELS.

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Previous mutagenic studies of channel gating have been based on *a priori* structural models of the channel (Stuhmer et al, 1989). Auld et al (1990) showed that a fortuitous mutant not expected to be involved in channel gating shifted the voltage dependence of activation. We have semi-randomly inserted oligonucleotide linkers into the rat IIa sodium channel cDNA in an effort to determine structure-function relationships in an unbiased manner. Mutations were introduced into the region proposed to be between segments S4 and S5 of domain II. When expressed in *Xenopus* oocytes, the mutant channels displayed shifts in the current-voltage relationship in the hyperpolarizing direction. In general, the mutants affected the voltage-dependence of activation. One mutant showed a shift in steady-state inactivation as well. Preliminary evidence suggests that the site mutated is part of or very close to the lining of the channel pore. Here, we describe a more detailed analysis of the mutants and the results of Hodgkin-Huxley model simulations to determine their actual effects on channel gating. Also we note the advantage of broader more general mutagenic approaches to study integral membrane proteins in the absence of a three dimensional structure.

M-Pos90

PRIMARY STRUCTURE OF A HUMAN CARDIAC SODIUM CHANNEL

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The predominant sodium channel in cardiac muscle is tetrodotoxin insensitive (TTX-I). We have previously cloned two rat skeletal muscle voltage-gated sodium channel alpha subunits, SkM1 and SkM2 which are tetrodotoxin sensitive (TTX-S) and TTX-I when expressed in oocytes, respectively. The SkM2 primary structure is identical to that of the rat cardiac sodium channel (RH1), (Rogart, R.B. et al., PNAS, 86, 8170-8174, 1989).

We have now screened 600,000 recombinants from a normal adult human heart cDNA library using a near full-length rat SkM2 probe. Twenty positive clones were plaque purified from which three overlapping clones were identified that encompass the entire coding sequence plus ~150 base pairs and ~2300 base pairs of of 5'- and 3'-untranslated (UT) region DNA, respectively. These clones were sequenced and the predicted amino acid structure of this human heart sodium channel (HH1) compared to those of rat SkM1 and SkM2 revealing approximately 72% and 92% overall amino acid sequence homology, respectively. Percent amino acid homology between the domain and interdomain regions of HH1 and the rat sodium channels is summarized below.

% Homology of HH1

Name	N-ter	D1	ID1-2	D2	ID2-3	D3	ID3-4	D4	C-ter
SkM1	57	70	21	79	28	80	86	86	45
SkM2	95	97	85	96	83	87	100	98	87

Antisense RNA transcribed from the 3'-UT region of HH1 was used as a subtype specific probe for Northern blot analyses. The probe hybridizes to a ~9.5kb transcript in human cardiac muscle RNA while no transcripts were detected in human quadriceps muscle RNA. A full length cDNA clone has been constructed for expression studies.

M-Pos91

CARDIAC AND NEUROBLASTOMA SODIUM CHANNELS HAVE DIFFERENT UNITARY CONDUCTANCES AND KINETICS

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Cardiac and nerve Na^+ channel isoforms are products of separate genes (Rogart et al. *PNAS* 86:8170-8174, 1989) and display a number of divergent characteristics. Some fundamental properties, e.g., unitary conductance (γ_{Na}), cannot be compared directly because data are not available under identical conditions. We compared Na^+ channels in cell-attached patches on freshly isolated, adult, rabbit ventricular myocytes and mouse NB2a neuroblastoma cells cultured with 10 μM retinoic acid for 2 d. Both cell types were bathed in (mM) 150 K aspartate, 1 EGTA, 5 HEPES (pH 7.4) at 10°C, and pipettes (Corning 7740) contained (mM) 280 NaCl, 2 $CaCl_2$, 10 HEPES (pH 7.4). γ_{Na} in NB2a was 22.9 ± 1.3 pS (n=5), significantly more than the γ_{Na} of 18.9 ± 0.9 pS (n=10) in heart. Extrapolated reversal potentials were 62 ± 4 mV in NB2a and 58 ± 4 mV in heart. Kinetic differences also were noted. In heart, the mean open time (MOT)-voltage relationship was markedly biphasic; MOT increased from 1.0 ms at -60 mV to 1.7 ms at -30 mV and decreased to 0.8 ms at 0 mV. In NB2a, MOT increased from 0.7 ms at -50 mV to 1.3 ms at -10 mV and decreased only slightly to 1.1 ms at 0 mV. Over this range, the O→I transition appears to be more voltage dependent in heart, and cardiac channel openings were more frequent at -50 and -60 mV. Rogart et al. (*Biophys J* 49:387a, 1986) reported that homogenized NB2a cells that were cultured with 10 μM retinoic acid for 2 d exhibit ~1:1 ratio of high and low affinity STX binding sites, a finding we confirmed on NB2a passages used for patch studies. In contrast, only one population of Na^+ channels was detected in patches made on the soma of NB2a cells. The NB2a Na^+ channels characterized by patch clamp corresponded to the high affinity binding sites. Inclusion of 100 nM TTX (n=10) or 50 nM STX (n= 5) in the pipette virtually abolished openings in NB2a cells; rare full-amplitude openings (e.g., 0.5-3 events/100 sweeps at -30 mV) in each of the patches exposed to STX or TTX verified the presence of Na^+ channels. We conclude that differences in cardiac and neural Na^+ channel γ_{Na} and kinetics reflect intrinsic properties of channel isoforms *in situ* rather than experimental conditions. These results and those of Weiss and Horn (*Ann NY Acad Sci* 479: 152-161, 1986) on cultured myotubes and myoblasts also suggest that γ_{Na} and TTX and STX affinities vary in parallel in natural Na^+ channel isoforms. Perhaps one protein modification alters both properties.

M-Pos92

FUNCTIONAL EXPRESSION OF RAT SKELETAL MUSCLE μ1 SODIUM CHANNELS IN A MAMMALIAN CELL LINE

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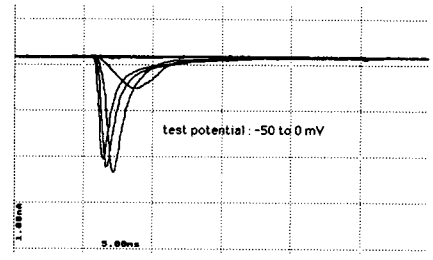
Characterization of cloned voltage-sensitive sodium channels expressed in stably transformed mammalian cell lines has been limited by the low abundance of functional channels on the cell surface (Scheuer et al., Science, 247, 854-857; C.U. & W.S.A., unpublished results).

We report reliable transient expression in a mammalian cell line of the μ1 skeletal muscle sodium channel. Voltage-activated, sodium selective currents of more than 2 nA were reliably obtained in a very high percentage of the cells in culture. These currents were blocked by low nanomolar concentrations of tetrodotoxin and the muscle-specific peptide toxin μ-conotoxin. Unlike currents expressed in *Xenopus* oocytes from μ1 cRNA, the currents show rapid inactivation kinetics. Studies with sequence specific antibodies to the μ1 channel (Ukomadu et al., Soc. Neurosci. Abstr. 16, 184 (1990) reveal that a core protein of M_r ~210 kDa is initially synthesized and then processed to a higher molecular weight product of M_r ~280 kDa.

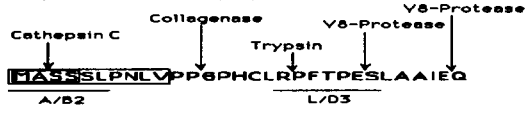
This approach promises to facilitate the characterization of normal and mutant forms of sodium channels in a mammalian cell background.



M-Pos93

EPITOPE MAPPING OF MONOCLONAL ANTIBODIES WHICH APPEAR TO DISTINGUISH SODIUM CHANNEL ISOFORMS IN ADULT SKELETAL MUSCLE. S. A. Cohen and R. L. Barchi. University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104.

Monoclonal antibodies A/B2 and L/D3 developed against purified adult skeletal muscle sodium channel protein produce different patterns of binding in immunocytochemistry of fast and slow muscle surface and T-tubular membranes, implying the presence of several channel isoforms in adult innervated muscle. Both monoclonal antibodies had previously been localized by proteolysis experiments to the amino-terminus of the sodium channel protein. Therefore, five partially overlapping peptides that together comprise the first 126 amino acids of the SkM1 sequence were synthesized and tested in RIA's against the antibodies. Both monoclonals bound to a peptide comprising the first thirty amino acids (I 1-30). For further localization, partial peptides comprising amino acids 25-30, 19-30, 13-30, and 7-30 were synthesized. The two monoclonals were again tested in RIA's against the original 1-30 peptide after preincubation with 1 uM of each of the partial peptides. Binding of L/D3 to I 1-30 was quantitatively inhibited by preincubation with partial peptides 19-30, 13-30, & 7-30 while A/B2 binding was blocked only with the intact 1-30 peptide, implying that L/D3's epitope encompasses aa 19-25 while A/B2's epitope encompasses aa 1-6; discontinuous epitopes can not be ruled out. Cathepsin C, an aminopeptidase, rapidly destroyed the A/B2 epitope, confirming its location within aa 1-10. V8-protease (cleaves aa 18-19) and trypsin (cleaves aa 23-24) rapidly destroyed the L/D3 epitope. Collagenase (cleaves aa 12-13) had no effect on antibody binding. Therefore, epitopes to both A/B2 and L/D3 are present in the SkM1 sequence and are in close proximity in the primary sequence. Their differential binding in immunocytochemistry may be due to the interaction of one or both of the epitopes with membrane cytoskeletal elements or channel beta subunits that varies with intracellular location.



Supported by NIH NS-08075, NS-18013, and the Pfizer Scholars Program for New Faculty

M-Pos94

DIVALENT CATION INTERACTIONS WITH VOLTAGE-GATED Na CHANNELS AT LARGE DRIVING FORCES. Beth B. Gangler and Bruce K. Krueger. Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Externally applied divalent cations produce voltage-dependent block of sodium channels (Woodhull, J. Gen. Physiol. 61:687, 1973). We have studied the nature of this block at extremely hyperpolarized membrane potentials where the channels are normally closed. Saxitoxin-sensitive, rat brain sodium channels were incorporated into planar lipid bilayers in symmetrical 500 mM NaCl and in the presence of both batrachotoxin and scorpion (*L. quinquestriatus quinquestriatus*) venom (O'Leary and Krueger, Molec. Pharmacol. 36:789, 1989). Under these conditions, the channels remain open from -190 mV to +130 mV. In the absence of divalent cations, the single channel current-voltage (i-V) relationship was linear with a conductance of 26 - 28 pS. Application of 15 mM Mg^{++} or Sr^{++} to the extracellular side caused voltage-dependent block that was well fit by the Woodhull model for a blocking site located about 30% of the way across the membrane electric field: fractional block increased monotonically as the membrane was hyperpolarized from -70 to -160 mV. In contrast, the i-V curve in 7.5 mM external Ca^{++} was not consistent with simple, voltage-dependent block. From -70 to -120 mV, Ca^{++} block was virtually identical to block by Mg^{++} and Sr^{++}, however, at potentials more negative than -130 mV, the block was relieved. From -150 to -180 mV, the single channel conductance was >20 pS. These results suggest that the open sodium channel is significantly permeable to Ca^{++}. As demonstrated by relief of block beyond -130 mV, very large hyperpolarizing potentials are necessary to drive the Ca^{++} inward through the channel. Supported by NIH grant NS16285 and a Howard Hughes predoctoral fellowship in biological sciences.

M-Pos95

SODIUM CHANNELS PURIFIED FROM SQUID MANTLE MUSCLES AND RECONSTITUTED INTO PLANAR LIPID BILAYERS.

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Voltage-dependent sodium channels have been purified and characterized from various vertebrate preparations. Although there seems to be a consensus for the main biochemical and biophysical properties of reconstituted sodium channels, it was worthwhile to investigate more primitive sources and in particular, sodium channels from the squid which has been subjected to intensive electrophysiological studies.

We began with membrane fragments from squid giant axons partially purified (25-fold) over a sucrose gradient but due to the limited availability of axonal tissue in the first instance, solubilization with Lubrol-PX was achieved with membrane fragments from mantle muscles. After ion-exchange (DEAE A-25) chromatography and ultrafiltration (Amico YM-100), the material was applied to a lectin (WGA) -Sepharose column. The elution with 100 mM N-acetylglucosamine delivered fractions with an average of 500 pmole TTX binding sites/mg protein.

The electrophoretic pattern of this 500-fold enriched preparation disclosed a major band at an apparent molecular weight of 260 KD, in a 5-20% gel, with no evidence for smaller subunits.

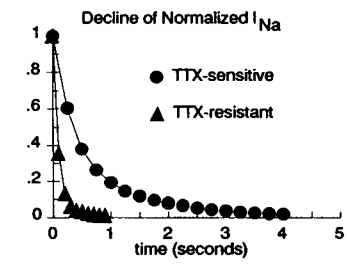
Aliquots of these fractions were fused with DOPE/POPC (7/3) virtually solvent-free lipid bilayers at the tip of patch-clamp pipettes. In symmetrical ionic conditions (0.5 M NaCl, pH: 7.4) and with 1 µM BTX in the pipette, a main single-channel conductance of 15 pS, with occasional substates and shifting between fast and slow gating modes, was retrieved. The mid-point of activation was -80 mV and the selectivity ratio PNa/PK = 7. Other bilayers yielded a much larger conductance, at 120-150 pS, as previously found with membrane fragments from squid giant axons.

The open configuration may well then be more flexible than accounted for by most current models.

M-Pos96

RATE OF LONG-TERM INACTIVATION DIFFERS IN TWO SUBTYPES OF Na CHANNEL IN VERTEBRATE SENSORY NEURONS. D.T. Campbell. Hatfield Marine Science Center, Oregon State University, Newport, OR.

Vertebrate DRG cells exhibit a wide range of action potential firing patterns. The largest cells can follow stimulus frequencies of several hundred Hz whereas small cells may be limited to frequencies of a few Hz. Differences in Na channel properties may contribute to these differences in action potential behavior: large cells exhibit only the fast-inactivating, TTX-sensitive Na channel subtype; whereas ~2/3 of small cells exhibit slowly-inactivating, TTX-resistant Na channels in addition to the TTX-sensitive channels. Repeated stimulation at 10-20 Hz with a 10 ms voltage clamp step to 0 mV causes little or no change in the TTX-sensitive I_{Na}, but causes a 50-75% decline in TTX-resistant I_{Na}. Long-term inactivation was studied using a pulse sequence in which a variable duration conditioning depolarization was followed by a 50 ms repolarization to -80 mV to remove short-term inactivation, followed in turn by a step to -10 mV to determine the amount of I_{Na} remaining. I_{Na} decreased ~exponentially with increasing duration of the conditioning pulse. At +10 mV, TTX-resistant I_{Na} inactivates 3-10 times faster (τ_{lt}=.1-.25 s) than the TTX-sensitive I_{Na} (τ_{lt}=.7-1.2 s). Recovery from long-term inactivation appears to be slightly faster for TTX-sensitive (τ=1-2.5 s) than for TTX-resistant (τ=2-3.5 s) I_{Na}, however determining its rate is complicated by the presence of even longer-term inactivation processes. Supported by USPHS grant NS22577.



M-Pos97

KINETIC FRACTIONATION OF GATING CURRENTS

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Previous attempts to "fractionate" ON gating currents (IgON) have employed the differential sensitivity of IgON components to immobilization by fast inactivation. About 1/3rd of the total gating charge (Q_{max}) appears to be "non-immobilizable". This "non-immobilizable" component (Ig_{ni}) shows rapid kinetics and is essentially complete within about 200 μs at positive potentials both in squid axons (Keynes, Greef & Forster, 1990, *P.R.S.* B 240:411-423) and crayfish axons (this poster). The relevance of fast Ig_{ni} to sodium channel gating has not been determined.

We demonstrate a *kinetic* fractionation technique which separates fast and slow components of charge movement in axons whose fast inactivation has been removed with chloramine-T. Following a 2 ms saturating depolarization, a 50 μs return to holding potential results in a truncated IgOFF. A second depolarizing step then shows only fast gating current (Ig_f). Apparently charge which returns rapidly at holding potential, also moves rapidly in IgON. However slow charge, which fails to return during the brief return to holding potential, is unavailable to contribute to secondary IgON. Ig_f is about 1/3rd of Qmax and appears kinetically identical to Ig_{ni}. However, Ig_f can "gate" as many as 90% of sodium channels during both deactivation and activation in chloramine-T treated axons.

(Supported by PHS grant #NS2115-04 and NIH RCMI grant #RR-03061)

M-Pos98

THE μI Na CHANNELS EXPRESSED IN OOCYTES HAVE AT LEAST THREE GATING MODES

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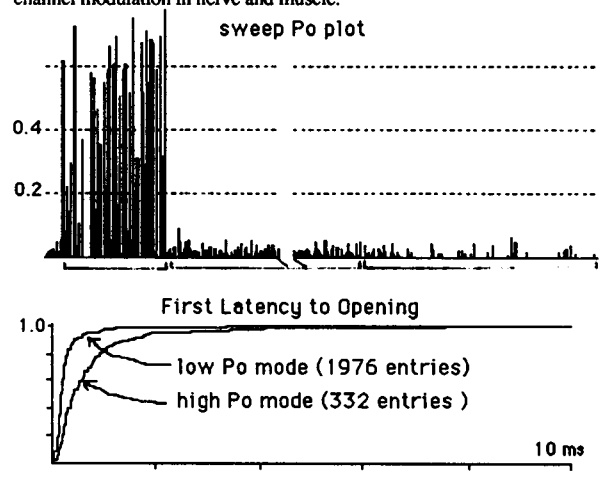
Cell attached patches containing a single μI Na channel were studied. A diary plot of channel open probability(Po) in each sweep shows that the channel switches between low Po (Po<0.1) and high Po(Po~0.5) modes. In the high Po mode, the channel opens in bursts and inactivates slowly; while in the low Po mode, it mostly opens only once and inactivates quickly. In addition, the first latency to opening is longer in the high Po mode. Complicating things further, the μI channel displays two distinctive behaviors in the low Po mode. In one, 80% of traces show openings, in the other, only 35% traces have openings. The former also has longer mean open time than the latter. The existence of these two low Po modes may be an artifact in oocyte, or it may provide a mechanism for Na channel modulation in nerve and muscle.



M-Pos99

PARALLEL AND SEQUENTIAL PROCESSES IN SLOW INACTIVATION OF SODIUM CHANNELS.

P.C. Ruben and J.G. Starkus. Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

Prolonged depolarization slowly immobilizes all gating charge (including the fraction which appears "non-immobilizable" by fast inactivation). Nevertheless, recovery from slow immobilization is rapid at -180 mV, and considerably faster than the recovery of sodium ionic current (I_{Na}). At -180 mV recovery of gating charge is complete within about 100 ms whereas less than 40% of sodium channels have recovered by this time. This result suggests that slow immobilization (of gating charge) is a separate process operating in parallel with slow inactivation (of I_{Na}).

By contrast, when holding potential (V_h) is used as the experimental variable, we can measure both the equilibrium availability of gating charge at positive test potentials (the $Q(V_h)$ curve) and the equivalent curve for sodium channels (the $F(V_h)$ curve). Careful comparison of the $Q(V_h)$ and $F(V_h)$ distributions indicates that both curves are indistinguishable in both slope and midpoint voltage. Thus slow immobilization and slow inactivation must be effectively coupled under equilibrium conditions.

We suggest that charge immobilization and slow inactivation result from separate mechanisms which are loosely coupled by electrostatic interactions between parallel voltage sensors.

(Supported by the American Heart Association, Hawaii Affiliate and PHS grant #NS21151-04 and NIH RCMI grant #RR-03061)

M-Pos100

VOLTAGE-SENSITIVE AND SOLVENT-SENSITIVE PROCESSES IN ION CHANNEL GATING: EFFECTS OF HYPEROSMOLAR MEDIA ON ACTIVATION AND DEACTIVATION KINETICS IN SODIUM CHANNELS.

J.G. Starkus, M.D. Rayner, P.C. Ruben and D.A. Alicata. Békésy Laboratory of Neurobiology, and the Department of Physiology, Univ. of Hawaii, Honolulu, HI 96822

Kinetic effects of osmotic stress on sodium ionic and gating currents have been studied in crayfish giant axons after removal of fast inactvation with chloramine-T. Internal perfusion with hyperosmolar media (addition of formamide or sucrose) slows sodium current activation but has no effect on tail current deactivation rate. Kinetics of ON and OFF gating currents are not affected by osmotic stress. These results provide confirmation for the separation of channel gating mechanisms into voltage-sensitive and solvent-sensitive processes recently proposed by Zimmerberg et al (1990 *Biophys. J.* 57:1049-1064) for potassium delayed rectifier channels. Additionally, the kinetic effects produced by hyperosmolar media seem qualitatively identical to the kinetic effects of heavy water substitution in crayfish axons (Alicata et al., 1990, *Biophys. J.* 57:745-758). However our observations are incompatible with models in which voltage-sensitive and solvent-sensitive gating processes are presumed to be either a) strictly sequential or, b) parallel and independent. We introduce a variant of the parallel model which includes explicit coupling between voltage-sensitive and solvent-sensitive processes. Simulations which demonstrate the characteristic properties noted in our data can be obtained using coupling energies as small as ~1/20th of kT.

(Supported by PHS grant #NS-21151-04, NIH RCMI grant #RR-03061 and American Heart Assoc. Hawaii Affiliate)

M-Pos101

LATE CURRENT THROUGH Na^+ CHANNELS MODIFIED BY FORSKOLIN

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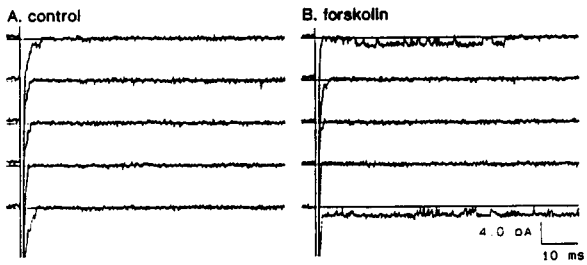
Recent experiments have shown that β-adrenergic stimulation inhibits cardiac Na^+ channels in the voltage-dependent manner (Am. J. Physiol. 256, C1131, 1989; 258, H977, 1990). We recorded canine ventricular cardiac Na^+ current in cell-attached patches with 280mM NaCl in the pipette and 150mM K^+ in the bath with forskolin (FSK) as an adenylate cyclase stimulator. Single channel currents were elicited by voltage steps from -150mV to 0mV. After application of FSK (15μM), the peak of the ensemble current was decreased by 13 ± 5.7% (n=5) and the decay was prolonged. Namely the first and second time constants (τ: 0.34 ± 0.01ms, 1.7 ± 0.6ms, n=3) were unchanged, but an additional third τ appeared (33.6 ± 5.7ms, n=3) with FSK, reflecting the enhanced incidence of late openings (see below). The open probability (P_o) histogram of the channels obtained during the late part of the sweeps (5-95ms) showed distinct augmentation of the channel activity with FSK. Average P_o increased from 1.3×10^{-4} to 9.5×10^{-4} (n=3). These observations suggest a role of cAMP-dependent phosphorylation in late Na^+ channel openings.



Consecutive sweeps in control (left) and FSK (right) demonstrate increase in P_o at late times with FSK. Peak currents (offscale) were 185pA and 163pA, respectively.

M-Pos102

MATHEMATICAL PERSPECTIVES OF THE RELATIONSHIP BETWEEN $\dot{V}_{max}$ AND I_{Na} IN CARDIAC MYOCYTES AT 37°C

Takafumi Anno, Akihiko Taniguchi, Masaki Shirakawa*, Shiro Usui*, and Junji Toyama

Res Inst of Environ Med, Nagoya Univ, Nagoya, *Dept of Infor & Comp Sci, Toyohashi Univ of Tech, Japan

The relationship of maximum upstroke velocity ($\dot{V}_{max}$) of cardiac action potential and peak sodium current (I_{Na}) has been shown to be nonlinear in low sodium gradient at low temperatures (<27°C)(Sheets et al.,1988). To examine such relationship under physiological conditions (37°C, 145mM $[Na]_o$), the upstroke of action potential and I_{Na} were recorded from enzymatically dispersed ventricular myocytes of guinea pig using the patch clamp technique. The upstroke was sampled at 1 MHz with 12 bit AD converter and differentiated by FIR digital filter. Action potential upstroke and phase plane plot (membrane potential vs upstroke velocity) at 37°C were compared with those by models of sodium channel (Beeler & Reuter, Drouhardt & Roberge). Based upon these models and sodium currents recorded at 37°C through the large cell-attached patch (Murray et al.,1989), a mathematical model structurally analogous to Noble-DiFrancesco was constructed for sodium currents. Our model could reproduce following experimental conditions: 1) voltage-dependence of availability for $\dot{V}_{max}$, 2) phase plane with different $\dot{V}_{max}$, 3) activation and inactivation kinetics fast enough to explain sodium currents at 37°C, 4) IV relationship and its descending limb over 40 mV. According to our simulation of voltage-dependent availability for $\dot{V}_{max}$ and I_{Na}, the relationship was considered to be fairly linear at 37°C.

M-Pos103

VERATRINE MODIFIED SODIUM CHANNELS IN HEART AND SKELETAL MUSCLE

P.P. Nanasi, A. Varro, S.H. Bryant, and D.A. Lathrop (Introduced by A.F. Martin) Dept. of Pharmacology & Cell Biophysics, Univ. of Cincinnati College of Medicine, Cincinnati OH 45267 and Dept. of Physiology, Univ. of Tromsø, Tromsø, Norway N-9001

Hille-Campbell triple vaseline gap voltage clamp and whole cell patch clamp techniques were used to examine the effects of veratrine (1 μg/ml) in isolated frog skeletal muscle fibers and in isolated rabbit ventricular myocytes, respectively. Veratrine revealed a slowly inactivating tetrodotoxin sensitive component of inward current in both preparations. Two major differences were found between cardiac and skeletal muscle. 1) In the cardiac myocytes the veratrine induced current displayed 2 exponential time constants for inactivation (128.3 ± 7.2 and 703.8 ± 49 ms) when activated by a test potential to -20 mV from a holding potential of -90 mV. In skeletal muscle, only one time constant for inactivation (509.4 ± 19 ms) was observed. 2) The veratrine-induced modification of sodium channels was strongly use-dependent in skeletal muscle, but not in cardiac myocytes. These data are consistent with the view that veratrine binding requires open sodium channels. The strong frequency-independent current component observed with veratrine in cardiac myocytes may be explained by the enhanced veratrine binding to the population of "window" sodium channels.

(Supported by NIH Grants NS-03178 and HL-37034)

M-Pos104

EFFECT OF ISOFLURANE ON SLOWLY INACTIVATING Na^+ CURRENT IN CANINE CARDIAC PURKINJE CELLS.

H Eskinder, F Supan, LA Turner, JP Kampine, and ZJ Bosnjak. Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226

In cardiac Purkinje fibers, Na^+ channel currents have a fast and slow component (Carmeliet, Pflugers Arch 408:18, 1987). The fast Na^+ current is responsible for the upstroke of the action potential while the slowly inactivating Na^+ current (s_INa) plays an important role in the plateau and repolarization phase. Inhalational anesthetic agents depress the fast Na^+ current in cardiac ventricular cells (Ikemoto Y et al., Jpn J Physiol 36:107, 1986). However, the direct effect of these anesthetics on s_INa is unknown. This study, therefore, examined the effect of isoflurane (0.51 and 1.04 mM) on s_INa in single canine cardiac Purkinje cells. Cells were dialized with pipette solution containing CsCl, and superfused with normal Tyrodes solution. Nifedipine (1 μM) and Ni^{++} (40 μM) were added to the external solution to block the inward Ca^{++} current, while veratridine (0.5 μM), a Na^+ channel agonist, was used to enhance the slowly inactivating component of the Na^+ channel current. Na^+ channel current was elicited by depolarizing pulses (400 ms) from a holding potential of -100 mV to stepwise (10 mV increments) more positive membrane potentials. The amplitude of TTX-sensitive Na^+ current was analyzed at 200 msec after voltage pulse initiation before, during and after the introduction of isoflurane. Slowly inactivating Na^+ current showed threshold activation around -80 mV and peak activation around -50 to -40 mV. Isoflurane dose-dependently depressed s_INa amplitude in the entire voltage range studied. Low and high concentrations of isoflurane suppressed peak s_INa by 36 ± 5 % and 54 ± 5 %, respectively. Reduction of s_INa by isoflurane may contribute to a decrease of action potential duration produced by inhalational anesthetic agents, and to a decrease of the functional refractory period for propagation of His bundle extra stimuli in the canine heart *in vivo*.

(Supported in part by NIH grant HL 39776)

M-Pos105

EVIDENCE FOR AN EXTERNAL BINDING SITE FOR COCAINE IN CARDIAC SODIUM CHANNELS.

William J Crumb Jr, Craig W Clarkson, Tulane University Medical School, New Orleans, LA

Recent studies have suggested the existence of an external binding site for local anesthetics on cardiac Na channels. In this study we tested this hypothesis by defining the effects of internally and externally applied cocaine on Na current (I_{Na}) at various internal pHs (6.6, 7.2, 9.2). I_{Na} was measured in guinea pig ventricular myocytes using the whole-cell variant of the patch clamp technique (16°C, $[Na]_i$=10 mM, $[Na]_o$= 25 mM). By manipulating pH_i, the fraction of cocaine (pKA=8.6) charged in the cell interior could be changed and the possible dependence of drug effect on drug form (charged/uncharged) could be studied. Cocaine (50 μM) applied externally (pH_o= 7.4; pH_i = 7.2) produced a significant use-dependent block evoked by thirty 20 ms pulses to -20 mV applied at 5 Hz, V_h = -140 mV (66 ± 4%, Mean±S.E., n=6, p<0.05). Estimated K_d values for cocaine binding to open and inactivated channels for externally applied cocaine were 19 and 8 μM respectively. However, internally applied cocaine (30-100 μM) at each pH_i produced neither significant use-dependent nor tonic block (n=5-7). Tonic block was estimated by comparison of the ratio of peak G_{Na}/C_m in the presence and absence of drug. Consistent with this lack of use-dependent effect, we could not define a significant time-dependent blocking effect of 30-100 μM internally applied cocaine on I_{Na} using a 2 pulse protocol with conditioning pulses to -20 mV ranging from 1 ms to 60 sec in duration and an interpulse interval of 0.3 sec (pH_i= 6.6, 7.2 & 9.2) (n=5-9 at each pH). We also could not define a significant drug-related slow component of recovery from block following a 10 sec depolarizing pulse in the presence of 100 μM internal cocaine. These results contrast with the observation of marked components of block development and recovery we have previously documented in the presence of 10-100 μM external cocaine (Biophys J 57:589-599, 1990). In contrast to cocaine, 50 μM QX572 applied internally did produce a significant use-dependent block of I_{Na} defined by a train of thirty 20 ms pulses applied at 5 Hz (28±7 %, n=4)(P<0.01 as compared to control). This suggests that the lack of effect of internally applied cocaine was not due to restricted drug access to the membrane. Our results indicate that application of high concentrations of internally applied cocaine do not produce a significant amount of Na channel block, whereas externally applied cocaine does. These data suggest that the Na channel binding site for cocaine in cardiac Na channels is more easily accessible from the external side of the membrane.

M-Pos106

GATING OF NON-INACTIVATING CARDIAC Na^+ CHANNELS: A 4 SUBUNIT ANALYSIS IN α-CHYMOTRYPSIN TREATED PATCHES.

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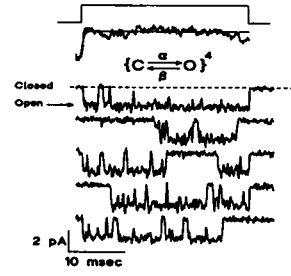
Voltage gated Na channels possess 4 putative transmembrane domains (S_4) containing numerous positively charged amino acids. These regions are postulated to comprise a portion of the channel gating voltage sensor. In addition, an intracellular amino acid loop connecting homologous domains III and IV is postulated be involved in channel fast inactivation. We have investigated channel gating after proteolysis of these intracellular regions. Guinea pig ventricular myocytes were obtained by collagenase dissociation. Giga-Ω seals were made on membrane patches using heat polished borosilicate glass pipettes. Patches were excised into a solution containing (in mM) CsF 110; NaF 10; CsCl 20; EGTA 10; HEPES 10. Extracellular (pipette) [Na] was 145 mM. Removal of fast inactivation was achieved by brief exposure of the intracellular surface to the proteolytic enzyme, α-chymotrypsin. Under these conditions, we analyzed channel gating with a simple 4 subunit activation gating model (Fig). Although a slow mode of gating was still present after removal of fast inactivation corresponding to a slow inactivation process, data which excluded this slow gating could be described by this model. Details of the analysis including the voltage dependence of the rate constants will presented. SUPPORTED BY NIH HL-40608.



M-Pos107

DEPRESSION OF I_{Na} BY TAURINE IN ISOLATED RABBIT CARDIAC MYOCYTES.

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Results obtained in our laboratory from Langendorff perfused whole hearts using the floating microelectrode technique suggested a reduction of I_{Na} by 20 mM taurine that also led to arrhythmias when depolarized by 10 mM K_o. To confirm the depression of I_{Na} we investigated the action of taurine at the cellular level with the patch clamp technique in isolated rabbit ventricular myocytes. Myocytes were obtained by enzymatic dispersion (collagenase) using the Langendorff retrograde perfusion technique. Experiments were performed at room temperature using 45 mM Na_o and the ionic channel blockers Cs^+ (5 mM) and Co^{++} (2 mM) in the extracellular medium (Tyrode, pH 7.4). Potassium in the pipette was replaced by Cs^+ (120 mM). A voltage dependent reduction of I_{Na} (30-60% with positive going membrane potentials) was observed in the presence of 20 mM taurine. Fitting a Boltzmann function to the voltage dependence of the steady state inactivation of I_{Na} revealed a significant shift of -4.3 mV (P<0.01, n=13) of the V_h parameter in presence of taurine. Such a shift of the voltage dependence of the steady state inactivation is compatible with a screening of surface charges by taurine at the cytoplasmic side of the membrane and/or a block of Na channels by taurine similar to the one known for local anaesthetics. (Supported by CRMC and FQMC, R.D. holds a FRSQ studentship.)

M-Pos108

LIDOCAINE INDUCED USE-DEPENDENT BLOCK OF HUMAN ATRIAL SODIUM CURRENT

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Use-dependence is an important characteristic of lidocaine (L) block of cardiac Na^+ channels. Bean et al. (1983) predicted that the voltage dependent increases in block following long pauses (tonic block) may affect use-dependent block (UDB). We, therefore, measured L (10-1000μM) induced UDB of human atrial Na^+ current (I_{Na}) of normal appearing, rod-shaped, striated, Ca^{2+} tolerant human atrial myocytes obtained by enzymatic isolation of right atrial appendage from 44 cardiac surgery patients. I_{Na} was recorded using whole cell patch clamp techniques at $[Na^+]_o$=5mM. The dose-response relationship at a holding potential (Vh) of -140mV indicated that L blocks I_{Na} by 1:1 binding (Kd=251μM). L induced UDB increased with concentration (10-1000μM), rate (0.5-3.0Hz) and pulse duration (3-100ms). During exposure to 200μM L, tonic block increased from 22±6% at Vh=-140mV to 47±5% at Vh=-120mV to 76±6% at Vh=-100mV (n=5). Conversely, UDB, at a pulse duration of 100ms, was greatest at Vh=-120mV and decreased proportionally at more positive Vh. The net result was that total block (tonic block+UDB) was nearly the same at Vh=-110 and -100mV. The time course of recovery from inactivation (RFI) at Vh=-140mV had fast and slow time constants of 7±2 and 75±15ms in the control solution (n=11) and 17±4 and 418±46ms in 20μM L (n=5) but only the slow phase (609±96ms) was present at L=200μM (n=6). The recovery rate of drug-affected channels was further reduced by depolarizing Vh. The results suggest that 1) L binds to both inactivated and activated Na^+ channels in human atrial cells; 2) L induces UDB is dependent on drug concentration, rate of stimulation, pulse duration and Vh; 3) at Vh positive to -110mV, the increase of UDB associated with the slowing of RFI was reduced by the development of tonic block at the same Vh.

M-Pos109

LIDOCAINE BLOCKS THE ACTIVATED STATE OF HUMAN ATRIAL SODIUM CHANNEL

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Lidocaine (L) induced use-dependent block of Na^+ current (I_{Na}) in mammalian heart is thought to reflect drug binding to activated and/or inactivated channels. All studies have been on animal models. There are no human heart data. We, therefore, used whole cell voltage clamp methods to study L effect on activated Na^+ channels in normal appearing, Ca^{2+} tolerant, enzymatically isolated human atrial cells. Specimens of right atrial appendage were obtained from 21 cardiac surgical patients. L (200μM) blocked I_{Na} in both a tonic and use-dependent manner. To determine the state-dependence of L block, the time course of block development during a depolarizing conditioning pulse was examined using a two-pulse protocol. The development of block had both fast and slow components at -20mV (τ_{fast}: 3.9±0.5ms, τ_{slow}: 173±20ms, n=11; mean±S.E.) but only one component at -80mV (τ: 387±20ms, n=5). To confirm L effects on the activated channel, we compared the amount of block after a single sustained (1-2,000ms) depolarizing pulse with that following short (3ms) repetitive pulses (30Hz). The amount of block was plotted against the total duration of depolarization during the conditioning pulses. Development of block at 100ms was significantly greater after short repetitive pulses (52±8% block) than following a single sustained pulse of the same duration (29±6% block, $p<0.01$, n=5). The voltage dependence of the fast component of block was studied using repetitive short (3ms) prepulses to various test potentials. The block exhibited voltage-dependence at potentials positive to -80mV and saturated at approximately -10mV (E_{mid}: -45.3±0.7mV). Voltage dependence of block was nearly identical to that of I_{Na} conductance (E_{mid}: -43.9 ±1.2mV, n=4). The results show that 1) there is substantial binding of L (200μM) to activated human atrial Na^+ channels and 2) drug binding to activated Na^+ channels appears to contribute to use-dependent block.

M-Pos110

EFFECT OF HYPOXIA AND SCORPION VENOM ON K CURRENTS IN CANINE PULMONARY ARTERY CELLS.
J.M. Post, E.K. Weir, S.L. Archer, N. Leblanc, and J.R. Hume. (Intro. by J. Peacock). Dept. of Physiology, Univ. of Nevada, Reno, NV 89557, and Dept. of Medicine, Minneapolis VA Medical Center, Minneapolis MN 55417.

Recent studies have shown hypoxia to reversibly inhibit K currents in carotid body cells (J. Gen. Physiol. 93: 1001) and scorpion venom (Leiurus quinquestriatus), a K channel blocker, to simulate hypoxia-induced increases in pulmonary arterial pressure (PAP) and contraction (FASEB J. 4: A813, 1990). The results suggest a possible involvement of K channels in hypoxic pulmonary vasoconstriction (HPV). Consequently, the effects of hypoxia and scorpion venom on K currents were investigated in freshly dispersed vascular smooth muscle cells from canine pulmonary artery. Using the whole cell patch clamp technique, application of voltage step or ramp protocols from a holding potential of -70 mV elicited time-dependent outward currents which reversed near the expected E_K. These currents contained a nisoldipine sensitive component suggesting involvement of Ca-activated K channels. After initial incubation of pulmonary cells in normoxic ($pO_2 \simeq 133$ mmHg) physiological saline solution (PSS), exposure to hypoxic ($pO_2 \leq$ 65 mmHg) PSS resulted in inhibition of outward K currents elicited by voltage step and ramp protocols. In a similar manner, application of scorpion venom (0.25 mg/ml) produced a rapid and reversible inhibition of these Ca-activated K currents. These results suggest that inhibition of K channels may initiate membrane depolarization and contribute to the increase in PAP pressure and vasoconstriction which results as a consequence of diminished alveolar oxygen availability. (Supported by VA, Minnesota Medical Foundation, NIH grant HL 30143 and the AHA).

M-Pos111

COMPARISON OF THE ACTIONS OF ACETYLCHOLINE AND LEMAKALIM (BRL 38227) IN THE GUINEA PIG CORONARY ARTERY.
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Acetylcholine (ACh) elicits endothelium dependent relaxation and hyperpolarization in the guinea pig coronary artery. The present study was undertaken to determine whether the hyperpolarization involves stimulation of ATP dependent K^+ channels. The contractile and electrical responses to ACh in isolated segments of coronary artery were compared to those of the putative ATP dependent K^+ channel agonist lemakalim (LEM). Both agonists produced concentration dependent relaxation of vessel segments contracted with the H_1 receptor agonist 2-(2-aminoethyl)pyridine. An IC_{90} of either vasodilator also produced 18-20 mV of membrane hyperpolarization. The magnitude of hyperpolarization with these two agonists was not significantly different. Glybenclamide (1-35μM), an ATP dependent K^+ channel antagonist, antagonized LEM induced relaxation but not ACh induced relaxation. In a like manner glybenclamide blocked the hyperpolarization produced with LEM but not with ACh. Glybenclamide (10-35 μM) also depolarized the smooth muscle membrane by 8-12 mV. Although both ACh and LEM relax and hyperpolarize the guinea pig coronary artery we conclude that they do so by different mechanisms. Whereas the actions of LEM are compatible with stimulation of ATP dependent K^+ channels in the smooth muscle membrane those of ACh must be due to some other mechanism.

M-Pos112

EFFECT OF CROMAKALIM AND LEMAKALIM ON Ca AND K CURRENTS IN COLONIC SMOOTH MUSCLE.
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The effects of cromakalim (BRL 34915) and its optical isomer lemakalim (BRL 38227) were investigated in intact tissue and freshly dispersed circular muscle cells from canine proximal colon. Cromakalim and lemakalim hyperpolarized resting membrane potential, shortened the duration and decreased the frequency of slow waves in intact tissue. Using the whole cell patch clamp technique, cromakalim, but not lemakalim inhibited L-type Ca channels. In addition, cromakalim, but not lemakalim was found to reduce peak outward K current. Nisoldipine also reduced the peak outward current, and in the presence of nisoldipine, cromakalim did not affect the peak outward current. The results suggest that cromakalim reduction of peak outward K current may be due to cromakalim inhibition of L-type Ca channels. Both cromakalim and lemakalim increased the magnitude of a time-independent K current. We conclude that the effects of cromakalim on membrane potential and slow waves in colonic smooth muscle may result from a combination of: 1) inhibition of L-type Ca channels, and 2) stimulation of a time-independent, background K conductance. In contrast, lemakalim appears to be a more pure K channel agonist because it stimulates a background K conductance without effects on Ca channels. The effects of these compounds on channel activity may explain the inhibitory effect of these compounds on contractile activity. (Supported by NIH grant DK 41315 and an AHA Grant-in-Aid).

M-Pos113

Mg^{++} AND ADP REDUCE THE ATP SENSITIVITY OF SKELETAL MUSCLE ATP-SENSITIVE K^+ CHANNELS RECORDED FROM SARCOLEMMAL BLEBS OF SPLIT FIBERS
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The regulation of skeletal muscle ATP-sensitive K^+ channels was studied using a new preparation of sarcolemmal blebs obtained by a purely mechanical procedure. These large hemispherical blebs appear spontaneously at the surface of a frog muscle fiber which has been split in half in a Ca^{++}-free relaxing solution. Using the patch-clamp technique in the excised inside-out configuration, we found that split-fiber blebs contained a high density of ATP-sensitive K^+ channels similar to those found in the surface membrane of other muscle and non-muscle cells. These channels were highly selective for K^+, had a conductance of ≈53 pS in 140 mM K^+, and were fully blocked by internal mM ATP.

Split-fiber blebs ATP-sensitive K^+ channels could be rapidly and reversibly blocked by glibenclamide (0.1-10 µM) in a dose-dependent manner. These channels were sensitive to ATP in the micromolar range in the absence of Mg. This sensitivity was noticeably reduced in the presence of millimolar Mg, most likely because of the ability of Mg^{2+} ions to bind ATP. Our data would therefore suggest that free ATP is a much more potent inhibitor of these channels than MgATP. Channel sensitivity to ATP was significantly reduced by ADP (0.3 mM) in a manner consistent with a competition between ADP, a weak inhibitor, and ATP, a strong inhibitor, for the same inhibitory binding sites.

These observations suggest that the mechanisms of nucleotide regulation of skeletal muscle and pancreatic ATP-sensitive K^+ channels are more analogous than previously thought.

M-Pos114

IONIC SELECTIVITY OF ATP-SENSITIVE K^+ CHANNELS FROM FROG SKELETAL MUSCLE INCORPORATED INTO PLANAR BILAYERS.

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We have found that plasma membrane vesicles prepared from bullfrog (Rana catesbeiana) skeletal muscle are a favorable preparation for routine incorporation and analysis of ATP-sensitive K^+ channels, K(ATP), in planar lipid bilayers. K(ATP) channels were identified by their unitary conductance and sensitivity to ATP. Under conditions of symmetrical 200 mM KCl, 10 mM Hepes, 1 mM EGTA and pH 7.2, single K(ATP) channels showed inwardly rectifying current-voltage behavior with a linear slope conductance of 62±3 pS at negative voltages. When applied to the cytoplasmic side, ATP (disodium salt, >30 μM) reversibly inhibited the channel currents. These channels exhibited "rundown". After incorporation the open state probability continuously decreased from 0.2-0.4 to zero over a period of 2 to 50 min. There was no apparent voltage-dependence of channel gating in the range of ±50 mV.

Under conditions of asymmetric external/internal KCl (mM) of 20/200, 40/200 and 200/80, reversal potentials shifted to -50±0.5, -34±1.0 and +20±3.0 (mV), and slope conductances at negative voltages were 53±2.5, 58±2.7 and 63 (pS), respectively. These reversal potentials are close to the expected values by the Nernst equation, indicating nearly ideal selectivity for K^+ over Cl^-.

We also measured single channel permeability ratios for alkali cations under bi-ionic conditions (200 mM external test ion/200 mM internal KCl). Permeability ratios were calculated from the reversal potentials using the GHK equation. The sequence of cation permeability relative to K^+ was $K^+(1)$, $Rb^+(0.478)$, $Cs^+(0.306)$, $Na^+(\leq 0.025)$ and $Li^+(\leq 0.022)$. This sequence is recognized as Eisenman's selectivity sequence IV and has been observed in other types of K^+ channels such as delayed rectifiers. (Supported by NIH AR38796 and HL38156).

M-Pos115

THE ATP-DEPENDENCE OF K_{ATP} CHANNEL KINETICS IN ISOLATED MEMBRANE PATCHES FROM RAT VENTRICLE

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The half-time ($t_{1/2}$) of current relaxations in response to step changes of [ATP] and of [K^+] ('concentration jumps') have been measured in inside-out membrane patches from rat cardiac K_{ATP} channels. A significant correlation between the $t_{1/2}$ of response to jumps of [K^+] and of [ATP] (Fig. 1A), suggests that diffusion-limited access of ATP to the membrane will be a major contributor to the time-course of such measured responses. We have attempted to fit the time-course of these current relaxations with possible models of K_{ATP} channel gating, taking diffusion into account. A single ATP-binding site model is inadequate to explain the data and a unit of two sequential binding sites ($\alpha\beta$ format), or a pair of such sequential units ($\alpha\beta\alpha\beta$ format, Fig. 1B), seems necessary.

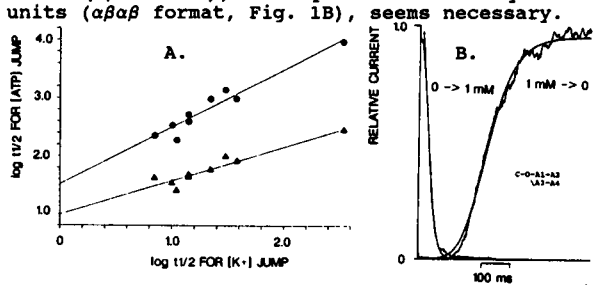

Fig. 1A. Log t1/2 (msecs) for current relaxation after [K^+] jump versus log $t_{1/2}$ for current relaxation after [ATP] jump (1 mM to zero, circles; zero to 1 mM, triangles). B. Time course of current changes following step from zero to 1 mM [ATP], and 1 mM to zero. Smooth curve is $\alpha\beta\alpha\beta$ model fit.

M-Pos116

"STOPPED FLOW" TIME RESOLUTION IN EXCISED PATCH CLAMP EXPERIMENTS?

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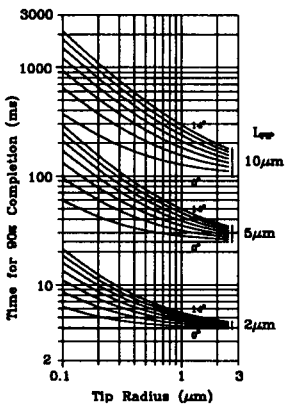
During giga-seal formation, a patch of membrane is drawn into the shank of the pipette, leading to a finite distance between the pipette tip and the majority of the patch. Thus while a change of solution at the pipette tip may be rapid, a delay occurs before the solution change is complete at the membrane surface. We have modelled the patch of membrane as a diffusion barrier at varying distances (L_{PIP}) from the opening of a right conical patch pipette. The figure shows the time taken for 90% solution change for varying values of L_{PIP} when the diffusing species has a diffusion coefficient of 10^{-5} cm²/s (each family of curves corresponds to cone half angles in the range 0° to 14°). Note how slowly the solution changes at the membrane compared to the speed at which solution can be changed at the pipette tip using the oil-gate technique (D. Qin & A. Noma. Am. J. Physiol. 257: H1624 -H1633. 1989). Channel activity during solution changes will reflect the convolution of d[Agonist]/dt at the membrane with the dose response function of the channel, so that channel activity may not simply reflect channel-agonist binding kinetics. These complications can be partly overcome by computer simulation methods. Supported by NIH HL 39733 and the Florida & Maryland Affiliates of the AHA.



M-Pos117

A POTASSIUM CHANNEL SENSITIVE TO ATP, CALCIUM AND VOLTAGE IN PORCINE CORONARY ARTERY.

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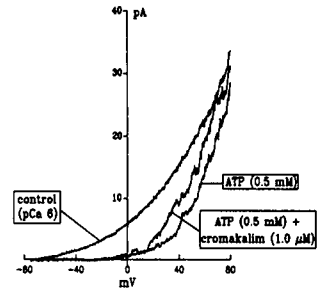
Potassium selective channels were studied in freshly dissociated porcine coronary artery smooth muscle cells using the inside-out variant of the patch-clamp technique. Voltage ramps in the range of -80 to +80 mV were applied at a rate of 25-40 mV/s. Ensemble current traces were elicited by averaging 50 consecutive current traces. The most abundant potassium channel ($K_{ATP,Ca}$ channel) had a conductance of 148 pS in a 5.4/140 mM K^+ gradient, at 0 mV, and was regulated by cytoplasmic ATP (0.05-3.0 mM), cytoplasmic Ca^{2+} (0.1-10 μM) and voltage. In $[Ca^{2+}]_i$ of 10 μM, channel activity was evident over the entire voltage range while in $[Ca^{2+}]_i$ of 0.1 μM, the threshold for channel activation was approximately +20 mV. ATP and AMP-PNP (0.5 mM) shifted the threshold for channel activation to more positive potentials, reducing the probability of channel opening (P_{open}), at 0 mV, by 87 and 92%, respectively. This inhibition was partially reversed by the addition of 0.5 mM ADP. ADP (2 mM) alone was a less effective inhibitor, reducing P_{open} by 46%, at 0 mV. As shown in the figure, cromakalim (1.0 μM) partially reversed the block induced by ATP (0.5 mM). The abundance and large conductance of the diversely modulated $K_{ATP,Ca}$ channel suggests that it may play a central role in the regulation of coronary artery tone.



(Supported by HL-35657; SDS is a Research Fellow of the American Heart Association, Florida Affiliate, Inc.).

M-Pos118

THE EFFECTS OF SODIUM 5-HYDROXYDECANOATE ON ATP-SENSITIVE POTASSIUM CHANNELS IN GUINEA PIG VENTRICULAR MYOCYTES.

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Glyburide (GLY) and sodium 5-hydroxydecanoate (5-HD), a proposed antiarrhythmic agent, are reported blockers of the ATP-sensitive K^+ channels (K_{ATP}). Recently we found that 5-HD (up to 1 mM), unlike GLY, was unable to reverse the vasorelaxant and cardiac action potential effects of cromakalim (CROM), an activator of K_{ATP} channels (McCullough and Normandin, J Mol Cell Cardiol, 22 [Suppl. 1]: S6, 1990). These results question whether 5-HD indeed blocks K_{ATP} channels. In the present study, we used whole-cell and single channel patch clamping techniques to study the effects of GLY and 5-HD on K_{ATP} in guinea pig ventricular myocytes. Consistent with previous observations, GLY (0.01-10 μM) blocked or reversed the CROM-induced increase in whole-cell outward K currents (n=6). 5-HD (100-500 μM) was ineffective at blocking these currents (n=6). We also investigated the effects of 5-HD on single K_{ATP} currents in inside-out patches from guinea pig ventricular myocytes. The K_{ATP} single channels had a conductance of 80-85 pS under conditions of symmetrical K^+ (inside: 140 mM KCl, 0.5 mM KH_2PO_4, 0.1 mM K_2-ATP, 10 mM K-HEPES, 3 mM EGTA, pH 7.2; outside: 150 mM KCl, 5 mM K-HEPES, 4 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.3). The probability of opening of these channels was increased by addition of 100 μM cromakalim. Exposure to 1 μM GLY completely inhibited channel opening. 5-HD (100 μM) reduced open probability to 0.14 ± 0.07 from a control value of 0.24 ± 0.07 (n=4, p<0.03). Our single channel results confirm earlier observations that 5-HD can block K_{ATP} channels (Notsu T, et at. J Mol Cell Cardiol, 21 [suppl 2]: S9, 1989). Furthermore, these studies may indicate that 5-HD modulates ATP sensitive K^+ channel activity at a site other than those at which glyburide or cromakalim interact to modulate channel activity.

M-Pos119

A Ca^{2+}-ACTIVATED K^+ CHANNEL FROM TRACHEAL SMOOTH MUSCLE IS MODULATED BY CROMAKALIM AND CYTOPLASMIC ATP.

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* Dept. of Pharmacodynamics und Toxicology, Univ. Graz, Austria. (intr. by Anton Hermann)

The effects of cromakalim on large conductance Ca^{2+}- and voltage-sensitive K^+ channels from rabbit tracheal smooth muscle were studied in inside-out patches. The observed channels had a slope conductance of 155 pS at 0 mV (K^+ gradient: 5.4/137 mmol/l), were sensitive to the Ca^{2+} concentration at the cytoplasmic side and open probability (P_o) increased with membrane depolarization. Ca^{2+}-dependent activation was very steep and occurred in the range of pCa 6.5 to 5.5. Addition of ATP (0.05-2 mmol/l) to the solution facing the cytoplasmic side of the patches reduced the channels' P_o, shifting the P_o-voltage curve to more positive voltages. Complete inhibition of channel activity was obtained with 2 mmol/l ATP at 0 mV and a pCa of 6. Cromakalim increased P_o in the presence of ATP, reversing the ATP-induced shift of the P_o-voltage curve. At an ATP concentration of 0.5 mmol/l and a pCa of 6, addition of 10 μmol/l cromakalim caused a 5.5-fold increase in the channels' P_o (0 mV). In the absence of ATP at the cytoplasmic side of the patches, cromakalim was ineffective even when channel activity was comparably low, the latter having been obtained by reducing the Ca^{2+} concentration.

Our observations suggest that the potassium channel activator cromakalim affects large conductance Ca^{2+}-activated K^+ channels in airway smooth muscle, in a manner depending on channel modulation by cytoplasmic ATP. Activation of this K^+ channel by cromakalim might be one mechanism underlying its bronchodilatory action.

M-Pos120

INVOLVEMENT OF G_s-ADENYLYL CYCLASE SYSTEM IN THE β-ADRENERGIC ACTIVATION OF Ca^{2+}-ACTIVATED K^+- CHANNELS IN GUINEA-PIG TAENIA COLI MYOCYTES.

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In freshly dispersed myocytes of the guinea- pig taenia coli, bathed in a low Ca^{2+}, 140 mM K^+ medium (pCa = 8), the β-adrenergic agonist isoproterenol (ISO, 2 μM) increases the open-probability (p_o) of the 150 pS Ca^{2+}-activated K^+- channels (Biophys. J. 55:471a '89). Cholera toxin (CTX) alone at 10^{-7} M increases the p_o of this channel also, probably via locking of G_s in its active form, and persistent activation of adenylyl cyclase. At 10^{-8} M, CTX suppresses the ISO- induced increase of p_o, no matter whether it is added before or after the application of ISO. Forskolin at 20-100 μM also increases the p_o, but not its analogue, 1,9-dideoxyforskolin, which lacks adenylyl cyclase stimulating effects. The kinase inhibitor, staurosporine (1 μM) suppresses the ISO-induced increase of p_o. In addition, Wiptide (10 μM), the protein kinase A inhibitor, applied intracellularly via diffusion from the patch electrode, suppresses the ISO-induced increase of whole-cell outward K^+-current during depolarization. All these observations indicate that ISO increases the p_o of the 150 pS Ca^{2+}-activated K^+-channels in the guinea pig taenia myocytes through the G_s-adenylyl cyclase system, most likely by phosphorylation of the channel protein. (Supported by NIH grants HD 00378 and DK 39731).

M-Pos121

THE SHAKING STACK MODEL OF ION CONDUCTION THROUGH THE CA^{++}-ACTIVATED K^+ CHANNEL.

Mark F. Schumaker, Dept. of Pure and Applied Mathematics, Washington State University, Pullman, WA 99164-2930.

We propose an ion conduction model which accounts for the high conductance and extreme selectivity of the Ca^{++}-activated K^+ channel - the shaking stack model. The motion of ions and their associated waters of hydration within the narrow, selective region of the channel is tightly coupled, forming a "stack". Ions can be gained or lost only singly, from the ends of the stack. Consistent with the results of Neyton and Miller (1988) on rat skeletal muscle channels, the stack would contain either 3 or 4 ions bound along a string of 4 ion binding sites. An important degree of freedom allows a stack of 3 ions to make a concerted shift from the first 3 ion binding sites to the last 3, eliminating a vacancy at one end of the stack and creating one at the other. Conduction occurs when an ion from solution on one side of the membrane occupies the fourth binding site, allowing the ion at the other end of the stack to come off into solution on the other side of the membrane. This conduction mechanism can achieve both high conductance and great selectivity. The concerted motion feature of the model results in higher conductance than would be obtained with single file diffusion if the rate constant for stack motion is assumed to be comparable to rate constants for individual ion movement. In addition, the shaking stack model does not require any substantial movement of chemical groups associated with the channel protein; only the stack motion itself and ion exchange with water at the ends of the stack are required. The concerted movement of the stack also has the effect of multiplying any difference in activation energy for ions to make a transition from one binding site to a neighbor. Slight differences in transition activation energies for different ion species would translate into larger differences for stack motion, resulting in enhanced selectivity. The shaking stack model is mathematically similar to a single vacancy conduction model with 2 binding sites. Results will be presented on the conductance, bi-ionic reversal potential, and flux ratio exponents associated with this model.

M-Pos122

EXPOSURE OF BOVINE AORTIC SMOOTH MUSCLE CELLS TO UTP CAUSES TRANSIENT ACTIVATION OF $P_{K,Ca}$ CHANNELS.

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The percent open time of $P_{K,Ca}$ channels within cell-attached patches may be increased from ~1% to peak values of 50-100% and then decline to control level (within 1-5 mins.) during exposure to UTP (.01-3 μM). Detectable transients are observed at 10-30 nM UTP in cells bathed in normal saline (in mM: 145 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, pH 7.0-7.3), however, in 150 mM K^+-saline threshold concentration of UTP is ~ 5 fold higher. Of the 4 other nucleotide triphosphates examined (ATP, CTP, GTP, and TTP) at concentrations up to 200 μM, only ATP was active but with a potency ~30 X < UTP. $P_{K,Ca}$ channels within excised patches did not respond to these nucleotides. In cell-attached patches bathed in Na^+, but not 150 mM K^+-saline, the transient increase in channel activity is accompanied by an increase in unitary current amplitude, most likely due to membrane depolarization. While this suggests involvement of a nucleotide-activated inward Ca^{2+} current, such as that described by Benham and Tsien (**Nature** 328, 1987). This could only account for a minor portion of the transient increase in P_{KCa} channel activity, since similar responses occur in 150 mM K^+-saline with 25 μM EGTA. In 1-2 mM Ca^{2+} containing salines repeated exposures to UTP or ATP elicit comparable responses with 5-15 mins. refractory periods. In the absence of Ca_o^{2+}, multiple responses cannot be elicited. This suggests that rechargeable intracellular Ca^{2+}-pools provide a major portion of a nucleotide-induced transient increase in cytosolic Ca^{2+} which in turn causes a transient increase in $P_{K,Ca}$ channel activity.

M-Pos123

PURIFICATION OF THE CHARYBDOTOXIN RECEPTOR FROM BOVINE AORTIC SMOOTH MUSCLE

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High affinity binding sites for charybdotoxin (ChTX) in bovine aortic sarcolemmal membrane vesicles can be solubilized in functional form in the presence of digitonin. A highly enriched preparation of ChTX receptor can be obtained after subjecting solubilized material to five different chromatographic steps (wheat germ lectin chromatography, Mono Q ion exchange chromatography, hydroxyl apatite chromatography, wheat germ lectin chromatography and sucrose density gradient centrifugation). Recovery of binding sites is 5-10% of the total activity found in membranes with an ~ 600-fold purification. This preparation displays all the properties that have been found in intact membranes for interaction of ChTX with its receptor. Thus, the K_d for [^{125}I]ChTX is unmodified, and the ability and potency of different agents to modulate binding (ie., ChTX, Iberiotoxin, TEA, K^+, Ba^{2+}, ionic strength) are unchanged. ChTX binding sites can be reconstituted into liposomes after digitonin removal in the presence of chaps. SDS-PAGE of the final preparation indicates the presence of a major band with an apparent M.W. of 35 kDa. This is consistent with crosslinking experiments utilizing [^{125}I]ChTX which indicate that a protein with the same apparent M.W. is specifically labeled. Since the apparent sedimentation coefficient of the functional ChTX receptor is high (approximately 30 S), this must represent a complex of the small molecular weight subunit. Efforts are underway to obtain amino acid sequence from the protein in order to clone it.

M-Pos124

PURIFICATION AND CHARACTERIZATON OF TWO NOVEL PEPTIDYL TOXINS DIRECTED AGAINST K^+ CHANNELS FROM VENOM OF NEW WORLD SCORPIONS.

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Venom of the scorpions *Centruroides limbatus* and *Centruroides margaritatus* contain activities that inhibit binding of [^{125}I]-charybdotoxin (ChTX) to both bovine aortic sarcolemmal and rat brain synaptic plasma membrane vesicles. Fractionation of these venoms by ion-exchange chromatography indicates that some of the fractions display preferential inhibitory activity against either the smooth muscle or brain binding sites. These receptors are believed to represent the high-conductance, Ca^{2+}-activated K^+ channel and a class of inactivating, voltage-dependent K^+ channel, respectively. The active fractions were subjected to further purification by reversed-phase high performance liquid chromatography techniques. Two peptides, one derived from *C. limbatus* and a second from *C. margaritatus*, have been purified to homogeneity and shown to inhibit binding of [^{125}I]ChTX to either smooth muscle or brain membrane sites, respectively, but do not cross react between receptors. In addition, *C. margaritatus* peptide, but not *C. limbatus* peptide, inhibits, at low nM concentrations, K^+ currents elicited by the rat lymphocyte KB3 clone expressed in *Xenopus* oocytes. The primary amino acid sequence of these two peptides has been determined by Edman degradation and shown to be unique. The peptide from *C. margaritatus* is homologous with noxiustoxin, while the peptide from *C. limbatus* is homologous with iberiotoxin. These two peptides, therefore, represent new tools with which to study the physiological role of specific classes of K^+ channels in defined tissues.

M-Pos125

EFFECTS OF INTRACELLULAR CALCIUM ON M CURRENT REVEALED BY INTRACELLULAR PERFUSION

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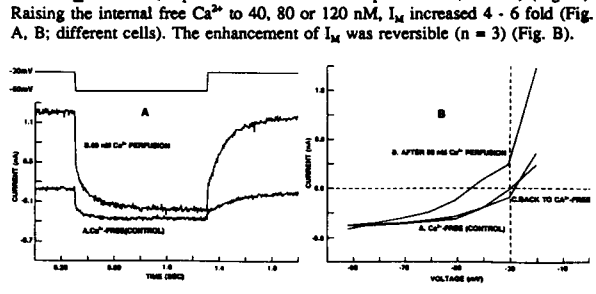
The role of intracellular free calcium in the regulation of M current (I_M) has been studied in dissociated bullfrog sympathetic ganglion B cells using whole cell voltage clamp and intracellular perfusion. Ringer contained 0.5 mM EGTA, 2.0 mM $MnCl_2$ and no Ca^{2+}. The intracellular solutions had 20 mM BAPTA or EGTA and 1.5 mM ATP. Electrode tip resistance was about 1 MΩ. Measurements of E_{Na} following changes of electrode Na^+ concentration showed that manipulation of intracellular ions could be completed within about 5 min. I_M was measured by 1 sec, -20 mV jumps from a holding potential of -30 mV. All recordings were made after at least 5 min of dialysis or perfusion. With Ca^{2+}-free internal solution, I_M was 0.1 ± 0.09 nA (amplitude of relaxation on repolarization; n = 35) (Fig. A). Raising the internal free Ca^{2+} to 40, 80 or 120 nM, I_M increased 4 - 6 fold (Fig. A, B; different cells). The enhancement of I_M was reversible (n = 3) (Fig. B).



Futher increase of free Ca^{2+} (300 nM) reduced I_M. Starting with 80 nM free Ca^{2+} and then changing to Ca^{2+}-free internal solution, I_M became very small or even disappeared after 5 - 10 min perfusion (n = 5). The I_M enhancement was much less prominent when ATP was excluded from the internal solution (n = 8). The time constant of deactivation of M channels was slower in 80 nM Ca^{2+} than in Ca^{2+}-free solution. Meanwhile the reactivation of M channels became faster in 80 nM Ca^{2+}. The data show that the magnitude of M current is affected by intracellular free Ca^{2+}. Possible involvement of surface potential shifts, Ca^{2+}-dependent protein phosphorylation and transduction pathways is under exploration.

M-Pos126

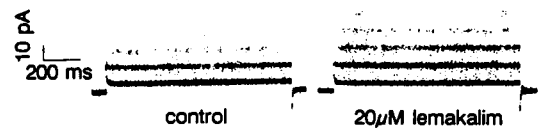
CYTOPLASMIC ATP INHIBITS AORTIC Ca^{2+}-ACTIVATED K^+ CHANNELS. **Craig H. Gelband and Cornelis van Breemen, Department of Pharmacology, University of Miami School of Medicine, Miami FL 33101 (Intro. by D.S. Weiss).**

We have previously characterized, using planar lipid bilayers, the effects of cromakalim, pinacidil, and glibenclamide, on the large conductance Ca^{2+}-activated K^+ channel (BK) of rabbit aortic smooth muscle, and now present evidence that this channel is inhibited by cytoplasmic ATP. In symmetrical 250 mM KCl (1 μM free Ca^{2+}), ATP (0.05-5 mM) inhibited single BK channel openings in a concentration-dependent manner when applied to the cytoplasmic face of the bilayer. At a membrane potential of +40 mV, P(open) decreased from a control value of 1.0 to 0.67 ± 0.03 and 0.05 ± 0.02 in the presence of 0.3 and 3.0 mM ATP, respectively (n=4). ATP decreased P(open) with a K_i of approximately 450 μM. P(open)-voltage relationships were fit by a Boltzmann relationship. ATP (500 μM) shifted the membrane potential where P(open) is 0.5, $V_{1/2}$ from -40.4 ± 2.0 to -13.7 ± 3.3 mV while having no effect on the slope of the activation curve (n=3). Experiments were performed to determine the interactions between various pharmacological agents and the aortic BK channel by the sequential addition of ATP, cromakalim, glibenclamide, and EGTA to the cytoplasmic solution. ATP (500 μM) reduced P(open) of single BK channels, while the addition of cromakalim (10 μM) increased P(open) to near control levels. Glibenclamide (10 μM) reversed the cromakalim stimulated increase in P(open). Addition of EGTA (2 mM) decreased P(open) demonstrating the Ca^{2+} sensitivity of the BK channel (n=4). Finally, in symmetrical 150 mM KCl (1 μM free Ca^{2+}), charybdotoxin (30 and 60 nM, extracellular) inhibited BK channel activity. At +40 mV, the percentage of channels blocked by 30 and 60 nM charybdotoxin was 25.8 and 58.3%, respectively (n=3). These data demonstrate that the BK channel may be a pharmacological site for putative vasodilators and constrictors and may play a role during ischemic events based on its sensitivity to changes in cytoplasmic ATP concentration. Supported by NIH HL-40184 and HL-07188.

M-Pos127

CROMAKALIM AND LEMAKALIM ACTIVATE A LARGE CONDUCTANCE, CALCIUM-ACTIVATED K^+ CHANNEL IN CANINE COLON CELLS. Susan M. Bowen, Andreas Carl, Craig H. Gelband, Kenton M. Sanders and Joseph R. Hume, University of Nevada School of Medicine, Reno, NV 89557.

Although cromakalim (BRL 34915) has been shown to activate an ATP-dependent K^+ channel in heart and pancreatic ß cells, it's mechanism of action in smooth muscle (SM) is controversial. ATP-dependent K^+ channels, large conductance, Ca^{2+}-activated K^+ channels, delayed rectifier K^+ channels and small conductance, Ca^{2+}-activated K^+ channels have all been suggested to be modulated by cromakalim and related compounds in vascular SM. We examined the effects of cromakalim and its (+) optical isomer, lemakalim (BRL 38227) on the activity of a 265 pS Ca^{2+}-activated K^+ channel in cell-attached and detached patches from canine colonic smooth muscle cells. In 3 of 4 inside-out patches (symmetrical solutions containing (mM): KCl 140, glucose 10, EGTA 1 and HEPES 10), 10 μM cromakalim increased channel mean open probability (Np(open)) from 0.06 to 0.09 at +50 mV. In cell-attached patches (pipette and bath solutions containing (mM): K-glutamate 140, KCl 10, $MgCl_2$ 2.3, glucose 5.5, EGTA 0.1 and HEPES 7.2) 20 μM lemakalim increased channel activity elicited by repetitive voltage clamp pulses applied from V_h = 0 to +50 mV (figure). In three patches mean Np(open) increased from 0.04 to 0.29. We conclude that cromakalim and lemakalim activate the large conductance Ca^{2+}-activated K^+ channel in mammalian colonic muscle. (Supported by NIH Grant DK 41315 and an AHA Grant-in-Aid).



M-Pos128

MECHANISM OF IBERIOTOXIN BLOCK OF THE HIGH CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNEL FROM AORTIC SMOOTH MUSCLE. K.M. Gianglacomo, M. L. Garcia and O.B. McManus. Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.

Iberiotoxin (IbTX), a peptide isolated from venom of the scorpion *Buthus tamulus*, exhibits 68% sequence homology to charybdotoxin (ChTX) and inhibits [^{125}I]ChTX binding to bovine aortic smooth muscle membranes (Galvez, *et al* 1990). IbTX also reversibly blocks high conductance calcium-activated potassium (BK) channels inserted into planar neutral lipid bilayers. Addition of IbTX to the external solution produced long non-conducting intervals (minutes) that were interspersed with periods of normal channel activity that presumably represent binding and unbinding of IbTX. The durations of the blocked and unblocked periods were described by exponential distributions. Increasing the IbTX concentration caused linear decreases in the mean unblocked times and no detectable changes in the mean blocked times. Thus, IbTX reversibly blocks the BK channel in an apparently bimolecular fashion with an inhibitor dissociation constant (K_i) of 1.5 nM in symmetric 150mM KCL at 40 mV. This tight interaction is derived from a slow dissociation rate constant (k_{off}),~2X10^{-3} s^{-1}, and a rapid association rate constant (k_{on}), ~10^6 M^{-1}s^{-1}. Block by IbTX was weakly voltage dependent; a 60 mV depolarization caused a 3-fold increase in k_{off}. Interaction of IbTX with the BK channel is also modified by ionic strength. When either external [KCl] or [NaCl] is varied from 25 to 300 mM, the K_i increases by ~10-fold due to a decrease in the k_{on}. When internal [KCl] is varied from 25 to 250 mM, the K_i also increases due to a ~10 fold increase in k_{off}. These findings suggest that electrostatic interactions promote association of IbTX with the BK channel while permeant potassium ions entering the channel from the intracellular side facilitate IbTX dissociation, as shown previously for ChTX (Anderson, *et al* 1988).

M-Pos129

STRUCTURAL REQUIREMENTS OF BOVINE PANCREATIC TRYPSIN INHIBITOR AND ITS HOMOLOGS FOR PRODUCTION OF SUBSTATES IN MAXI K(Ca) CHANNELS. G. W. J. Moss*, D. Laheru†, S. Wooden†, D. P. Goldenberg† and E. Moczydlowski*. *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510. †Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Mamba snake dendrotoxins and bovine pancreatic trypsin inhibitor (BPTI) are members of a family of ~60 residue proteins that we have found to induce discrete substate events in maxi K(Ca) channels when present on the "intracellular" side in the planar bilayer model system. Toward understanding this protein/channel interaction, a goal of our current experiments is to identify BPTI residues that make contact with the channel. Since BPTI is a potent Kunitz protease inhibitor for which the 3D structure of the trypsin-inhibitor complex is known, we asked whether the same contact residues (11-19, 34-39) of BPTI are involved in the K(Ca) channel interaction. In this regard we have found that the trypsin-BPTI complex is ineffective at producing substates in the single channel assay. Since most of the inhibitor's surface remains exposed to water in the BPTI-trypsin complex, this suggests that the trypsin contact region may be required for channel binding. However, we have also found that synthetic Kunitz inhibitor domain from the human amyloid precursor protein (which is a potent trypsin inhibitor and highly homologous to BPTI in the trypsin contact region) does not produce channel substates even at a concentration of 15 µM, as compared to the K_d of ~0.3 µM for BPTI. This implies that a functional trypsin contact site alone is not sufficient for binding to the channel. In addition, we have started to assess the contribution of individual residues in the protein/channel interaction using mutants of BPTI produced via a cloned bacterial expression system. Examination of seven BPTI mutants with replacements of single residues suggests that the association rate for substate production is correlated with net positive charge of the homologs: implying an important electrostatic effect in this process. In addition, one mutation outside the trypsin contact region Gly28→Lys was observed to lengthen the mean substate dwell time in some channels (range of 1.2-8.3-fold for 6 channels). These results suggest that residues both within and far from the trypsin contact site can influence the protein/channel interaction. (Supported by NIH AR38796, HL38156 and GM42494).

M-Pos130

CHARACTERIZATION OF Ca-ACTIVATED K-CURRENT IN RAT NEUROHYPOPHYSIAL NERVE TERMINALS. Gang Wang and José R. Lemos. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Since Ca^{2+}-activated K^+-currents play an important role in the patterning of activity in neuronal cells bodies, we studied this current in isolated rat neurohypophysial nerve terminals using the whole-cell patch clamp technique. In the presence of 4-amino pyridine, which blocks I_A in these terminals (Thorn et al., 1990), with 10 mM Ca^{2+} in the extracellular saline and 10 μM Ca^{2+} in the pipette solution, a large, voltage-dependent, slowly developing, outward current was elicited by depolarizing steps from holding potentials of either -90 mV or -50 mV. This current did not exhibit appreciable inactivation over the range of step durations (< 500 ms) tested. The threshold for activation of the current was -50 mV. It had a reversal potential of -77.9 mV, consistent with a potassium current under these conditions. When depolarizing to more than +50 mV, the amplitude of the current did not increase further, but reached a plateau. It's amplitude could be reduced by lowering either extracellular or intracellular Ca^{2+} or by rundown of Ca^{2+} currents. Thus it appears to be a Ca^{2+}-dependent K^+-current.

The outward current was not affected by 4-AP (7 mM), but could be blocked by low concentrations of tetraethylammonium (1 mM) and Ba^{2+} (0.2 mM). Neither apamin (80 nM) nor charybdotoxin (100 nM), both of which are considered specific blockers of Ca^{2+}-activated K^+-channels, could block the neurohypophysial terminal Ca^{2+}-activated K^+-current. Under current clamp, tetraethylammonium (1 mM) blocked the after-hyperpolarization of evoked action potentials in the terminals.

These results suggest that the outward K^+-current recorded in rat neurohypophysial nerve terminals is characteristic of Ca^{2+}-activated K^+-channels with the exception of the insensitivity to both charybdotoxin and apamin. (Supported by NSF grant BNS 8919790).

M-Pos131

RECONSTITUTION OF A Ca^{2+} ACTIVATED K^+ CHANNEL FROM AIRWAY SMOOTH MUSCLE INTO ARTIFICIAL MEMBRANES.
D. Savaria and E. Rousseau (Intro. by G. Bkaily), Dept. of Physiol. and Biophys., Fac. of Medicine, Univ. of Sherbrooke, Que. Canada J1H 5N4.

Microsomal fractions were prepared from canine airway smooth muscle (ASM) by differential and percoll gradient centrifugations. Vesicles population were characterized by bradykinin, ouabain and dihydropyridine bindings. Vesicles derived from the surface membrane were fused into planar lipid bilayers made of PE, DOTAP*, PC (5:3:2) in the presence of either KCl or K-Gluconate buffers (pH = 7.2). In our experiments the cis-chamber corresponds to the extracellular face. The presence of a K^+ channel which displays a high conductance 222 ± 18 pS in 150 mM K-gluconate was currently recorded. The open probability of this channel is voltage dependent, Po increasing with depolarizing potentials (-60 mV to +60 mV). Steps decreases in free trans Ca^{2+} concentrations (from 1 mM to 0.1 μM) reduce the Po values (at + 30mV) from 0.92 to 0.06. This effect is totally reversible. Cumulative acidifications of the trans-chamber from (7.2 to 6.2) induce a modification of the gating behaviour resulting in lower Po values. Furthermore these unitary electrical activities were inhibited by submicromolar CTX (cis) and 1 mM Ba^{2+} (trans). All of these properties are characteristic of a GK_{Ca}. Time analysis of single channel recording revealed the presence of 2 open and 2 closed time constants suggesting that the channel kinetic might be described by different models involving 2 open and 2 closed states. Our reconstitution experiments confirm that ASM surface membranes contains a GK_{Ca} as previously reported by McCann and Welsh (1986). This conductance might facilitate the repolarization of these smooth muscle cells. On the other hand, our approach would ease the characterization of other types of channels from ASM intracellular membranes.

*DOTAP: 1-2 Bis (oleoyloxy)-3 (trimethylammonio) propane is a cationic lipid used to increase the fusion rate.

E. Rousseau is a scholar of C.H.F.

M-Pos132

A PUTATIVE PYRIMIDINERGIC RECEPTOR IN BOVINE AORTIC SMOOTH MUSCLE CELLS: PERTUSSIS TOXIN AND PHORBOL ESTER SENSITIVITY. **M. Sanchez, G.M. Katz, T. Bale and J.P. Reuben. Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.**

UTP exhibits high selectivity over other nucleotides in evoking a transient increase in $P_{K,Ca}$ channel activity within cell-attached patches and this increase in channel gating is apparently due to release of Ca_i^{2+} (Sanchez et. al. these abstracts). Selectivity over other nucleotides (eg. UTP/ATP ≅ 30), threshold concentrations in the 10-30 nM range, and lack of dependence on Mg^{2+} for eliciting the transient response suggests a pyrimidine receptor. Attempts to identify coupling steps in the receptor-signalling process have revealed blockage of the response in $P_{K,Ca}$ channel activity by pertussis toxin and by a phorbol ester (PMA). Acute exposure (~ 3 hrs.) of a cell bathed in 150 mM K^+-saline to a high dose of toxin (200 ng/ml) causes progressive decline of peak % open time from › 50% to ‹ 2% with 20 mins. intervals in between exposure to 5 μM UTP. Cells exposed to toxin (20-200 ng/ml) for long periods (4-24 hrs.) before obtaining on-cell patches in a 150 mM K^+-saline did not respond to 5 μM UTP (n=8), while 6 of 7 control cells exposed to carrier-free toxin responded to 5 μM UTP with typical transient. The UTP-induced transient increase in unitary channel current amplitude (membrane depolarization) that is always observed in cells bathed in 5 mM K^+-saline was converted by toxin-treatment to a sustained increase through out the period of UTP exposure. While these data implicate a G-protein in the coupling process, subsequent steps have not been identified. However, the phorbol ester (0.1 to 1.0 μM) causes blockage of the increase in $P_{K,Ca}$ channel activity. The phorbol-induced increase in protein kinase C activity may decouple activated G-protein from phospholipase C (Smith et. al., **J. Biol. Chem.** 262, 6121, 1987) and thus implicating these enzymes in the signalling process.

M-Pos133

DIFFERENT Ca^{2+}- AND ATP-SENSITIVITIES OF Ca^{2+}-ACTIVATED K CHANNELS IN MEMBRANE PATCHES AND ARTIFICIAL BILAYERS. **M.L.Hall, A.Carl, B.W.Frey, C.L.Gentry, J.L.Kenyon, K.M.Sanders. Dept. of Physiology, University Nevada School of Medicine, Reno, NV 89557, USA**

We studied the large conductance Ca^{2+}-activated K channel from enzymatically dispersed smooth muscle cells from the circular layer of canine colon with the patch clamp technique. The properties of these channels were compared with those of a large conductance Ca^{2+}-activated K channel from a microsomal preparation from the same tissue incorporated in artificial lipid bilayers (5:3:2 mixture of PS:PE:PC).

In membrane patches, the channels had a slope conductance of 265±30 pS (n=31) in 140/140 mM KCl and were voltage dependent with a slope factor of 16 mV (n=3) for an e-fold increase in open probability. The holding potential for half maximal open probability ($V_{1/2}$) was 107±18 mV with 10^{-7}M $[Ca^{2+}]$ and -27±31 mV with 10^{-6}M $[Ca^{2+}]$ at the cytoplasmic site of the membrane. ChTX applied to the outer surface of the membrane blocked these channels and external TEA reduced the single channel amplitude with a voltage dependent K_D of 100-600 μM in low extracellular Ca^{2+} and 1 mM in high extracellular Ca^{2+}.

In artificial bilayers, the channels had a slope conductance of 270±22 pS (n=9) in 140/140 mM KCl and were blocked by TEA (trans) with a K_D of about 600 μM (n=6). These channels were substantially less sensitive to Ca^{2+} than those in native membranes : $V_{1/2}$ was +100 mV with 10^{-6}M Ca^{2+} and +60 mV with 10^{-5}M Ca^{2+} (n=6).

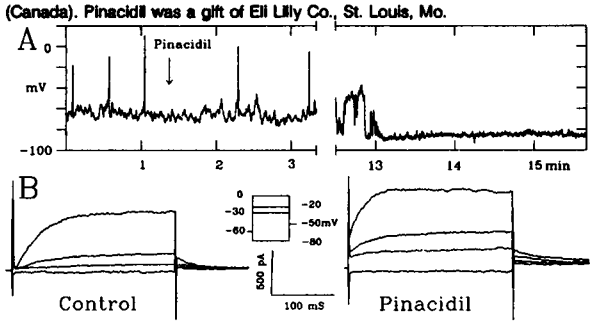
Recently Ca^{2+}-activated K channels in several smooth muscle preparations have been found to be inhibited by internal ATP. In our experiments, application of ATP up to 2 mM had no effect in membrane patches (n=10). However, 2 mM ATP (cis) in the bilayer reduced p(open) from .94 to .14 (n=3) at -25 mV and 10^{-5}M Ca^{2+}. (Supported by NIH-DK41315)

M-Pos134

PINACIDIL, A K^+ CHANNEL OPENER, APPEARS TO ENHANCE Ca^{2+}-ACTIVATED K^+ CURRENT IN ISOLATED SMOOTH MUSCLE CELLS

Alastair L. Miller & William C. Cole, Department of Physiology, Cardiovascular Sciences, University of Manitoba, St. Boniface Research Centre, Winnipeg, Manitoba, Canada, R2H 2A6.

The identity of the K^+ current(s) in smooth muscle cells (SMC) affected by K^+ channel opening drugs, such as pinacidil, is controversial. We isolated SMC from guinea-pig taenia coli and rabbit portal vein and monitored alterations in electrical activity and membrane currents via the whole-cell mode of the patch clamp technique. Bath and pipette solution contents, respectively; (in mM) NaCl 120, KCl 4.2, $NaHCO_3$ 25, KH_2PO_4 0.6, $MgCl_2$ 1.2, $CaCl_2$ 1.8, HEPES 10, pH 7.4 and K-gluconate 110, KCl 30, $MgCl_2$ 0.5, HEPES 5, EGTA 0.1, K_2ATP 5.0 or 10.0, pH 7.2). In current clamp (Fig. A), pinacidil (50-100 μM) first abolished spontaneous APs and then caused resting membrane potential (~-60 mV) to hyperpolarize by 20-25 mV and approach E_K. In voltage clamp (Fig. B), pinacidil caused; 1) a slight outward shift in holding current, 2) increased voltage-dependent outward K^+ current, i.e. both time-independent (I_{bkgd}) and time-dependent (I_t) K^+ current, 3) increased and decreased time constants for activation and deactivation of I_t, respectively, 4) a negative shift of 15 mV for 50% activation of I_t via analysis of tail currents, and 5) a negative shift by ~10 mV of the reversal potential for the steady-state I-V relation. The characteristics of the pinacidil-sensitive component strongly resemble Ca^{2+}-activated K^+ current in many SMC preparations. Further experiments will be performed to test the Ca^{2+} sensitivity of response to pinacidil in detail. Supported by Manitoba Heart and Stroke Foundation. WCC is a Scholar of the MRC (Canada). Pinacidil was a gift of Eli Lilly Co., St. Louis, Mo.



M-Pos135

GATING AND BLOCK OF Ca^{2+}-ACTIVATED POTASSIUM CHANNELS IN NATIVE ENDOTHELIAL CELLS FROM RABBIT AORTA.

J. Rusko, F. Tanzi, C. van Breemen and D.J. Adams. Dept. of Molecular & Cellular Pharmacology, University of Miami School of Medicine, Miami, FL.

Calcium-activated K channels in freshly dissociated endothelial cells from rabbit aorta were studied using the patch clamp technique. Unitary Ca^{2+}-activated K currents were recorded in whole-cell and excised membrane patches using an intracellular pipette solution containing (mM): 126 KCl, 5 NaCl, 1.2 $MgCl_2$, 0.7 K_2EGTA, 11 glucose, 10 HEPES-KOH, pH 7.4. The reversal (zero-current) potential of the unitary currents was dependent on the extracellular K^+ concentration and a single channel conductance of ~250 pS was obtained in symmetrical 140 mM K^+ solutions (23°C). In inside-out membrane patches, the Ca^{2+} sensitivity of this channel was examined at +20 mV and the open probability (P_o) determined to be <0.01 at pCa 7, ~0.5 at pCa 6 and >0.9 at pCa 5. Rundown to 10% of normal channel activity was consistently observed to occur within ~ 5 min at pCa 6 but was absent at pCa 7 and pCa 5. In the whole-cell recording configuration, spontaneous transient outward currents (s.t.o.cs; >50 pA at +20 mV) and unitary Ca^{2+}-activated K currents were observed under resting conditions and the amplitude and frequency of these currents were voltage dependent. Bradykinin (1 μM) evoked a biphasic increase in the P_o of Ca^{2+}-activated K channels and the amplitude and frequency of s.t.o.cs. Ca^{2+}-activated K channels and s.t.o.cs were inhibited by extracellular tetraethylammonium (TEA^+; 0.5 mM), tetrabutylammonium (TBA^+; 0.5-5 mM) and charybdotoxin (100 nM) and completely blocked by 5 mM TEA^+. Bath application of Ba^{2+} (10 mM), 3,4-diaminopyridine (1 mM) or apamin (0.1-1 μM) failed to inhibit unitary Ca^{2+}-activated K currents or s.t.o.cs. Furthermore, the removal of extracellular Ca^{2+} (0 Ca^{2+} + 1 mM EGTA) both in the presence and absence of bradykinin stimulation failed to inhibit Ca^{2+}-activated K channel activity suggesting that Ca^{2+} release from intracellular stores may sustain Ca^{2+}-activated K currents in freshly dissociated endothelial cells. The conductance, Ca^{2+} sensitivity and pharmacological profile of this channel is consistent with the large conductance Ca^{2+}-activated K (maxi-K) channel observed in other preparations. It is suggested that the simultaneous activation of Ca^{2+}-activated K channels underlie the s.t.o.cs observed in freshly dissociated endothelial cells. The activation of Ca^{2+}-activated K channels in endothelial cells maintains an electrochemical gradient for Ca^{2+} entry which may serve to promote the sustained release of vasodilators in response to neurohumoral and physical stimuli. (Supported by NIH grant # HL39831).

M-Pos136

K^+ CHANNELS FROM THE SEA URCHIN SPERM (*S. purpuratus*) RECONSTITUTED ON BLACK LIPID BILAYERS.

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K^+ channels are implied in many cellular functions such as secretion and membrane resting potential. In sea urchin sperm, it has been recognized that K^+ fluxes play an important role, probably in chemotaxis and during the acrosome reaction (AR). Elevating (K^+) in sea water to 20 mM or adding TEA (10 mM) strongly inhibits the AR (Schackmann and Shapiro, Dev. Biol. 81:145).

Liévano and coworkers (Dev. Biol. 112:253) using the tip dip technique reported the presence of K^+ channels in the plasma membrane of *S. purpuratus* sea urchin sperm. Here we describe the characterization in black lipid membranes of two K^+ channels of 100 and 130 pS of unitary conductance. Both channels were found in plasma membranes isolated from sperm flagella. The two channels were sensitive to 10 mM TEA, only if the blocker was added to the *trans* side. The 130 pS channel follows the permeability sequence $K^+ > Rb^+ > Na^+ > Li^+$, and the 100 pS one $K^+ > Rb^+ > Na^+$. In addition, the 100 pS channel was blocked by noxiustoxin in the *cis* side. This scorpion toxin blocks K^+ channels in the squid axon (Carbone *et al.*, Pflügers Archiv. 408:423). The 130 pS channel is slightly voltage dependent with an estimated effective gating charge between 0.5 and 1, displaying longer openings at negative holding potentials (voltage applied in *cis* compartment). This work was supported by grants from WHO, CONACYT and a fellowship to A. Darszon from the John Simon Guggenhein Memorial Foundation.

M-Pos137

DM-9384, A NEW COGNITION-ENHANCING AGENT, FACILITATES LONG-LASTING (TYPE II) Ca CHANNEL CURRENTS IN NG108-15 CELLS.
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The newly-developed cognition enhancer, DM-9384 (a cyclic derivative of GABA), is capable of increasing transmitter release from GABAergic neurons in the brain (Watabe et al., Soc. Neurosci. Abstr. 15, 601, 1989). There, Ca channels responsible for the transmitter release might be potentiated by the drug. In the present study, we have investigated the effect of DM-9384 on Ca channels in neuroblastoma-glioma hybrid (NG108-15) cells. Like neuroblastoma (N1E-115) cells the hybrid cells have two types of Ca channels, one of which (type II) is known to be facilitated by intracellular cAMP (Narahashi et al., J. Physiol. 383, 231-249, 1987). The type II channels are supposed to be identical to those involved in the transmitter release. Ca channel currents as carried by Ba^{2+} (50 mM) were recorded using a whole-cell variation of the patch-clamp technique.

Transient currents through type I channels were induced at potentials positive to -50 mV and monitored at -20 mV. Long-lasting currents through type II channels were induced at potentials positive to -20 mV and monitored at +10 mV. In many cases, DM-9384 (1 μM) applied to the bathing saline remarkably increased the amplitude of type II currents to more than 200% without altering their time course. Type I currents remained unchanged. The action of the drug seemed independent of the holding potential in the range between -80 and -40 mV. In the cells treated with dibutyryl cAMP (1 mM), DM-9384 failed to enhance type II currents. Type II currents once increased by DM-9384 were not further enhanced significantly by dibutyryl cAMP. It is concluded that DM-9384 facilitates the activity of type II Ca channels, possibly by involving protein kinase A-dependent channel phosphorylation.

M-Pos138

CALCIUM CURRENT IN ACUTELY DISSOCIATED CA1 HIPPOCAMPAL NEURONS: BLOCKADE BY (-)- BUT NOT (+)-PENTOBARBITAL.
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Barbiturate block of Ca^{2+} channel current in enzymatically dissociated CA1 neurons from the hippocampi of adult guinea-pigs was examined using whole-cell patch clamp techniques with 3 mM Ba^{2+} as the charge carrier. Na^+ current was blocked with tetrodotoxin and K^+ currents were eliminated by using tetraethylammonium and *N*-methyl-D-glucamine as the predominant extracellular and intracellular cations, respectively; the pipette solution also contained Cs^+-BAPTA and Mg^{2+}-ATP. I_{Ba} was evoked with depolarizing command steps from a holding potential of -80 mV to a test potential of -10 mV. (-)- and (+)-Pentobarbital (PB) and phenobarbital (PHB) were applied by rapid superfusion. (-)-PB reversibly depressed peak I_{Ba} with an IC_{50} of 3.6 μM. In contrast, (+)-PB was ineffective at concentrations as high as 1 mM. PHB also inhibited I_{Ba} but it was weaker than (-)-PB (IC_{50} ≥50 μM). The inhibition of I_{Ba} by (-)-PB (an anion at physiological pH) was voltage-dependent with the fractional block increasing at positive membrane potentials. Analysis according to the method of Woodhull indicated that the (-)-PB blocking site senses 52% of the transmembrane electrostatic field [K_D(0 mV), 6.6 μM]. The time course and voltage-dependence of activation of I_{Ba} was unaffected by (-)-PB, although the rate of deactivation of the current was accelerated by the drug. Inactivation during a 1 sec step was well fit by the sum of two exponential functions; (-)-PB enhanced the rate and extent of this inactivation. The major effect of (-)-PB was to decrease τ_{slow}. Steady-state inactivation of I_{Ba} showed an inverted U-shaped voltage dependence which is compatible with a 3-state cyclic model of channel gating with rate constants depending only upon voltage (see S.W. Jones & T.N. Marks, *J. Gen. Physiol.* 94: 169-182, 1989). Analysis of the barbiturate block according to this scheme suggested that (-)-PB may specifically slow the rate of transition of the channel from the inactivated to the open state. We conclude that (-)-PB produces a potent, stereoselective block of the low voltage activated, partially inactivating Ca^{2+} current in hippocampal CA1 neurons. This Ca^{2+} current has characteristics that are similar to N-type Ca^{2+} currents in other preparations. N-type Ca^{2+} channels may be the predominant Ca^{2+} channel type responsible for Ca^{2+} influx and neurotransmitter release from nerve terminals, and it has been suggested that blockade of these Ca^{2+} channels could contribute to the sedative and hypnotic effects of barbiturates. Since these pharmacological actions of PB reside in the (-)-enantiomer, our results are compatible with this idea.

M-Pos139

DUAL ACTION OF IMIPRAMINE ON NEURONAL CALCIUM CURRENT.
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In addition to its well described antidepressant and antimuscarinic action, imipramine (IP) is known to suppress calcium current (I_{Ca}) in tumor cells (Brain Res. 476:140, 1989). Since I_{Ca} is also modulated by muscarinic mechanisms (Proc.Nat.Acad.Sci. 84:4313, 1987), the preceding effects are likely to bestow a complex action of IP upon Ca^{2+} channels. To investigate this possibility, we used the whole cell recording technique to analyze the effects of IP on I_{Ca} of dorsal root ganglia (DRG) cultured from 12-14 day embryos of short-sleep mice. Cells were bathed in a medium (22° C and containing (mM): TEA (125), TTX (0.002), CsCl (5), 4-AP (5), & $CaCl_2$ (5)) and voltage-clamped to a holding potential of -70 mV with a pipette containing Tris phosphate (65) and base (100), EGTA (11), $MgCl_2$ (2), CsCl (15), & ATP (2). To activate I_{Ca}, potential was stepped to 30 mV in increments of 10 mV. IP's (30 uM) action on I_{Ca} depended upon the visual detection of synaptic connections. Specifically, for DRG which clearly made synaptic contact with either other DRGs or spinal cord neurons, atropine (ATR, 3nM) enhanced the amplitude of I_{Ca} by 40.4%. Further exposure to IP caused I_{Ca} to increase by 50.9% of the value recorded with ATR. In marked contrast, for those DRG which did not show such synaptic contacts, ATR enhanced I_{Ca} by only 6.8% and IP now suppressed I_{Ca} by 28%. Our data suggest that tonic release of acetylcholine (ACh) onto DRG with synaptic contacts causes suppression of I_{Ca}. ATR enhances I_{Ca} by antagonizing the interaction of ACh with a muscarinic receptor subtype of unknown identity. IP potentiates this action of ATR. In contrast, IP suppresses I_{Ca} in cells lacking tonic muscarinic input. (NIAAA grant R01 AA08025)

M-Pos140

SPECIFIC INHIBITION OF N-TYPE CALCIUM CHANNELS BY μ-OPIOID RECEPTOR ACTIVATION IN HUMAN NEUROBLASTOMA CELLS.
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Differentiated human neuroblastoma cells (SH-SY5Y) express N- and L-type calcium channels (Reuveny and Narahashi, Biophys. J. 57, 520a, 1990) with single channel conductances of 13 and 20 pS, respectively. Internal application of guanine nucleotides specifically inhibits the N-type calcium channel currents in a pertussis toxin-sensitive manner (Reuveny and Narahashi, Neurosci. Soc. Abs. 16, 677, 1990). The study to be reported was aimed at identifying the receptor involved in this inhibition. Following application of the specific μ-opioid receptor agonist, morphiceptin (1 μM), the N-type calcium channel currents were completely inhibited without any change in the L-type calcium channel currents. The inhibition was naloxone (1 μM) sensitive, indicating involvement of a specific opioid receptor. Preincubation of the cells with 150 ng/ml pertussis toxin for 6 hr abolished the effect of μ-opioid receptor activation on the N-type channels. Upon washout of morphiceptin, the amplitude of N-type current increased 50 - 100% above the control levels. This rebound phenomenon was seen even after a short exposure (30 sec) to the agonist. We conclude that μ-opioid receptors in SH-SY5Y cells modulate the N-type calcium channels via G_i/G_o-proteins. Supported by NIH grant NS14144.

M-Pos141

GABA REDUCES CALCIUM CURRENT OF RETINAL BIPOLAR NEURONS. Ruth Heidelberger and Gary Matthews. Department of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794-5230.

Retinal bipolar neurons are non-spiking interneurons that relay information from photoreceptors in the outer retina to amacrine and ganglion cells in the inner retina. In the retina of the goldfish, one class of bipolar cell has a bulbous synaptic terminal 8-10 μm in diameter, in which we have been studying the regulation of calcium influx using both patch-clamp and fura-2 techniques. Previously, we reported that GABA inhibited depolarization-induced Ca-influx into the terminal, acting via a large GABA-activated chloride conductance ($GABA_A$) to clamp the membrane potential more negative than the activation range of the calcium current (Heidelberger & Matthews, 1990, *Society for Neuroscience Abstracts, 16*, 465). To determine if GABA might have an additional effect on Ca-current, independent of its action via Cl-conductance, we measured whole-cell Ca-currents in bipolar cells acutely isolated after papain digestion of goldfish retina. The $GABA_A$-mediated Cl-conductance was blocked with 100 μM picrotoxin. With 300 μM GTP in the pipette solution, application of 3-10 μM GABA to the synaptic terminal reversibly reduced the Ca-current to 71 ± 3% of the control level (mean ± s.e.m.; N=21). The $GABA_A$ agonist muscimol (5 μM) could not mimic this action of GABA (I_{Ca} in muscimol = 106 ± 3% of control; mean ± s.e.m.; N=13), suggesting that $GABA_A$ receptors were not involved. Without GTP in the pipette solution, the effect of GABA on Ca-current was less (I_{Ca} in GABA = 90 ± 6% of control; N=11), indicating the possible involvement of a G-protein-coupled GABA receptor. To determine if a $GABA_B$ receptor might be responsible, as reported for salamander bipolar cells (Maguire *et al.*, 1989, *PNAS, 86*, 10144), we examined the effect of baclofen on Ca-current; however, even at high concentration (0.5-1 mM), baclofen had little effect (I_{Ca} = 94 ± 3% of control; N=12). Thus, if a $GABA_B$ receptor underlies the reduction of Ca- current by GABA, its pharmacology in fish retina is different from that in other systems.

We have identified two mechanisms by which GABA affects Ca-influx into the synaptic terminal of bipolar cells: a GTP-dependent reduction in Ca-current and a $GABA_A$-mediated increase in Cl- conductance that prevents membrane voltage in unclamped cells from reaching the activation range of Ca-current. Because the GABA-induced Cl-conductance is large compared to other conductances of the bipolar cell and because the effect of GABA on Ca-current is small (~30% reduction in peak current), it is likely that the $GABA_A$ effect is the more important of the two in intact cells.

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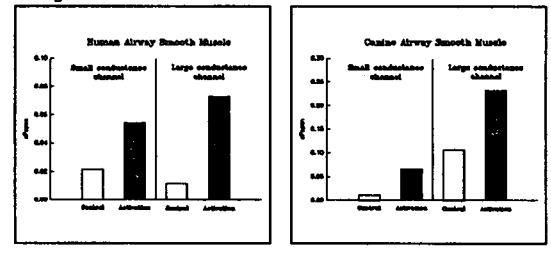
M-Pos142

AGONISTS INCREASE OPEN-STATE PROBABILITY OF SINGLE Ca^{++}-CHANNEL CURRENTS IN AIRWAY SMOOTH MUSCLE CELLS. M. Tomasic, J.P. Boyle, J.F. Worley and M.I. Kotlikoff. Dept. of Animal Biology, School of Veterinary Med., Univ. of Penna., and Dept. of Pharm., West Virginia Univ.

Two unitary conductance Ca^{++} channels were identified in on-cell single channel recordings from both cultured human airway smooth muscle (HASM) and fresh enzymatically dissociated canine airway smooth muscle (CASM), using 80mM Ba^{++} as the charge carrier. Values for the large and small conductances were 8.9±1.7pS and 24.8±2.0pS, respectively, in HASM, and 9.0±0.6pS and 25.3±1.2pS respectively, in CASM. In both cell types, the open state probabilities of both channels were dependent on transmembrane potential, and were sensitive to bath addition of dihydropyridines. While the large conductance channel has been previously characterized in airway smooth muscle as an "L" type Ca^{++} channel, the low conductance channel has only been described in other smooth muscle tissues.

Addition of methylcholine (CASM, 100μM final concentration; n=3) or bradykinin (HASM, 1 to 4μM final concentration; n=5) caused a 2 to 6 fold increase in the open-state probability (nP_o) of both channel types. nP_o for control and agonist-exposed states were determined at 0mV (-70mV holding potential) for all CASM experiments and between -10 and 10mV in human cell experiments. The data represented in the figure for HASM are the average nP_o at these pipette potentials.

These experiments demonstrate the presence of a small conductance Ca^{++} channel in airway smooth muscle not previously described. Additionally, these data show the nP_o of both channel types can be increased through the bath addition of contractile agonists.



M-Pos143

PEPTIDE-INDUCED REDUCTION IN CALCIUM CURRENTS IN RAT NUCLEUS BASALIS AND LOCUS COERULEUS NEURONS. J.J.Grigg, K.Koyano, S.Nakajima and Y.Nakajima. Dept. of Anat. and Cell Biol. and Dept. of Pharmacol., Univ. of Illinois, College of Med. at Chicago, Chicago IL 60612.

Dynorphin A (DYN) reduces Ca-currents in dorsal root ganglion cells (MacDonald and Werz, J. Physiol., 377:237, 1986). We now report that DYN inhibits voltage-dependent Ca-currents in cultured cholinergic neurons from the nucleus basalis of Meynert. Under the whole-cell patch clamp, DYN (1 μM) reduced Ca-currents (23.4 ± 3.2 %, mean ± s.e.m., n=9) in these neurons. A comparable degree of inhibition was achieved with (±)ethylketocyclazocine (kappa 1,2,3 agonist; 50 μM) and U50,488H (kappa 1,2 agonist; 10 μM), but not with [D-pen^{2,5}]-enkephalin (delta agonist; 1 μM) or U69,593 (kappa 1 agonist; 10 μM). Unexpectedly, the inhibitory effect of U50,488H(10 μM) was not antagonized by a high concentration of naloxone(50 μM), suggesting either the kappa receptor mediating this effect is not very sensitive to naloxone or this effect is not mediated through an opioid receptor. Methionine-enkephalin (10 μM), a mu agonist, decreased Ca-currents in nucleus basalis and locus coeruleus neurons. This effect, in contrast with the effect by the kappa agonists, was completely blocked by NAL (50 μM), indicating that it is mediated through a mu receptor.

We have also observed that substance P (0.2 μM) decreased Ca-currents in both nucleus basalis and locus coeruleus neurons. Neurotensin (1 μM) also inhibited the Ca-current in nucleus basalis neurons. The degree of the inhibitory effect by subsbance P or neurotensin is greater than that by the kappa or the mu agonists. Supported by NIDA grant (DA05701).

M-Pos144

NMDA RECEPTOR AGONISTS SELECTIVELY BLOCK N-TYPE CA^{2+} CHANNELS IN RAT HIPPOCAMPAL NEURONS. N.I. Chernevskaya, A.G. Obukhov & O.A. Krishtal. Bogomoletz Institute of Physiology, Ukrainian Academy of Sciences, Kiev, U.S.S.R. 252024.

Rat hippocampal neurons are known to possess at least three types of voltage-gated Ca^{2+} channels: a low-treshold T-type channel, and two high-treshold, L- and N-type channels. N-type channels are thought to play a role in excitatory synaptic transmission from the CA3 input to CA1 neurons, since ω-conotoxin (ω-CgTX), but not dihydropyridines, block transmission in this pathway. We have used standard patch clamp techniques to study the effect of excitatory amino acids (EAA) on the Ca^{2+} currents of neurons freshly dissociated from the CA1 and CA3 regions of rat hippocampus.

We find that the EAAs, glutamate and aspartate, block a component of Ca^{2+} current. The component of Ca^{2+} current blocked by EAAs was identified as N-type based on the following criteria: 1. It is a high-treshold current; 2. It inactivates during a maintained depolarization; 2. It can only be elicited from negative holding potentials; and 4. It is the same component blocked by adenosine and ω-CgTX, both thought to be specific blockers of N-type current.

The block of Ca^{2+} channels appeared to occur through NMDA receptors since both aspartate and NMDA are effective while the non-NMDA-agonists, kainate and AMPA, were ineffective. Block is prevented by the specific NMDA receptor agonist, APV. Ca^{2+} entry through the NMDA receptor does not appear to be necessary since the block occurred in the presence of kynurenic acid which prevents NMDA channel opening by interacting with the modulatory glycine receptor site. Block was equally effective with either Ca^{2+} or Ba^{2+} as the charge carriers through the Ca^{2+} channel.

We postulate the existence of a regulatory complex which controls N-type Ca^{2+} channels via functionally interconnected NMDA and adenosine receptors. Whether this complex operates through physical interconnections or through networks of second messengers awaits further experiments. However, it suggests presynaptic regulation of transmitter release by EAAs.

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M-Pos145

PROSTAGLANDIN E_2 ACTIVATES CALCIUM CURRENTS IN DORSAL ROOT GANGLION NEURONS. G.D. Nicol and M.R. Vasko. Dept. of Pharmacology & Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Chemical mediators of pain such as bradykinin or capsaicin activate nociceptive sensory neurons presumably by enhancing calcium currents. This activation of sensory neurons is potentiated by various prostaglandins especially PGE_2. Although the mechanism of action for these effects of PGE_2 is unknown, PGE_2 appears to enhance the release of the putative sensory transmitter, substance P. We sought to investigate the mechanism by which PGE_2 potentiates the release of substance P. Embryonic avian dorsal root ganglion (DRG) neurons were harvested and grown in culture for 3-5 days. We utilized the whole-cell patch clamp technique to examine the calcium currents in these neurons. The neurons were perfused with a solution containing (in mM), 105 CsCl, 20 TEA, 10 $BaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, and 1 uM TTX. The pipette solution contained (in mM), 50 CsCl, 50 NMG, 10 TEA, 10 EGTA, 2 $MgCl_2$, 10 HEPES, and 4 ATP. Membrane potentials were held at either -60 mV or -90 mV and depolarized with 10 mV steps from the holding level. In control recordings the mean peak ICa was -384 pA (range -156 to -499 pA, n=4) and -488 pA (range -354 to -562 pA, n=3) for neurons held at -60 mV and -90 mV, respectively. The addition of 1 uM PGE_2 to the perfusate increased ICa in 7 of 9 neurons. The mean ICa after PGE_2 was -622 pA (range -421 to -854 pA) for neurons held at -60 mV and was -704 pA (range -595 to -767 pA) for neurons held at -90 mV. This enhancement was 1.8-fold and 1.5-fold greater than that of the control ICa. Neither arachidonic acid nor it's lipoxygenase product, leukotriene B_4 (1 uM) enhanced ICa. These results suggest that PGE_2 has direct actions on sensory neurons to augment calcium entry into the cell. This elevation of intracellular calcium could account for the enhanced activation of sensory neurons and the increase in transmitter release produced by PGE_2. (Supported by PHS 2 S07 RR 5371 and the Arthritis Foundation)

M-Pos146

INHIBITION OF PROLACTIN SECRETION FROM INDIVIDUAL LACTOTROPES BY ω-CONOTOXIN AND NIFEDIPINE. J. Horta, R. Félix, A. Navarrete, M. Hiriart, and G. Cota. Dept. of Physiol., CINVESTAV-IPN, and Dept. of Bioenergetics, Institute of Cell Physiology, UNAM, México, D.F.

We reported previously that the rate of basal prolactin secretion of an individual lactotrope is positively correlated with the surface density of high-threshold, fast deactivating Ca channels in its plasma membrane (Hiriart et al., *Biophys. J.* 57:518a '90). We report here that prolactin secretion can be inhibited by ω-conotoxin GIVA (CgTx), a blocker of high-threshold Ca channels in neurons, and nifedipine (NIF), a well known modulator of L-type Ca channels in a wide variety of neuronal and nonneuronal cells. Prolactin secretion from single lactotropes in pituitary cultures of adult male rats was detected and quantified using the reverse hemolytic plaque assay. In this assay, the extent of hemolysis (plaque area) induced by an individual lactotrope provides an index of the cumulative amount of prolactin released by that cell. Measurements of mean plaque area indicated that the total amount of prolactin released from the pituitary cells during 1 h at 37°C decreases to ~55% of its control value by adding 5 μM CgTx to the culture medium. Comparatively, prolactin secretion in the presence of NIF (2 μM) decreased by ~60%. Lactotropes are markedly heterogeneous with respect to the basal rate of prolactin secretion and can be grouped in two main subpopulations, small-plaque (SP) cells and large-plaque (LP) cells. Both CgTx and NIF reduced the fraction of lactotropes forming large plaques and induced a corresponding increase in the fraction of cells forming small plaques. Neither CgTx nor NIF significantly reduced the total number of plaque-forming cells. This strongly suggests that inhibition of prolactin secretion by CgTx or NIF is not uniform among the individual lactotropes. Instead, these Ca channel modulators seem to preferentially affect the subpopulation of LP lactotropes.

M-Pos147

CHANGES IN Ca CHANNEL ACTIVITY AND PROLACTIN SECRETION DURING THE ONTOGENY OF RAT LACTOTROPES. Ricardo Félix, Jorge Horta, Araceli Navarrete, Angel Marin, and Gabriel Cota (Intro. by Fidel Ramón). Department of Physiology, CINVESTAV-IPN, México, D.F. 07000.

We have used the reverse hemolytic plaque assay to quantify prolactin secretion from single lactotropes in pituitary cell cultures of 10-day-old and adult male rats. The frequency distribution of prolactin plaque areas in cell cultures derived from adult rats was well fitted by the sum of two Gaussian curves, which is consistent with the existence of two main subpopulations of lactotropes that differ in secretory activity, small- and large-plaque (SP and LP) cells. Plaque area was 857±75 μm^2 (mean±SEM, 4 determinations) for SP cells and 3944±454 μm^2 for LP cells. SP lactotropes comprised ~7.8% of all pituitary cells, whereas the percentage of LP lactotropes was ~11.7%. In contrast, the frequency distribution of plaque sizes in cultures of neonatal rat pituitaries was well fitted by a single Gaussian curve; prolactin secretors in these cultures accounted for ~5.2% of all cells and formed plaques 866±93 μm^2 in area. Ca channel currents in lactotropes were studied using whole-cell recording with patch electrodes (20 mM external Ba, pulses from -80 to +20 mV). Current density through FD Ca channels in SP cells was 3-4 times smaller than that in LP cells, whereas the current density carried by SD Ca channels did not significantly differ between the two lactotrope subpopulations. Lactotropes from neonatal rats also expressed the two types of Ca channels; both SD and FD current densities in these cells were practically the same as those recorded from SP lactotropes. Thus, the secretory and Ca channel activity of adult-rat SP lactotropes closely resembles that of neonatal rat lactotropes. The results raise the possibility that the ontogenic appearance of cells secreting large amounts of prolactin per unit time (i.e., the LP cells) is associated with an increased expression of FD Ca channels in the lactotropes.

M-Pos148

SOMATOSTATIN INHIBITS VOLTAGE-SENSITIVE CALCIUM CURRENTS BY DIFFERENT MECHANISMS IN PITUITARY AND INSULINE-SECRETING CELLS. Carla Marchetti and Giampaolo Brambilla. Istituto di Cibernetica e Biofisica, C.N.R., Genova, Italy.

Somatostatin (SS) inhibits hormone secretion in pituitary cells and in insulin-secreting pancreatic β cells. We have studied the effect of SS on the voltage-sensitive calcium current in an ACTH-secreting cell line (AtT20/D16) and in an insulin-secreting cell line (HIT-T15). In AtT20 cells, previous work demonstrated that the calcium current is reversibly depressed by SS through the activation of a PTX-sensitive G-protein. With barium as the charge carrier, we found that SS (0.6 μM) also prolonged the time to peak of the maximum current (V=+20 mV). Both effects were partially reversed when test depolarizations were preceded by a depolarizing prepulse. This voltage-driven recovery was dependent on the prepulse length (20-50 ms) and amplitude (maximum recovery for V≥+60 mV) and on the prepulse-pulse delay. The control current was not affected by prepulses. In HIT cells, in either 5 mM Ca or Ba, the same dose of SS depressed the current at +20 mV by 25%, without altering its time course. Depolarizing prepulses did not remove the inhibition, but caused a further decrease of the current in calcium, both with and without SS, an effect interpreted as calcium-dependent inactivation. In addition, in ≥50% of the cells the current recovered spontaneously in minutes, but no inhibition was ever observed when the pipette contained GDP-β-S. We propose a double action of SS on the calcium current:a voltage-independent inhibition and a voltage-dependent effect on channel activation. Both effects are mediated by a G-protein, but might occur combined or separately in different cell types.

M-Pos149

CALCIUM CHANNEL MODULATION BY DIHYDROPYRIDINES STUDIED WITH PERFORATED PATCH RECORDING.

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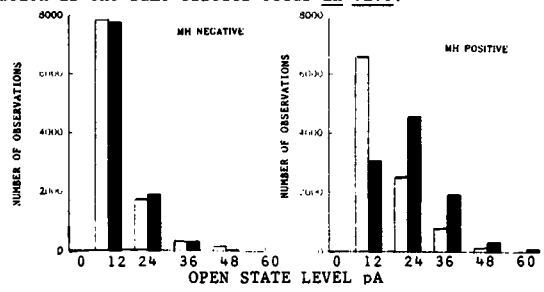
The perforated patch-clamp technique was used to study the influence of dihydropyridines on calcium currents in GH3 pituitary cells. This newly developed method allows the recording of stable calcium currents for up to 120 minutes in virtual absence of run-down. Effects induced by the well known dihydropyridines Bay K 8644 and Nimodipine, as well as the new dihydropyridine (calcium antagonist) Lacidipine, were studied. Calcium currents were elicited by voltage steps, from a holding potential of either -90 mV (Low Voltage Activated, LVA) or -40 mV (High Voltage Activated, HVA), up to 12 mV. Dihydropyridines were applied and removed in a few seconds by a fast perfusion method based on two or more pipettes alternatively facing the cell. In accordance with experiments performed on several dihydropyridines (calcium antagonist) with conventional whole-cell patch-clamp, also with perforated patch-clamp 1 μM Nimodipine partially blocks HVA calcium channels. Furthermore, we observed that Nimodipine block is at least partially reversible. The same concentration of Lacidipine completely abolishes HVA current. No measurable recovery was observed after one hour from the exposition to Lacidipine. A consistent enhancement of LVA calcium currents was observed exposing the cell to calcium agonist Bay K 8644 (1 μM), in the absence of any measurable increase of HVA currents. Bay K 8644 was still able to increase LVA calcium currents when HVA channels were preventively blocked by Lacidipine.

M-Pos150

HALOTHANE EFFECT ON CALCIUM RELEASE CHANNEL FROM HUMAN MALIGNANT HYPERTHERMIA SKELETAL MUSCLE

Nelson, T.E., Dept. Anesthesiology, Univ. Texas Health Science Center, Houston, TX (Introduced by Phil Palade).

Halothane is a potent inhalational anesthetic that can trigger the malignant hyperthermia (MH) syndrome in genetically predisposed patients. Normal and MH human single calcium release channels were reconstituted from sarcoplasmic reticulum (SR) vesicles into planar lipid bilayers (7:3, POPE:POPC, 50 mg/ml decane) and the effect of halothane was compared. Each patient had diagnostic contracture tests on biopsied vastus lateralis muscle and heavy SR vesicles were isolated from extra muscle. Release channels were selectively measured in solutions containing (mM) 250 cis/50trans Cs_2MeSO_4, 10 CsHEPES (pH7.4), and pCa=5. Halothane, 4-32 uM increased Po and mean open time while decreasing mean closed time of the MH release channel and had no effect on normal channels. Open state levels were measured in the presence and absence of halothane. MH channels had a greater percentage of larger open state levels than normals and halothane increased the effect for MH channels while no effect was observed for normal channels. The net result of this action of halothane on MH calcium release channels would be an increased myoplasmic Ca^{2+} concentration if the same effects occur in vivo.



M-Pos151

HALOTHANE SELECTIVELY INHIBITS TWO COMPONENTS OF CALCIUM (Ca^{2+}) CURRENT IN CLONAL (GH_3) PITUITARY CELLS.

J. Herrington and C.J. Lingle, Dept. of Anesthesiology, Wash. Univ. Sch. of Med., St. Louis, MO 63110. (Intro. by E. McCleskey).

The action of halothane (HAL) on the isolated voltage-dependent Ca^{2+}, K^+, and Na^+ current of GH_3 cells was studied using standard whole-cell clamp techniques. HAL was most effective at inhibiting the high threshold Ca^+ current (HVA), studied by stepping to + 10 mV from -30 mV. The K_d estimated from the peak current was 0.85 mM, with inhibition being 100% at about 3.0 mM. However, the inhibition at the end of a 190 msec step to + 10 mV is greater than at the peak, with a K_d of approximately 0.5 mM. Thus, halothane appears to be more effective at reducing the non-inactivating component of HVA current. The low threshold, transient Ca^{2+} current (LVA), activated by stepping to -30 mV from a holding potential of -90 mV, was also sensitive to HAL, with an estimated K_d of 1.3 mM and 100% inhibition at about 5 mM. The inhibition of LVA Ca^{2+} current by HAL was associated with an apparent acceleration of activation, deactivation, and inactivation. Halothane had no effect on the voltage-activated inactivating component of K^+ current (studied from a holding potential of -80 mV in the absence of extracellular Ca^{2+}) at concentrations up to 1.2 mM. The non-inactivating component of K^+ current (isolated by holding at -40 mV) was inhibited by 25-40% by 1.2 mM at + 40 mV, with little inhibition at 0 mV. Peak Na^+ current was unaffected at 1 mM HAL, while 2.5 mM HAL produced about 50% inhibition of peak current and shifted steady-state inactivation approximately -10 mV. These results indicate that the two components of Ca^{2+} current exhibit a greater sensitivity to halothane than any of three other voltage dependent currents in GH_3 cells. Supported by NIH-DK-37109.

M-Pos152

CITRATE DECREASES Ca CURRENT, BUT INCREASES Na CURRENT THROUGH Ca CHANNELS IN CARDIAC MYOCYTES.

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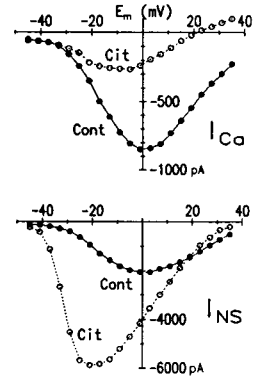
We have recently shown that extracellular citrate decreases contraction and I_{Ca} in cardiac muscle by a direct effect on Ca channels, rather than by Ca buffering *per se* (*Biophys. J.* 57:117a, 1990). Buffering extracellular Ca with citrate (10mM) immediately reduces myocyte shortening despite a constant extracellular [Ca] (2 mM). Here we examine the effects of citrate on whole cell current through L-type Ca channels in voltage-clamped rabbit ventricular myocytes. I_{Ca} was isolated by using impermeant ions in the dialysate and superfusate (Cs, TEA). Citrate (10 mM) rapidly and reversibly depressed peak I_{Ca} (from 1.1 ± 0.06 to 0.37 ± 0.04 nA, mean ± S.E.M., n=11). The apparent reversal potential for I_{Ca} was also shifted by citrate especially at low $[Ca]_o$ (by~25 mV at 0.5 mM Ca), indicating a possible change in Ca channel selectivity (see leak subtracted I_{Ca} in Fig.). We also studied the effects of citrate on current through the Ca channel in the absence of Ca, using Na as the charge carrier (I_{NS}). Citrate rapidly and reversibly increased peak I_{NS} (from 2.2 ± 0.3 to 5.7 ± 0.8 nA, n=24). Steady state activation and inactivation curves for I_{Ca} and I_{NS} were also examined. Citrate shifted the voltage for ½-maximal activation and inactivation (V^*) towards negative potentials for both I_{Ca} (by ~6 & 7 mV) and I_{NS} (by ~18 & 4 mV). Citrate also increased the steepness of the E_m-dependence for activation of I_{NS} by ~70% (from k=8.8 ± 0.4 to 5.3 ± 0.9 mV/e-fold change, n=5). As I_{NS} inactivates very slowly compared to I_{Ca}, this large change in activation (of both k and V^*) produced by citrate is likely to underly the augmentation of I_{NS}. The decrease in I_{Ca} may reflect a combination of changes in Ca channel gating (i.e. decreased "window current") and a possible decrease in selectivity for Ca *vs* monovalent cations. These results indicate that studies using citrate as a pure Ca buffer should be interpreted with caution.



M-Pos153

"BUILD-UP" OF THE CARDIAC CA CURRENT : EFFECTS OF INTERNAL CELL DIALYSIS.

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CRBM, CNRS LP84Ø2, INSERM U249 - BP5Ø51
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Dialyzing cells with an artificial intracellular solution induces the well-known slow rundown of the L-type Ca current (ICa). We describe here the build up of ICa that, prior to the rundown step, develops immediately after establishing the whole-cell patch-clamp recording configuration. On freshly isolated rat ventricular cells, we have identified 3 distinct phases. Less than a few seconds after breaking tight seals (> 1 GΩ), no inward, but a small outward current is recorded which is rapidly blocked (< 1 min) by Cs+ ions dialysing from the recording pipette (phase 1, P1). At the same time, ICa increases from nearly zero current to reach its maximum peak amplitude within 2 - 3 min (second phase, P2). We have determined that P2 is independent of P1 and of several parameters including voltage loss, holding potential, frequency of stimulation, nature of permeating ion (Ca2+, Ba2+, Na+), presence of EGTA and ATP in the pipette and pH. In addition, in the same time that Ica reaches its maximal peak amplitude, its rate of decay, which is initially monoexponential and slow, increases dramatically (third phase, P3), revealing a fast and a slow component (see Richard et al., 199Ø, Am. J. Physiol. H1872-H1881).

These results suggest that internal cell dialysis, prior to inducing the rundown process, relieves some form of inhibition to which Ca channels in the intact cells were subject. The wash-out of diffusible intracellular regulator(s) of Ca channel activity may possibly be involved.

M-Pos154

Preferential Recruitment of One Subtype of Cardiac L-type Ca^{2+} Channel by Bay K 8644. Noritsugu Tohse, Laura Conforti, Nicholas Sperelakis - Department of Physiology & Biophysics, University of Cincinnati, College of Medicine, Cincinnati, OH 45267-0576

We previously showed that the slow (L-type) Ca^{2+} channels in young (3-day-old) embryonic chick heart cells naturally exhibited long-lasting openings in the absence of any added Ca^{2+} channel agonist (Am. J. Physiol. 259, H639-H642, 1990). Therefore, we examined the effects of the Ca^{2+} agonist Bay K 8644 on the Ca^{2+} slow channels. Bay K 8644 (5 μM) enhanced the openings of the Ca^{2+} channels, including the maximal number of simultaneous openings. In 7 cells, Bay K 8644 increased the ensemble-averaged current by 3.9 (± 0.9)-fold (mean ± SE) and the maximal number of simultaneous openings from 2.6 (± 0.4) to 4.4 (± 0.9). Bay K 8644 had no effect on the unitary conductance of the channel (26 pS in control, 25 pS in Bay K 8644). In the open-close kinetic analysis, Bay K 8644 had relatively little effect. Mean open times were 4.2 ms and 5.2 ms, in control and Bay K 8644, respectively. Therefore, these results suggest that Bay K 8644 increases the number of functional Ca^{2+} channels or recruits silent Ca^{2+} channels. Furthermore, we observed two types of ensemble averaged current: non-inactivating current (L_A channel) and inactivating current (L_B channel). Time-to-peak current for the inactivating current (13 ± 2 ms, n = 9) was faster than that for the non-inactivating current (26 ± 4 ms, n = 11). Estimation of the number of channel sub-types indicated that the percentage of L_B channels was increased by Bay K 8644 (from 52% to 78%), indicating that Bay K 8644 preferentially activates the L_B channels. Because the activation of the L_B channels may produce the faster activation and inactivation of the Ca^{2+} current, our results can explain the kinetic change in macroscopic current produced by Bay K 8644, including acceleration of activation and inactivation. (This research was supported by grant HL-31942).

M-Pos155

HEPARIN BLOCKS CALCIUM AND SODIUM CURRENTS IN MAMMALIAN VENTRICULAR MYOCYTES.

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Heparin is a polysacharide, naturally occuring in the liver, spleen and lungs. Its concentration in human plasma is 1.5 μg/ml. Recently, it was shown that heparin binds to a novel domain on the α_1 subunit of the Ca^{2+} channel in heart and skeletal muscle and thereby enhancing I_{Ca} (Knaus et al., J. Biol. Chem: 265, 11156, 1990). We tested the effect of 10 μM of bovine and porcine heparin of high (HMW) and low molecular weights (LMW) on both sodium and calcium channels of myocytes isolated from guinea pig and rat ventricles using the whole-cell patch clamp technique. Unlike the previous report, we found only a suppressive effect of all types of heparin tested (porcine HMW 15-18,000; porcine LMW 4-6,000; bovine LMW 5,000 Sigma) on both calcium (22 cells) and sodium (7 cells) channels. The effect of heparin on I_{Ca} was rapid in its onset (<5s) and was complete within 50s (HMW) and 120s (LMW). The degree of suppression varied from 25-60% in different cells. Suppression of I_{Na} was 30-80%, depending on heparin-type used. The effect was immediate and complete. The effect of heparin on I_{Ca} was difficult to reverse, while the effect on I_{Na} reversed readily. The suppressive effect of heparin on I_{Ca} was present in TTX and zero Na^+ containing solution and was independent of the presence of intracellular cAMP. The steady-state inactivation of I_{Ca} or I_{Na} were not significantly affected by heparin. Since non-permeant PKC inhibitor, phorbolester-12,13 diacetate, has been shown to inhibit I_{Ca} (Hockberger et al., Nature, 338: 360, 1989), we examined whether it could mask the heparin effect on I_{Ca}. The suppressive effects of heparin and phorbolester were additive and independent of the presence of internal cAMP (0 to 50 μM). We conclude that heparin may indeed bind to a novel Ca^{2+} channel site, but its effect was neither specific nor agonistic to the Ca^{2+} channel. (Supported by NIH grant HL 16152.)

M-Pos156

PURIFIED BRAIN RECEPTOR FOR THE SECOND MESSENGER INOSITOL1,4,5 TRISPHOSPHATE (IP_3) FORMS A LOW CONDUCTANCE CALCIUM PERMEABLE CHANNEL ACTIVATED BY IPS_3.

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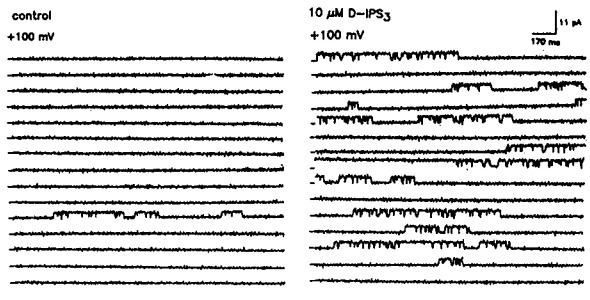
IP_3 receptors were purified from rabbit brain by a modification of the procedure of Chadwick et al, (1990). Receptors were bound to heparin-agarose, WGA-Sepharose and centrifuged on a 10-30% sucrose gradient. A 260 kDa polypeptide was enriched in the peak [^{3}H]IP_3 binding fractions. When incorporated into planar bilayers, the purified IP_3 receptor formed a 27 pS channel activated by the non-hydrolyzable analog, D-myo-inositol 1,4,5-trisphosphorothioate (IPS_3). This channel differed from the brain ryanodine receptor which had a conductance of 107 pS. Reversal potential in trans 50 mM Ca^{2+} and cis 250 mM Cs^+ was -10 mV indicating a low Ca^{2+} selectivity. The number of openings per trace increased more than 20-fold when 10 μM IPS_3 was present in the cis solution. The ryanodine receptor blocker ruthenium red (10 μM) had no effect on IPS_3-activation of channels. A 30 pS IPS_3-activated channel was also recorded in the SR of rabbit skeletal muscle suggesting that the same IP_3 receptor may be present in skeletal muscle. Supported by NIH, MDA, AHA.



M-Pos157

ROLE OF VOLTAGE-DEPENDENT Na^+ AND Ca^{2+} CURRENTS IN COUPLING GLUCOSE STIMULATION TO INSULIN SECRETION IN CANINE PANCREATIC ISLET B CELLS. David M. Pressel and Stanley Misler, The Jewish Hospital of St. Louis and the Program in Neurosciences, Washington University Medical Center, St. Louis, MO 63110.

Glucose-induced electrical activity in canine pancreatic islet B cells is distinct from that in rodent islets, though both display Ca^{2+}-dependent insulin secretion. Under conditions which stimulate insulin secretion, canine islet B cells undergo isolated Na^+_o-dependent action potentials which often give way to a depolarized "wobble" or plateau potential, rather than undergoing the regular bursts of Ca^{2+} action potential seen in rodents. Here, we present evidence to reconcile the species difference in electrical activity with the similarity of Ca^{2+} dependence of secretion. (1) Increasing glucose concentrations produce increasing membrane depolarization and increasing frequency of Na^+_o-dependent action potentials until a background membrane potential ($\sim$ -40 mV) is reached where Na^+ currents are inactivated. (2) High threshold, voltage dependent Ca^{2+} currents are present which are readily activated over the voltage excursion of the action potential. They inactivate slowly (over seconds) during prolonged depolarizations to membrane potentials near the plateau potential and, hence, are in a position to contribute to them. (3) Tetrodotoxin blocks an early transient phase of glucose-stimulated insulin secretion, but not a subsequent prolonged plateau. BAY K 8644 and elevated K^+ enhance the plateau phase secretion. The transient phase of secretion often corresponds in time to the period of initial high frequency action potential activity. These results suggest that, in canine B cells, electrical activity and its underlying Ca^{2+} currents regulate Ca^{2+}-dependent insulin secretion much as it does in rodents. That is, following exposure to glucose, the early train of Na^+-dependent action potentials allows pulsatile Ca^{2+} entry and, thus, promotes an early transient phase of insulin secretion. The subsequent sustained plateau potential maintains prolonged Ca^{2+} entry and steady insulin release. Support: PHS DK37380 and HL07275.

M-Pos158

IDENTIFICATION OF A PHENYLALKYLAMINE BINDING REGION WITHIN THE α1 SUBUNIT OF SKELETAL MUSCLE CA2+-CHANNELS

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The α1 subunit of skeletal muscle Ca^{2+}-channels has been specifically photoaffinity labeled with the phenylalkylamine receptor-selective verapamil derivative [N-methyl-3H]LU49888 ((-)-5-[(3-azidophenethyl)[N-*methyl*-3H]methylamino]-2-(3,4,5-trimethoxyphenyl)-2-isopropylvaleronitrile). Proteolytic fragments generated by different endoproteases were probed by immunoprecipitation with several sequence-specific antibodies to determine the site of labeling within the primary structure of α1. These results restrict the site of photolabeling by [N-methyl-3H]LU49888 to the region between tyr_{1350} and asp_{1390}. This segment of α1 contains transmembrane helix S6 of domain IV and the beginning of the long intracellular C-terminal tail. Preliminary experiments show in addition that one site of specific photolabeling with the dihydropyridine-receptor-selective drug [3H]azidopine occurs within 25-30 kDa of the site of phenylalkylamine labeling.

Because the phenylalkylamine receptor site is only accessible from the intracellular side of the Ca^{2+}-channel (Affolter H. & Coronado R., Biophys.J. 49: 767-771, 1986; Hescheler J., Pelzer D., Trube G. & Trautwein W., Pflügers Arch. 393: 287-291, 1982) we propose that the intracellular end of helix IVS6 and the adjacent intracellular amino acid residues play an essential role in formation of the phenylalkylamine receptor site. This region of the α1 subunit may be involved in the formation of a putative calcium binding site of the channel (Babitch J., Nature 346: 321-322, 1990). The action of the phenylalkylamines as open channel blockers suggests that this region may also be part of the intracellular opening of the transmembrane pore of the Ca^{2+}-channel.

M-Pos159

PHOSPHORYLATION OF CALCIUM CHANNEL α1 SUBUNITS IN SKELETAL MUSCLE CELLS. Y. Lai, M. Seagar, M. Takahashi, and W.A. Catterall (Intro. by Benson M. Curtis) Department of Pharmacology, University of Washington, Seattle, Washington 98125.

Skeletal muscle dihydropyridine-sensitive calcium channels are *in vitro* substrates for cAMP-dependent protein kinase. In the present work, α1 subunits were isolated from cultured skeletal muscle cells by immunoprecipitation with a specific monoclonal antibody under conditions where proteolysis and dephosphorylation were prevented. Two forms of α1 subunit, 200 kDa and 160 kDa, were identified by back phosphorylation *in vitro* with cAMP-dependent protein kinase, specific immunoprecipitation, and phosphopeptide mapping. Treatment of cells with forskolin, isoproterenol, calcitonin gene related peptide, or 8 bromo-cAMP to increase intracellular cAMP reduced ^{32}P incorporation into all phosphopeptides *in vitro* by 60-80%, indicating that increases in cAMP caused endogenous phosphorylation of all sites on both $\alpha1_{200}$ and $\alpha1_{160}$ to nearly maximal levels. The extents of basal and stimulated phosphorylation *in vivo* were estimated by back phosphorylation methods to be 35% to 40% and 83% to 86%, respectively. In muscle cells metabolically labeled with ^{32}P, 3 mol phosphate were incorporated into α1 subunits. Forskolin stimulated ^{32}P incorporation into α1 subunits 1.6 fold. Taken together, our results show that skeletal muscle cells contain two forms of the α1 subunit which both are basally phosphorylated on cAMP-dependent phosphorylation sites and are further phosphorylated in response to agents that increase intracellular cAMP.

M-Pos160

NOVEL SUPPRESSION OF CALCIUM CHANNELS BY 2,3-BUTANEDIONE MONOXIME (BDM). G.-J. Huang and J.J. McArdle. Dept. Pharmacol., UMDNJ-N.J.Med.Sch. Newark, N.J. 07103

BDM is a chemical phosphatase. When added to the recording solution bathing 3-4 week old cultures of murine dorsal root ganglia, BDM (20 mM) reduced peak whole cell calcium current (I_{Ca}; V_h= -70mV) by 50%. In cell attached patches where barium served as the charge carrier, BDM reduced the frequency, conductance, and open time of single channel events. For a multi-channel patch BDM suppressed all openings in response to command voltages from a V_h of -40 mV, whereas openings persisted when V_h was -70mV. Thus, BDM suppression of L-type calcium channels does not require direct access to the outer membrane surface. While I_{Ca} recovered fully within 10 min of removing BDM from the recording medium, simultaneous exposure to Bay K-8644 (0.5-5uM) did not reverse the suppression. Likewise, pre-treatment with an activator of protein kinase C (TPA, 10-200 nM) did not antagonize the BDM effect. In contrast, either isoproterenol (50 uM) or 8-bromo-cyclic-AMP (cAMP; 10 mM) completely reversed the BDM suppression of I_{Ca}. While cAMP restored the ensemble calcium current to normal by increasing the probability of single channel opening, the duration of the open state was reduced relative to that seen in the control. However, the total time the channel spent in the open state was normal. This change of gating kinetics may reflect a rapid sequence of phosphorylation-dephosphorylation brought about by the simultaneous action of cAMP and BDM on the channel protein. We suggest that BDM modulates phosphorylation of a calcium channel site, normally under the influence of cAMP-dependent protein kinase, which controls moment to moment function. (NIAAA grant R01 AA08025)

M-Pos161

MODULATION BY PHOSPHORYLATION OF RECONSTITUTED SKELETAL MUSCLE Ca^{2+}-CHANNELS IN LIPOSOMES CONTAINING FLUO-3. L.M. Gutierrez, C.F. Chang, C. Mundina-Weilenmann and M.M. Hosey. Dept. Pharmacology Northwestern Univ. Med. Sch., Chicago, Il.

The absence of extensive biochemical studies of the activity of purified voltage-dependent dihydropyridine-sensitive Ca^{2+} channels is due in part to the lability of these proteins and the lack of convenient biochemical assays. We developed approaches that allow for the reconstitution of partially purified skeletal muscle Ca^{2+} channels in liposomes containing the Ca^{2+}-sensitive dye Fluo-3. The purification of channels in the presence of the activator Bay-K 8644 resulted in the isolation of stable channels, that, after reconstitution, exhibited Ca^{2+} flux sensitive to depolarization achieved by valinomycin in the presence of a K^+ gradient. The channels also exhibited dose-dependent activation by Bay-K 8644 and inhibition by the antagonists (+)PN 200-110, diltiazem and verapamil. Furthermore, the Ca^{2+} channels could be stereoselectively modulated by the enantiomers of the dihydropyridine (S) 202-791. Using this system we have analyzed the effects of phosphorylation induced by cAMP-dependent protein kinase (PKA) and Ca^{2+}-phospholipid dependent protein kinase (PKC) on channel activity. The channels were phosphorylated in the native skeletal muscle membranes and subsequently purified and reconstituted. Under conditions in which the α_1 subunit was the main target of either kinase, we observed that both kinases increased the rate and extent of Ca^{2+} uptake compared to activity obtained with the non-phosphorylated channels. The results demonstrate that phosphorylation of the skeletal muscle L-type Ca^{2+} channels by either PKA or PKC results in channel activation. The reconstitution assay developed provides a convenient biochemical assay of channel activity. (Supported by NIH HL 23306 to M.M.H. and Spanish M.E.C. fellowship to L.M.G.).

M-Pos162

FUNCTIONAL EFFECTS OF cAMP-DEPENDENT PHOSPHORYLATION OF DHP-SENSITIVE Ca^{2+}-CHANNELS IN INTACT SKELETAL MUSCLE.
Chan Fong Chang, Cecilia Mundina-Weilenmann, Luis M. Gutierrez and M. Marlene Hosey. Dept. Pharmacology, Northwestern University Medical School, Chicago, IL (Introduced by N. Owen)

Evidence from physiological studies suggests that dihydropyridine-sensitive Ca^{2+} channels in skeletal muscle are regulated by cAMP-dependent phosphorylation. However, no biochemical evidence has demonstrated phosphorylation of the channels in *intact* cells. In recent studies using the technique of back-phosphorylation, we observed that in situ treatment of newborn chick skeletal muscle with isoproterenol resulted in the selective phosphorylation of the α_1 subunit of the channel protein. This effect appeared to be mediated by cAMP, as it was mimicked by cAMP elevating agents, but not by activators of protein kinase C or Ca^{2+} ionophores. In the present study, we examined the effects of cAMP elevating agents on the phosphorylation of Ca^{2+} channels in intact newborn chick skeletal muscle and the functional consequences of this phosphorylation. Functional effects of cAMP-dependent phosphorylation on channel activity were studied by reconstituting purified channels into liposomes containing fluo-3. Depolarization and dihydropyridine-sensitive Ca^{2+} influx was observed in these preparations. Channels isolated from skeletal muscles incubated with isoproterenol exhibited an increased rate and extent of Ca^{2+} influx compared to control preparations. These effects could be mimicked by phosphorylating the channels with cAMP-dependent protein kinase *in vitro*. These results provide the first biochemical demonstration that the α_1 subunit of the DHP-sensitive Ca^{2+}-channels is the primary target of cAMP-dependent phosphorylation in intact muscle, and that the observed phosphorylation leads to activation of channel activity. (Supported by NIH HL 23306 to M.M.H. and Spanish M.E.C. fellowship to L.M.G.)

M-Pos163

PHOSPHORYLATION ALTERS THE VOLTAGE–DEPENDENT GATING OF SKELETAL MUSCLE Ca CHANNELS IN LIPID BILAYERS. C. Mundiña–Weilenmann, *J. Ma, *E. Ríos and M.M. Hosey. Northwestern and *Rush Universities, Chicago, IL.

A "whole cell" approach was taken to study the effects of phosphorylation on voltage–dependent gating of the DHP– sensitive Ca channels of skeletal muscle. DHP receptors from rabbit T membrane vesicles were incorporated into planar lipid bilayers voltage–clamped at –80 mV. The recording solutions were closer to physiological than in previous work; *cis* (intracellular), in mM: 200 KCl, 3 ATP Mg, 0.3 μM BAY K; *trans*: 50 NaCl, 100 $BaCl_2$. No channel activity was observed at potentials of either –80 mV or constant 0 mV. Channel events appeared when bilayers were pulse depolarized at potentials above –40 mV. The P_o increased with voltage following a Boltzmann with P_{max}=7.8 %, V_T=–26 mV and K=7 mV. Through calculation of test–minus–control currents and ensemble averaging, the kinetics of channel gating could be resolved. The averaged current was fitted as $I = I_{max} m^3 h$, a "Hodgkin–Huxley" time dependence, with τ_m=118 ms and τ_h=1.24 s at V=–10 mV and 22°C.

Addition of the catalytic subunit of protein kinase A (250 nM) to the intracellular side, led to a 50% increase in P_{max} and a shift of V_T by –7 mV. The gating kinetics were modified, the inactivation rate decreased significantly (τ_h=2.5 s) and the activation rate increased slightly (τ_m=109 ms). The availability (fraction of non–blank sweeps) increased significantly, from 72% to 79%. The distribution of open times in both reference and PKA was a sum of two exponentials. The time constants were not changed by PKA, but the proportion of long over short events increased from 0.38 (reference) to 0.50 in PKA. These results are consistent with observed effects of β–adrenergic stimulation and PKA on Ca currents in skeletal muscle cells (Arreola et al., J. Physiol. 1987; García et al. Pflügers Arch. 1990) and the effect on Ca channel gating currents in cardiac myocytes (Bean, Biophys. J., 1990).

Phosphorylation presumably adds negative charges near the cytoplasmic end of the voltage–sensitive segments of the protein. The shift in P_o *vs.* V toward *negative* V and the changes in gating kinetics suggest that phosphorylation causes changes in structure and function more complex than predicted by a simple addition of fixed charges. Supported by MDA, AHA and NIH.

M-Pos164

INCREASE OF L-TYPE CALCIUM CURRENT IN GUINEA-PIG VENTRICULAR MYOCYTES BY PHOSPHODIESTERASE INHIBITORS AND N-ETHYLMALEIMIDE.
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The effects of two non-selective phosphodiesterase inhibitors, papaverine (100 µM) and isobutylmethyl xanthine (IBMX; 100 µM), and of the alkylating agent N-ethylmaleimide (NEM) on the L-type Ca current (I_{Ca}) of guinea-pig ventricular cells were studied with the whole-cell voltage-clamp technique at 22-24°C. The phosphodiesterase inhibitors increased peak I_{Ca} 5-10 fold in a way similar to isoproterenol, forskolin or intracellular cAMP.

Papaverine but not IBMX had the following additional effects on I_{Ca}: 1) a shift of the end-of-pulse current in the outward direction (consistent with an increase of I_{Cl}), 2) an apparent increase of I_{Ca} inactivation as revealed by (i) an increase in the relaxing component of I_{Ca}, (ii) a negative shift of the I_{Ca} inactivation curve at potentials between -40 and 0 mV and (iii) a suppression of the relief from inactivation at potentials between 0 and 110 mV.

The stimulatory effect of papaverine and IBMX on I_{Ca} could be reversed by CCh (100 µM) or prevented by dialysis with GDPβS (300 µM). Neither CCh nor GDPβS inhibited papaverine enhancement of I_{Ca} inactivation. NEM (50 µM), which is known to inhibit G_{i1}, produced a comparable increase of I_{Ca} that could not be prevented with GTPγS or GDPβS.

It is suggested that part of papaverine and IBMX action is mediated by G proteins, perhaps a direct inhibition of G_{i1}. For papaverine, there is in addition a depolarization-dependent Ca antagonist action.

M-Pos165

cAMP AND cGMP BLOCK CA^{2+} CHANNELS IN VASCULAR SMOOTH MUSCLE. Peyrow M. and Bkaily, G. Department of Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

In cardiac muscle, cAMP and cGMP appear to have opposite effects. In vascular smooth muscle, on the other hand, both cyclic mucleotides appear to relax vascular smooth muscle. In this study we recorded the macroscopic and microscopic Ca^{2+} currents using patch-clamp technique in aortic vascular smooth muscle (VSM) of rabbit in order to verify if both cAMP and cGMP block the Ca^{2+} channels. We report here that agents that increase cAMP as well as 8-Br-cAMP block the inward Ca^{2+} current by decreasing the probability of opening of this channel. Also, agents that increase [cGMP], as well (such as nitroprusside) as 8-Br-cGMP block the inward Ca^{2+} current by decreasing the probability of opening of this channel. Recently, we reported that increasing cAMP also blocks the delayed outward rectifier potassium current in VSM and that increasing [cGMP] increases this current by increasing the probability of opening of this channel. In conclusion, it is possible that the relaxation of VSM by increasing $[cGMP]_i$ could be due to blockade of Ca^{2+} current and to the increase of K^+ current as well as to phosphorylation of myosin light chain kinase. However, the vasorelaxation induce by increasing $[cAMP]_i$ could be mainly due to blockade of I_{Ca}. This work was suspported by MRCC (MA 8920) and Dr. Bkaily is a Merck Frosst-FRSQ professor and M. Peyrow is a fellow of CHF.

M-Pos166

INHIBITION OF ELECTRICAL SLOW WAVES AND CALCIUM CURRENTS IN SMOOTH MUSCLE BY PHOSPHATASE INHIBITORS. S.M. Ward, F. Vogalis, D.P. Blondfield, H. Ozaki, N.G. Publicover, K.M.Sanders. Dept. Physiology, University of Nevada School of Medicine, Reno, NV. 89556. (Intro. P.Nemeth)

In has been reported in many studies that phosphorylation causes an enhancement in voltage-dependent Ca^{2+} current. Blockade of dephosphorylation causes further enhancement of Ca^{2+} current. The effects of calyculin-A, a phosphatase inhibitor isolated from the marine sponge *Discodermia calyx*, and okadaic acid on the electrical activity of colonic and gastric muscles were studied. Calyculin-A reduced the amplitude and duration of slow waves, primarily by inhibiting the plateau component. Okadaic acid, also reduced the amplitude and duration of gastric slow waves. The plateau component of slow waves appears to be due to the balance between dihydropyridine-sensitive Ca^{2+} current and at least 2 voltage-dependent components of outward current. Suppression of the inward current or enhancement of the outward current could lead to premature repolarization and shortening of the slow wave. Therefore, we studied the mechanism of action of calyculin-A by studying its effects on inward currents of isolated gastric and colonic myocytes. Calyculin-A (10^{-7} M) reduced the amplitude of the transient component of inward current by $69\pm6\%$ in gastric cells and $51\pm7\%$ in colonic cells. The sustained phase of the inward current was also reduced by $66\pm14\%$ in gastric cells ($n=7$) and $57\pm24\%$ in colonic cells ($n=4$). Okadaic acid had similar effects. Reduction of the sustained phase could account for the reduction in the plateau phase of slow waves. These data suggest that phosphorylation of Ca^{2+} channels of gastrointestinal smooth muscles may inhibit Ca^{2+} currents. This mechanism may provide an important means of regulating the currents responsible for excitation-contraction coupling in these muscles. (Supported by DK 32176 and DK 41315)

M-Pos167

EFFECTS OF GENISTEIN ON K^+ AND Ca^{2+} CURRENTS IN SINGLE ATRIAL CELLS. A. de S. Otero and A.F. de Amorim. Dept. of Physiology, University of Virginia, Charlottesville VA 22908.

The actions of genistein, a reported inhibitor of tyrosine protein kinases (Akiyama et al., J.Biol. Chem. 262:5592, 1987), were studied in single frog atrial myocytes by the whole cell patch clamp technique. In the presence of 3 μM tetrodotoxin, external application of 100 μM genistein led to large (60%) decreases in the inward currents elicited by 250 ms pulses from a holding potential of -85 mV to either -135 mV or -5 mV; the changes in the whole cell current-voltage relationship induced by genistein were compatible with a partial block of the background K^+ (I_{K1}) and Ca^+ (I_{Ca}) currents. Also, the outward current flowing through muscarinic activated K^+ channels ($I_{K(ACh)}$), as measured at -5 mV, was reduced by 30%. All effects of genistein were fully reversible upon washout. The generalized inhibition of membrane currents by genistein is presumably non specific, and may be partly responsible for its cytotoxic effects at high concentrations. At 1-3μM, genistein had virtually no effect on I_{K1} and I_{Ca} , but was still able to decrease steady state outward $I_{K(ACh)}$ by 18%. Moreover, 3μM genistein reduced , also reversibly, the rate of agonist-independent activation of $I_{K(ACh)}$ by ATPγS and GTP analogs (GTPγS and GMP-PNP) by 80%, but did not affect fully activated, GTP analog-supported $I_{K(ACh)}$. The results suggest that genistein affects the function of the G protein, G_k, that couples muscarinic receptors to K^+ channels in atrial cells.

Supported by AHA, Virginia Affiliate, and by NIH HL37137.

M-Pos168

PARADOXICAL AGONIST EFFECTS OF THE DISULFONIC STILBENE DERIVATIVE SITS ON THE CARDIAC CHLORIDE CURRENT

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The anion exchange inhibitor 4-acetamido-4'-isothiocyano-2,2'-disulfonic acid stilbene (SITS) has been shown to directly block Cl^- channels. Therefore, we examined the effect of this compound on the whole cell Cl^- current observed in isolated cardiac ventricular myocytes. Antagonists, ion substitution, and holding potential were used to eliminate currents carried via other conductance pathways. Isoproterenol (ISO) was used to activate the otherwise latent Cl^- conductance. Following stimulation of the Cl^- current with 1 μM ISO, subsequent exposure to ISO plus SITS (0.1 to 1.0 mM) increased, rather than decreased, the current without affecting its voltage dependence or reversal potential. When myocytes were exposed to SITS prior to ISO, variable responses were observed. In six experiments, SITS alone activated a current that reversed at the Cl^- equilibrium potential and exhibited the same time and voltage dependence as the ISO-induced Cl^- current. In three of these cells, the SITS-induced current was only transiently observed. In five other experiments SITS alone had no effect. In six of the eight experiments in which there was no maintained SITS-induced current, subsequent exposure to SITS plus ISO activated the Cl^- current, however, washout of SITS attenuated the magnitude of the current even though ISO was still present. These results indicate that SITS alone can activate the Cl^- current in cardiac myocytes. The fact that SITS also facilitated the activation of the Cl^- current by ISO suggests that the effect of SITS may be the result of an indirect effect on the regulatory pathway. Any evidence of a SITS induced block of the Cl^- current was obscured by its agonist properties. [Supported by NIH (HL30143 and HL45141) and AHA]

M-Pos169

OKADAIC ACID MODULATES CHLORIDE CURRENT ACTIVATED BY cAMP-DEPENDENT PROTEIN KINASE IN GUINEA PIG VENTRICULAR MYOCYTES. Minoru Horie, Tzyh-Chang Hwang and David C. Gadsby. *Laboratory of Cardiac Physiology, The Rockefeller University, New York, NY 10021.*

The influence of okadaic acid, an inhibitor of types 1 and 2A protein phosphatases, on the cAMP-dependent protein kinase (PKA)-mediated Cl conductance was examined in cardiac myocytes. Whole-cell currents were recorded, at ~37 °C, in myocytes voltage-clamped and internally dialyzed with wide-tipped pipettes (~1 MΩ) and an intra-pipette perfusion device. Extracellular solutions were nominally Ca-free and K-free, and contained 1 mM Cd and usually 1 mM Ba, to minimize Ca-channel and K-channel currents. With 100 μM GTP and 20 mM Cl in the pipette, and 150 mM extracellular [Cl], bath application of isoproterenol (ISO; 1 μM) or forskolin (FSK; 1 μM) activated typical, outwardly-rectifying, Cl current: the Cl current disappeared completely in less than 1 min, or 3 min, of washing out the ISO, or FSK, respectively. In the absence of ISO or FSK, switching to a pipette solution containing okadaic acid (1-10 μM) caused minimal changes in membrane currents even after as long as 15 min . However, subsequent application of ISO or FSK in the presence of okadaic acid (in some cases after only 5 min exposure to okadaic acid) activated Cl currents which were 20-60% (n=7) larger than the initial control responses to ISO or FSK. Similarly, introduction of okadaic acid in the continued presence of ISO or FSK, after waiting 3-16 min to ensure steady-state activation of the Cl conductance, resulted in a further 40-100 % (n=5) enhancement of the Cl currents. Moreover, okadaic acid considerably delayed, and slowed, the deactivation of the Cl current following wash-out of the ISO or FSK, and some 20-60% (n=6) of the Cl current persisted (for tens of min) in the absence of ISO and FSK. This persisting current component was insensitive to intracellular application of a synthetic peptide inhibitor (150 μM) of PKA: that concentration of the inhibitor was sufficient to completely abolish ISO- or FSK-induced Cl current in other cells in the absence of okadaic acid. Dephosphorylation by an okadaic acid-sensitive protein phosphatase appears to be essential for complete deactivation of the PKA-activated Cl conductance. *(Supported by NIH HL-14899 and HL-36783, and the Irma T. Hirschl Trust. We thank A. Nairn and P. Greengard for PKA inhibitor and Y. Tsukitani for okadaic acid).*

M-Pos170

G-PROTEIN MODULATION OF A CARDIAC DELAYED RECTIFIER K^+ CURRENT BY A MEMBRANE DELIMITED PATHWAY. Wai-Meng Kwok, Lisa C. Freeman, Robert S. Kass., Dept. of Physiology, University of Rochester, Rochester, NY, 14642.

G-proteins can regulate ion channels indirectly by altering cytoplasmic second messengers, or directly by a membrane delimited mechanism. Indirect pathways are known to couple β-adrenoceptors (βAR) to the delayed rectifier K^+ (I_k) channel in heart ventricular cells. Using excised membrane patches from guinea pig ventricular myocytes in the inside-out configuration, we demonstrate that a membrane-delimited pathway also exists, and contributes to regulation of the cardiac delayed rectifier K^+ (I_k) channel. Pipette solutions (extracellular side) contained (mM): 132 N-methyl-glucamine; 0 to 5 KCl; 2 $MgCl_2$; 1 $CaCl_2$; 5 dextrose; 0.2 $CdCl_2$; 10 HEPES, pH 7.3. Bath solutions (cytosolic side) contained (mM): 140 K-aspartate; 1 $MgCl_2$; 1 $CaCl_2$; 11 EGTA; 5 ATP; 5 HEPES, pH 7.3. Currents were measured in response to 2 second pulses applied at 6 or 14 second intervals from a holding potential of -30 mV. Experiments were conducted at 32-37°C. Discrete single channel openings were not resolvable. Instead, a macroscopic patch current similar to whole cell I_k was recorded. Addition of GTP or GTPγS (100 or 200 μM) to the cytoplasmic side caused I_k to increase markedly in 60% of the patches (n=9); I_k increased by at least 18% and up to 120%. Enhancement of I_k by guanine nucleotides was also observed in the presence of βAR stimulation by 10 μM isoproterenol (n=4); I_k increased by at least 10% and up to 280%. GTP effects could be reversed by 200 μM GDPβS (n=3). These data show that the ventricular I_k channel is modulated by a direct, membrane delimited G-protein pathway, and that this mechanism may contribute to βAR stimulation of I_k.

M-Pos171

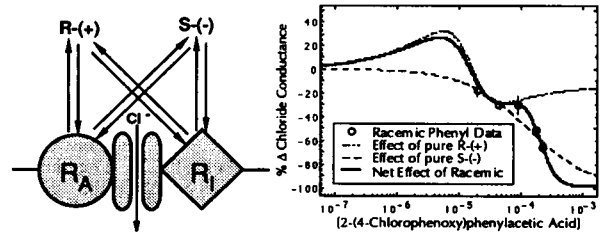
CELL SWELLING ACTIVATES A MEMBRANE Cl CHANNEL IN CANINE CARDIAC MYOCYTES G.-N. Tseng, Dept Pharmacology, Columbia U., New York.

Cell swelling (Swell) activates Cl and K channels in epithelial cells and lymphocytes. In cardiac cells, Swell occurs under compromised conditions, e.g. myocardial ischemia. The effects of Swell on membrane conductance (g_m) were studied in canine ventricular and Purkinje cells. Recording conditions were designed to largely suppress currents through membrane Na, K and Ca channels or generated by electrogenic transport systems. Swell was induced by either reducing the osmolarity of external solution to 80% of control or raising osmolarity of internal solution to 200% of control, the latter by an intracellular dialysis technique, and the steady-state I-V relation was monitored. Swell induced a time- and voltage-independent current. A Cl channel blocker, 9-AC, decreased Swell-induced current by 90% without changing its reversal potential (E_{Rev}) (n=4). Reducing $[Cl]_o$ from 148 to 0 mM, substituted by aspartate (Asp), caused a decrease in the Swell-induced outward current and a positive shift in E_{Rev}. Increasing $[Cl]_i$ from 20 to 145 mM (Asp as the main internal anion at 20 mM $[Cl]_i$) caused a positive shift in E_{Rev}. These observations suggested that Cl is a charge carrier for Swell-induced current. However, the change in E_{Rev} when altering $[Cl]_o$ or $[Cl]_i$ was less than that predicted by the Nernst equation, and suggested that Asp may also pass through the channel or other types of channel were also activated by Swell. With a low external divalent cation concentration ($[Mg]_o$ = 0.5 mM), the I-V of Swell-induced current was linear. Adding Ca or Mn (2-5 mM) decreased Swell-induced current and made the I-V outwardly rectified, suggesting a modulatory role of divalent cations in ion permeation through Swell-activated Cl channel (g_{Cl}). The mechanism of activation of g_{Cl} by Swell was investigated. The activation of g_{Cl} did not require Ca_i and was not sensitive to temperature changes (22-36°C) nor affected by 100 uM H-8 (a protein kinase inhibitor) in the pipette solution. However, 10 uM dihydrocytochalasin B (DCB) increased g_m with an I-V relation and E_{Rev} similar to that induced by Swell. Following DCB treatment, Swell did not induce further increase in g_m. These observations suggested a role of cytoskeleton in the activation mechanism. In conclusion, Swell activates a membrane Cl channel whose ion permeation properties and activation mechanism may be different from those of the cAMP-activated Cl channel described for cardiac myocytes.

M-Pos172

MODELING THE EFFECTS OF OPPOSITELY ACTING ENANTIOMERS OF CLOFIBRIC ACID ANALOGS ON CHLORIDE CONDUCTANCE IN RAT SKELETAL MUSCLE. R. Wagner, A. De Luca*, D. Tricarico*, D. Conte-Camerino* and S.H. Bryant, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 and *Dipartimento Farmacobiologico, Facolta di Farmacia, Universita di Bari, 70126-Bari, Italy.

Enantiomeric pairs of 2-(4-chlorophenoxy)-X, where X=propionic acid, have been shown to produce opposite actions on resting chloride conductance (G_{Cl}) of rat skeletal muscle fibers (Conte-Camerino et al., *Pflugers Archiv.* 413:105-107,1988). At concentrations below 10 μM the S(-) enantiomer blocks while the R(+) enantiomer increases G_{Cl}; above 10 μM both enantiomers block G_{Cl}. We now show that the concentration-response data of the R-(+), S-(-), and racemic derivatives of three different compounds (X=phenylacetic, propionic, and valeric acids) can be fitted to a functionally antagonistic, two receptor site model. This model assumes that 1) S-(-) derivatives act as agonists at an R_I (inactivating) receptor, which decreases G_{Cl}, 2) R-(+) derivatives act as agonists at both R_A (activating) and R_I receptors, thereby producing a biphasic response, 3) S-(-) derivatives bind to the R_A receptor, but produce no direct effect at this receptor (i.e. act as true competitive antagonists at R_A).



Data sets for R-(+), S-(-), and racemic concentration-response curves were nonlinear least squares fitted to the three model equations simultaneously to obtain estimates of the parameters. The IC_{50} & EC_{50} values obtained, ranging from 5 to 65 μM, set upper limits on the dissociation constants represented in the model. Although the goodness-of-fit of all of the data sets to the model was excellent, we cannot exclude the possibility that other models might fit the data equally well.

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M-Pos173

CHANGES IN MEMBRANE CAPACITANCE ARE CORRELATED WITH ACTIVATION OF OSMOTICALLY-INDUCED Cl^- CONDUCTANCE. Sarah S. Garber[1] and Michael. D. Cahalan[2], [1]Univ. of Alabama, Birmingham, Birmingham, AL, 35294 and [2]Univ. California, Irvine, Irvine, CA 92717; [1,2] Membrane Biophysics, Max Planck Inst., Göttingen, Germany.

In Jurkat and other T lymphocytes, osmotic swelling induces an outwardly-rectifying Cl^- conductance (g_{Cl}). We have used the whole-cell (w/c) patch-clamp technique to simultaneously monitor changes in osmotically-induced g_{Cl} and membrane capacitance (C_m) in Jurkat T lymphocytes. A lock-in amplifier was used to measure increases in C_m and assess a possible relationship between vesicle fusion events and changes in membrane conductance. Pipette solutions contained 140 mM Cs glutamate, 0.1 mM fura 2, 0.5 mM ATP, and $[Ca^{++}]_i$ was buffered to $<10^{-9}$ M or 300 nM with 10 mM EGTA. At 26°C and low $[Ca^{++}]_i$, 10/20 cells were observed to swell spontaneously upon establishing the w/c recording configuration. In 9 of these cells, *both* g_{Cl} and C_m increased. In the 10 cells that did not swell, *neither* parameter increased. Increases in g_{Cl} and C_m ranged from a chord conductance of 0.4 to 4.5 nS and 0.2 to 1.0 pF, respectively. The correlation was less apparent at lower temperatures (19-21°C). At 26°C, with internal solutions containing either ≥0.1 mM 8Br-cAMP or cAMP, 7/10 cells became swollen, with 6 cells showing increases in *both* g_{Cl} and C_m. Three out of 10 cells did not swell and showed *no* increase in either parameter. In the presence of 300 nM $[Ca^{++}]_i$ alone (26°C), 5 cells became swollen, with 4 showing an increase in *both* g_{Cl} and C_m. One possible explanation for the correlated increases in C_m and g_{Cl} is that swelling may promote fusion of intracellular vesicles containing Cl^- channels. However, the causal relation between swelling, C_m and g_{Cl} remains to be explored.

This work was supported by the National Cystic Fibrosis Foundation to SSG and NIH grant #NS14609 to MDC. We thank Dr. Erwin Neher for encouragement and support.

M-Pos174

DUAL CURRENT MODULATION BY DOPAMINE AND FMRFAMIDE IN CULTURED *LYMNAEA* V.D4 CELL BODIES. S. Barnes, N. Syed, A.G.M. Bulloch and K. Lukowiak (Intro. by W. Wonderlin), Department of Medical Physiology and Neuroscience Research Group, University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1.

Cell V.D4 responds to exogenous application of either FMRFamide or dopamine with slight hyperpolarization and no rebound spikes, but produces rebound spikes following hyperpolarization when dopamine and FMRFamide are applied sequentially in this order. To see why, we examined the underlying conductance changes associated with FMRFamide and dopamine application in isolated V.D4 cells under whole cell patch clamp. Individual V.D4 cells were removed from young *Lymnaea stagnalis* and maintained for 24-48 hours in culture conditions which inhibit neurite outgrowth, at which time the medium was switched for recording to saline normally containing (in mM) 51.3 NaCl, 1.7 KCl, 1.5 $MgCl_2$, 4.1 $CaCl_2$ and 5 HEPES (pH 7.9) but as specified, either 1) low Ca^{2+} (0 mM), high Mg^{2+} (15 mM), 2) high Ca^{2+} (41 mM), 3) choline (51 mM, no Na^+), 4) 30 mM TEA or 5) 0.1 or 1 mM Cd^{2+} was substituted. The patch pipette contained (in mM) either 50 KCl or 50 CsCl, along with 5 EGTA, 5 $MgCl_2$ and 5 HEPES. I_{Na}, I_A, $I_{K(V)}$, $I_{K(Ca)}$, and I_{Ca} were readily observed.

Internally dialysed with the KCl solution, VD4 responds to 10 uM dopamine under voltage-clamp in normal saline with increased outward current at all potentials positive to -40 mV, but to 100 nM FMRFamide with increased outward current only in the range between -40 mV and +30 mV. With the ionic substitutions described above, we found that dopamine increases the amplitude of $I_{K(V)}$ while simultaneously decreasing I_{Ca}. Consequently, $I_{K(Ca)}$ may be reduced as well. At 100nM, FMRFamide only decreases I_{Ca}, but at 1 uM some increase in $I_{K(V)}$ was seen. These actions suggest that rebound spiking is supported when a specific balance is struck between 1) deeper spike repolarization due to increased voltage-gated K^+ current activation (as caused by 10 uM dopamine) and 2) a reduced calcium component of the action potential and/or consequent reduced calcium activated K^+ current (as induced by 100 nM FMRFamide). Complexities of the timecourse of drug washout, cell recovery and inhomogeneity of receptor and target ion channel distribution on the cell soma and processes could also play a role.

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

M-Pos175

PARATHYROID HORMONE (PTH) MODULATES GATING OF A STRETCH ACTIVATED NON-SELECTIVE CATION CHANNEL (SA-cat) IN UMR-106.01 OSTEOBLAST-LIKE CELLS. Randall L. Duncan (Intro. by Stanley Misler), Renal Division, Jewish Hospital, St. Louis, MO 63110.

PTH modulates synthesis of bone matrix proteins by osteoblasts and osteoblast-like cell lines. An early response of osteoblasts to PTH stimulation is plasma membrane depolarization. Previously, we have demonstrated that UMR-106.01 cells contain voltage-independent, 18pS SA-cat channels. Enhanced opening of these channels could result in a stimulus-induced depolarization. Here we present evidence which may link the PTH depolarization with PTH activation of SA-cat channels. Whole cell current clamp recordings from cells in colonies of more than 20 cells demonstrate a PTH-induced depolarization of UMR-106.01 cells from a resting membrane potential (Vm) of -38.1 $\pm$ 1.0mV (n=42) by an average of 5.9mV with the peak depolarization reached within 1-2 min after PTH (50nM) addition to the bath. PTH elicited this response in 71% of the cells tested while Vm was not affected by PTH in the rest of the cells tested. Depolarization is blocked by 10μM Gd^{3+} but not by Ni^{2+} (100μM), nitrendipine (1.5μM) or Ba^{2+} (5mM). The depolarization can not be mimicked by addition of 8-br-cAMP (100μM) although cAMP is a second messenger of PTH stimulation of other osteoblastic functions. SA-cat channels activated in the cell-attached patch by pulses of pipette suction demonstrate enhanced activity after bath addition of 50nM PTH. The time course of enhanced activity of these channels parallels the time course of the PTH-induced Vm depolarization. The activity of these channels is likewise blocked by 10μM Gd^{3+}. In addition to the PTH-stimulated increase in activity there is an increase in channel conductance suggesting that PTH may unveil different conductance states of the channel. Preliminary kinetic analysis of the SA-cat channel indicate no change in the open times of the channel in response to PTH stimulation. Our results suggest that PTH modulation of SA-cat channel activity may produce osteoblast membrane depolarization, which, in turn, may be important in the transduction of the PTH signal. (Support: Shriner's grant 15952)

M-Pos176

THE MODULATION OF THE MITOCHONDRIAL OUTER MEMBRANE CHANNEL, VDAC, BY A HIGHLY CONSERVED PROTEIN FROM MITOCHONDRIAL FRACTIONS (Intro. by Richard H. Racusen)
Mingyao Liu and Marco Colombini, Lab. of Cell Biology, Dept. of Zoology, University of Maryland, College Park, MD 20742

Soluble protein preparations obtained from the mitochondrial fractions of three very different sources: Neurospora crassa, rat livers, and potato tubers were discovered to greatly enhance the voltage sensitivity of the mitochondrial outer membrane channel, VDAC. The active ingredient, referred to as the VDAC modulator, increased the rate of voltage-dependent channel closure by about 10 fold. In addition to finding that the modulator extracted from mitochondria of one species can act on VDAC from the same species, we further discovered that the modulator from one species increased the voltage sensitivity of VDAC channels obtained from other species. Preliminary experiments show that the VDAC modulator tightly binds to an anion-exchange column, indicating that the modulator is a negatively charged protein. However, the modulator activity can't be mimicked by another negatively-charged protein, BSA. Therefore, the modulator may be a protein specifically designed to interact with and regulate the voltage gating behavior of VDAC channels in mitochondria. The highly conserved property of this protein in the long process of evolution suggests an important physiological role in regulating the metabolism of mitochondria, and consequently the cell's energy production. (Supported by ONR grant # N00014-90-J-1024)

M-Pos177

PHORBOL ESTERS MODULATE A CHLORIDE CURRENT IN HUMAN MONOCYTE DERIVED MACROPHAGES. F. Jow and D.J. Nelson, University of Chicago, Dept. of Neurology, Chicago, IL 60637.

Upon exposure to phorbol myristate acetate (PMA), macrophages undergo a "respiratory burst" during which oxygen is converted to superoxide anion and hydrogen peroxide, metabolites which play an important role in macrophage microbicidal and tumoricidal activity. Although the signal transduction process is not well understood, stimulus-induced changes in membrane potential have been proposed to be closely associated with respiratory burst activation. PMA-induced respiratory burst activity is neither enhanced in high-K^+ solutions nor impaired in external solutions in which Na^+ was completely replaced with choline. We therefore sought to determine whether PMA-induced membrane potential changes could be due to the modulation of a chloride conductance. Whole-cell voltage clamp experiments were carried out on human monocyte-derived macrophages (HMDMs) using isosmotic pipette and bath solutions containing N-methyl-D-glucamine as a Na^+ and K^+ replacement. Bath perfusion of 30-80 nM PMA led to a 10 fold increase in an outwardly rectifying Cl^- current (I_{Cl}) activation at 100 mV (n=12) with no change in current reversal potential from the theoretically predicted value for E_{Cl} of -31 mV. The membrane permeant analog of diacylglycerol (1-oleoyl-2-acetylglycerol, OAG, 100 μM) produced current activation of similar magnitude (n=2). Perfusion of the cells with the inactive phorbol, 4α-phorbol-12,13-didecanoate (74 nM) failed to produce current activation (n=5). The specific inhibitor of protein kinase C (PKC_{19-36}) when added to the pipette solution (5 μM) reversed the PMA-induced enhancement of I_{Cl}. Dialysis of the cells with 5 mM ATPγS resulted in an increase in I_{Cl} (n=3) presumably due to the dephosphorylation-resistant thiophosphorylation. These results support the conclusion that PMA-dependent phosphorylation of I_{Cl} in monocyte-derived macrophages increases current activation and may be the basis for stimulus-induced membrane potential changes which accompany the PMA-induced respiratory burst activation. Supported by NIH RO1 GM36823.

M-Pos178

PROTEIN KINASE C ACTIVATES A H^+ (EQUIVALENT) CONDUCTANCE IN THE PLASMA MEMBRANE OF HUMAN NEUTROPHILS. Arvind Nanda and Sergio Grinstein (Intro. by Amira Klip). Division of Cell Biology. The Hospital for Sick Children, Toronto, M5G 1X8, Canada.

Activation of the respiratory burst in neutrophils is associated with a substantial intracellular production of acid. To prevent acid accumulation, protons are extruded from the cell, partly via Na^+/H^+ exchange. However, a sizeable fraction of the acid produced is exported even in Na^+-free media, implying the existence of acid extrusion processes other than Na^+/H^+ exchange. The present studies were designed to identify these alternative H^+ extrusion mechanisms. In Na^+-free, high K^+ or NMG^+ media, cytosolic pH decreased upon stimulation with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), largely due to H^+ production by the NADPH oxidase. To limit this production of H^+, the oxidase was inhibited using diphenyleneiodonium (DPI). Under these conditions, addition of TPA induced only a modest acidification. In DPI-treated cells suspended in high K^+ medium containing 1 μM valinomycin (a K^+ ionophore), stimulation with TPA caused an intracellular *alkalinization* of approximately 0.2 pH unit. The alkalinization is not due to tightly coupled, endogenous K^+/H^+ exchange, since it is only apparent in the presence of the ionophore. Moreover, a similar response occurred upon stimulation of DPI-treated cells suspended in a high Na^+ medium containing a potent inhibitor of Na^+/H^+ exchange and 200 nM gramicidin D (a Na^+ ionophore). These observations suggest that alkalinization is due to electrogenic efflux of H^+ (equivalents) coupled to the uptake of alkali cations through the ionophores. Accordingly, alkalinization failed to occur when DPI-treated cells suspended in NMG^+ medium containing valinomycin were stimulated with TPA. Moreover, when DPI-treated cells suspended in a high K^+ medium with valinomycin were stimulated with TPA, their intracellular pH became highly sensitive to external pH perturbations. In both high K^+ and high Na^+ media, the alkalinization response was insensitive to bafilomycin A_1, a potent inhibitor of vacuolar-type H^+-transporting ATPases. The dependence of the phorbol ester-induced pH changes on the electrochemical H^+ gradient and their insensitivity to bafilomycin A_1 are consistent with translocation of H^+ through a passive conductive pathway. Because this pathway is not apparent prior to the addition of TPA, we conclude that a H^+ conductance, possibly a H^+ channel, is activated in neutrophils by protein kinase C.

M-Pos179

ACTIVATION AND INHIBITION OF NON-SELECTIVE CATIONIC CHANNELS IN HUMAN NEUTROPHILS

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Although non-selective cation channels found in a broad variety of cells have been recognized as regulators of activation, excitation, and secretion, kinetics and modulation of their macroscopic currents have not been studied in human neutrophils. By means of whole-cell, voltage-clamp and single-channel, ramp-clamp we studied the kinetics, activation and inhibition of the channels. In activated cells, channels showed significant voltage-dependence and channel activation followed a double-exponential function with higher rates of activation. From macroscopic conductance-voltage plots an estimated maximal activated conductance of 10 nS was derived suggesting a minimal channel density of 400 channels per neutrophil. In resting cells, the derived minimal channel density was 200 channels per neutrophil. The chemotactic oligopeptide, formylmethionylleucyl-phenylalanine (fMLP), the Ca^{2+} ionophore, A23187, and phorbol myristate acetate (PMA) augmented the whole-cell, non-selective cationic currents and enhanced their activation rates. The Ca^{2+}-chelator 1,2-*bis*(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetate (BAPTA) markedly diminished resting, and PMA-induced currents but only partially decreased A23187-, or fMLP-induced currents. Further reduction of resting, A23187-, and PMA-induced currents was caused by the adenosine analogs 5'*N*-ethylcarboxamidoadenosine (NECA) and *N*6-cyclohexyladenosine (CHA). Dibutyryl cAMP (DBcAMP), NECA and CHA significantly reduced resting and fMLP-induced currents, but DBcAMP had no significant effect on A23187-, or PMA-induced currents. Thus, activation and inhibition of non-selective cation channels in human neutrophils involve Ca^{2+}-independent, indirect Ca^{2+}-dependent, and cAMP-dependent mechanisms.

M-Pos180

EFFECTIVE PARTICIPATION OF Na^+/H^+ EXCHANGE ON BILE ACID INDEPENDENT BILE FORMATION IN GUINEA PIG HEPATOCYTES.

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Under physiological conditions, intracellular pH is thought to be maintained by excluding H^+ throughout the bile acid independent bile formation in hepatocytes. To clarify the participation of buffering systems, we examined the effects of intracellular acidification with the application of different internal pH solutions by measuring the delayed rectifier outward current in guinea pig hepatocytes under whole cell voltage clamp techniques. When the pH of the pipette solution was lowered from the control of 7.3 to 6.8 (mild acidic), no outward current change was observed. On the contrary, following perfusion with more acidic pipette solution (pH=6.2), the outward current was suppressed. This may indicate the limitation of intracellular buffering activity against the excessive intracellular acidification. When the outward current was recorded in Na^+ free external solution, this current was suppressed even with a mild acidification (pH=6.8), suggesting the existence of Na^+/H^+ exchange system. Furthermore, 1mM amiloride applied in the bath solution suppressed the outward current. Another possible buffering mechanism may have operate to exclude continuously H^+ which entered from the pipette. The mechanism is efflux of Cl^- from the cell, which is sensitive to the extracellular HCO_3^- concentration (Cl^-/HCO_3^- exchange). Since we did not use HCO_3^- buffer in our experiments, this mechanism could be ignored. Therefore we conclude that Na^+/H^+ exchange will effectively participate in the bile acid independent bile formation by excluding continuously H^+ which is generated in its process.

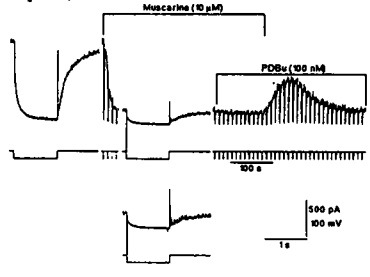
M-Pos181

Pre-closure of M-channels by muscarine prevents the action of phorbol ester. N.V. Marrion & P.R. Adams (Intro. by R. Kullberg). Howard Hughes Medical Institute, SUNY at Stony Brook, NY, 11794.

To date, the only second messenger system known to suppress the M-current (I_M) is activation of protein kinase C (PKC). Using whole-cell recording from dissociated bullfrog sympathetic neurons, the phorbol ester 4-β-phorbol 12,13-dibutyrate (PDBu) suppressed I_M in a concentration-dependent manner. This irreversible inhibition required low concentrations of PDBu (K_i 31 nM), was slow to develop, and affected over 80% of elicited M-current.

Pre-closure of M-channels with muscarine protects them from the action of PDBu. If PDBu was applied for 120 seconds and then removed in the continued presence of muscarine (10 μM, suppressing >90% of M-current), the muscarine-evoked suppression of I_M reversed upon washout of agonist. Neither closing the channels with voltage or blocking the M-current with Ba^{2+} prevented the action of PDBu.

Reversing the order of washout of muscarine and PDBu produced the effect shown in the figure below. After I_M had been suppressed by muscarine (10 μM), PDBu (100 nM) was added. After 120 seconds in PDBu, muscarine was now removed. As muscarine's effect reversed the current transiently recovered but then was rebound suppressed by the continued presence of PDBu. Even after 30 minutes wash from PDBu I_M had not recovered (lower panel).



This illustrates that while M-channels were closed by muscarine PDBu was ineffective. As M-channels reopened, after removal of muscarine, they could be re-closed by the action of PDBu. Thus, M-channel closure by muscarine protects the channel from the effects of PKC activation.

M-Pos182

GLIBENCLAMIDE AND BRL 38277 HAVE OPPOSING EFFECTS ON MEMBRANE POTENTIAL AND ON A TIME-INDEPENDENT K^+ CURRENT IN ARTERIAL MUSCLE.

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Cromakalim relaxes arterial muscle and causes membrane hyperpolarization. Its effects are reversed by sulphonylurea drugs, such as glibenclamide. Using the patch-clamp technique in the whole-cell mode, we have studied the effects of the active enantiomer of cromakalim (BRL 38277) and glibenclamide on isolated rabbit pulmonary arterial cells. Cells were bathed in a physiological salt solution (1.8 mM Ca) and the recording pipette contained in mM: 139 KCl, 1 EGTA, 15 HEPES, 1 ATP, 0.5 GTP, pH 7.2. Drugs were applied by pressure ejection. In current clamp, BRL 38277 hyperpolarized the membrane at concentrations inducing muscle relaxation (5-20 μM). At 10 μM, it hyperpolarized cells from the resting potential of -60 ± 3 mV to -70 ± 2 mV (n=6), with glibenclamide (50 μM) subsequently repolarizing them to beyond the control (-54 ± 2 mV). Furthermore, glibenclamide itself caused a prolonged depolarization from -60 ± 1 mV to -52 ± 1 mV (n=6), associated with a reduction in membrane noise. Thereafter, BRL 38277 (10 μM) caused a larger hyperpolarization of 17 ± 1 mV (n=5). Voltage clamp experiments revealed that BRL 38277 increased an instantaneous current, which was activated during depolarizing steps to -70 to +40 mV. This current is likely to be carried by K^+, since it reversed close to the K^+ equilibrium potential. At negative potentials (<-30 mV) the BRL 38277-activated current was time independent. Moreover, glibenclamide suppressed this BRL 38277-sensitive, time-independent current to the control level or beyond. Although these drugs have been previously shown to act on ATP-sensitive K channels, our results imply that the time-independent current, regulated by BRL and glibenclamide, is active at the resting potential, even in the presence of 1 mM intracellular ATP.

Supported by the British Heart Foundation and the Royal Society.

M-Pos183

ON THE MECHANISM OF NUCLEOTIDE DIPHOSPHATE-INDUCED OPENINGS OF THE ATP-SENSITIVE POTASSIUM CHANNEL IN VENTRICULAR CELLS OF GUINEA-PIG - A FUNCTIONAL TRANSDUCER UNIT HYPOTHESIS. R.T. Tung and Y. Kurachi, Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic, Rochester, MN 55905.

Depletion of intracellular ATP causes activation of ATP-sensitive potassium channels (K_{ATP}) in cardiac myocytes. To elucidate the regulatory mechanisms of K_{ATP} by intracellular nucleotides, effects of nucleotide diphosphates (NDPs) on K_{ATP} were examined in guinea-pig ventricular cells using the patch clamp technique. Upon formation of inside-out patches in the ATP-free internal solution, K_{ATP} appeared and then decayed spontaneously. This channel run-down was probably due to de-phosphorylation of the channel. We found that millimolar concentrations of various NDPs (e.g., UDP, IDP, CDP and GDP), applied to the internal side of the patch membrane after run-down, dramatically activated the channel. NDP-induced openings of K_{ATP} were Mg^{2+}-dependent and inhibited completely by ATP (100 μM) and glibenclamide (1 μM, a specific K_{ATP} inhibitor). Nucleosides, nucleotide mono- and tri-phosphates did not activate K_{ATP}. This suggests that K_{ATP} may have a novel Mg^{2+}-dependent site specific to NDPs which induces openings of the de-phosphorylated channel, in addition to the Mg^{2+}-independent ATP-binding inactivation site and phosphorylation site. Pinacidil (a K_{ATP} opener) activated the phosphorylated but not the de-phosphorylated K_{ATP}. Pinacidil also enhanced the de-phosphorylated K_{ATP} openings vigorously in the presence of various NDPs. These suggest that NDP-binding to a specific site has similar effects to channel phosphorylation, i.e. to keep the channel in an operative state and to enhance the channel openings by pinacidil. We hypothesized a functional transducer unit between the ATP-binding site (A-site) and the channel gate, where the phosphorylation site (P-site) is located. When the unit is phosphorylated, the signal from A-site can be transduced to the gate. Since NDP-binding to the de-phosphorylated channel may play a role similar to the channel phosphorylation, the NDP-binding site (NDP-site) is also assumed to be located in the same unit as the P-site. NDPs bind to a specific NDP-site and, possibly cause a conformational change of the unit similar to phosphorylation. This model offers a new approach to elucidate the molecular mechanisms underlying the regulation of cardiac K_{ATP} by various agonists, drugs and G-proteins in physiological and pathophysiological conditions.

M-Pos184

DOPAMINERGIC MODULATION OF INWARD-RECTIFYING K CHANNELS IN RAT PITUITARY MELANOTROPES. Gabriel Cota, Arturo Yañez, and Angel Marin. Dept. of Physiology, CINVESTAV-IPN, México, D.F 07000.

It is well known that dopamine (DA) inhibits hormone secretion in pituitary pars intermedia cells (melanotropes), an effect that is mediated by D-2 dopaminergic receptors. Stimulating these receptors decreases the intracellular levels of cAMP, increases a K^+ conductance in the plasma membrane, hyperpolarizes the membrane, and reduces the frequency of spontaneous action potentials. In order to identify the type of K channel stimulated by DA, we have analyzed the effect of the catecholamine on the "resting" membrane conductance of the melanotropes using whole-cell recording with patch electrodes (2-3 MΩ). The external//internal solutions contained (in mM): 125 NaCl, 25 KCl, 2 $CaCl_2$, 0.2 $CdCl_2$, 0.001 TTX, and 5 glucose//125 K-aspartate, 20 KCl, 1 $MgCl_2$, 1 Mg-ATP, 1 EGTA, and 0.05 GTP (pH 7.3). Under these recording conditions the ionic current evoked by 60-ms voltage clamp steps (HP -60 mV) showed a marked inward rectification. The reversal potential of the current was close to the expected E_K, and was not altered by raising the internal Cl^+ concentration to 107 mM. Addition of DA (2 μM) to the external solution increased the amplitude of the currents in response to voltage steps by 30-80%. The DA-induced current also showed inward rectification and its reversal potential was close to E_K. The DA response was drastically reduced, or completely abolished, by omitting GTP in the internal solution or by preincubating the cells with pertussis toxin (PTX, 0.2 μg/ml, ~12 h), but was not significantly affected by the addition of 1 mM cAMP to the internal solution. We conclude that DA increases the conductance of the membrane to K^+ ions in the melanotropes by stimulating the activity of inward-rectifying K channels through a PTX-sensitive G protein; inhibition of the intracellular cAMP levels does not seem to be required for this action of DA.

M-Pos185

EXPRESSION OF VOLTAGE-DEPENDENT ION CHANNELS DURING EARLY DEVELOPMENT OF EMBRYONIC MUSCLE OF *XENOPUS LAEVIS*. A.E. Spruce, C. Kim and W. Moody, Dept. of Zoology, University of Washington, Seattle, WA. 98195

Previous studies of the appearance of ion channels in developing skeletal muscle have concentrated on the Acetylcholine receptor (AChR) channel (Blackshaw & Warner, Nature 266:217). Voltage-dependent channels have been studied in much less detail at early developmental times, particularly in acutely dissociated cells. The aim of our experiments is to characterize the earliest appearance of currents in muscle cells isolated from *Xenopus* embryos. The induction of ectoderm by endoderm to form mesoderm takes place in the blastula stage. It is not until during gastrulation that muscle-specific proteins begin to be expressed (Gurdon et al, TIG 5:51). However, in whole-cell current recordings from cells isolated from the gastrula stage, voltage-dependent currents were not detected. At the neurula stage, dorsomedial mesoderm was dissected out of the embryo and dissociated. The first recordings were made within 1 hr. In cells showing early morphological signs of muscle differentiation, voltage-dependent currents were expressed first at the neural fold stage (Stage 14, Nieuwkoop & Faber). At that time, an inward-rectifying K^+ current was seen in a few of these cells. It was not until 3-1/2 hours later (just after the time of somite segregation) that delayed rectifier K^+ and transient CA^{2+} (activation threshold, -40mV) currents were seen. This is also the time that AChR activation has been found (Owens & Kullberg, J. Neurosci. 9:108). Prior to the stage of muscle contractions in the intact embryo, a Na^+ current was seen only rarely. Thereafter, a majority of muscle cells exhibited Na^+ currents. The number of cells with voltage-dependent currents increased markedly over this developmental period. Supported by NSF BSN-8910254.

M-Pos186

MUSCARINIC SUPPRESSION OF M-CURRENT IN RAT SCG NEURONS DEPENDS ON THE INTRACELLULAR FREE CALCIUM CONCENTRATION. Alistair Mathie, Laurent Bernheim, David J. Beech & Bertil Hille, Department of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

The M-current of rat superior cervical ganglion (SCG) neurons is suppressed by activation of muscarinic receptors. The dose-response curve for the specific muscarinic agonist oxotremorine-M (oxo-M), recorded using whole-cell voltage-clamp, has a K_D of 0.42 μM with a Hill coefficient of 0.93 and suggests that oxo-M maximally inhibits the current at concentrations above 10 μM.

The ability of oxo-M to suppress M-current depends on the intracellular Ca concentration. The intracellular free Ca concentration with the different intracellular solutions used in these experiments was estimated from values measured with fura-2 in separate experiments. After 12 min dialysis with 0.1 mM BAPTA in the pipette (free Ca around 70 nM) 10 μM oxo-M suppressed M-current by 82±3% (mean±s.e.m., n=10); however, 12 min dialysis with 20 mM BAPTA (free Ca around 12 nM) reduced the suppression to 15±8% (n=9). Adding 10 mM Ca to 20 mM BAPTA to give a free intracellular Ca of around 150 nM restored the mean suppression by oxo-M to 81±7% (n=6) and suppression was 76±7% (n=4) with 0.1 mM BAPTA and 20 mM dinitroBAPTA, a non-chelating derivative, in the pipette. The amplitude of the M-current at -30 mV was relatively unaltered by these changes being, respectively, 6.8±0.9 pA/pF (n=12), 8.7±1.4 pA/pF (n=9), 6.9±1.4 pA/pF (n=6) and 7.2±1.7 pA/pF (n=4).

Recording through nystatin-perforated patches and therefore, in the absence of artificial control of intracellular Ca, gave M-currents of 6.3±1.4 pA/pF at -30 mV and a mean suppression by 10μM oxo-M of 86±6% (n=5) suggesting that M-current suppression is large under normal conditions.

Supported by NIH grant NS08174, Fogarty International Research Fellowship FO5 TWO4457 (AM) and the McKnight Foundation.

M-Pos187

EFFECTS OF GTP ON THE SINGLE-CHANNEL KINETICS OF THE CARDIAC MUSCARINIC K+-CHANNEL. Chaya Nanavati and Yoshihisa Kurachi. Departments of Physiology and Biophysics, and Cardiology, Mayo Foundation, Rochester, MN 55905.

The cardiac muscarinic receptor is coupled to its effector, the inwardly-rectifying muscarinic K+-channel, $i_{K.ACh}$, via guanine nucleotide binding proteins (G-proteins). With a constant concentration of acetylcholine in the patch pipette, channel activation by application of intracellular GTP occurs in a concentration dependent manner (Kurachi etal. 1990. Pflugers Arch. 416:216-218). To elucidate the mechanisms underlying the dose-dependent increase in activation, we studied the effects of different concentrations of GTP on $i_{K.ACh}$ kinetics using single-channel and noise analysis techniques. All experiments were performed on inside-out patches from neonatal rat atrial cells. The $i_{K.ACh}$ channel was characterized by a single open state (τ_o=1 ms) and three closed states (τ_{c1}=1 ms, τ_{c2}=12 ms, and τ_{c3}=160 ms). The simplest kinetic model for this channel has four states:

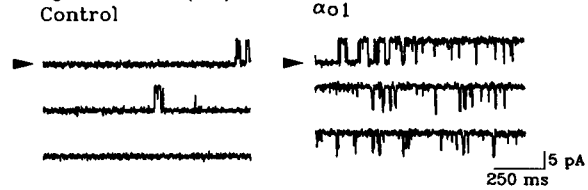
$$C_3 <----> C_2 <----> C_1 <----> O$$

We found that channel open time, which is related to the rate constant for transitions from O to C_1, was independent of GTP concentration. To overcome the ambiguities arising from multiple-channel containing patches, we also used noise analysis. The power spectrum was described by the sum of two Lorentzians with corner frequencies at 90 and 470 Hz. These frequencies represented transitions between the C_2—C_1, and C_1--O states. The corner frequenies were independent of GTP concentration, but the power itself increased with increasing GTP concentration. These data suggested that transitions between states C_2, C_1, and O were unaffected by GTP. One interpretation is that GTP increases the number of functionally active channels by increasing the rate for transitions between C_3 and C_2 and / or decreasing the rate for transitions between C_2 and C_3 thus increasing the open probability of the channel. We are grateful to Dr. David Clapham for the support of these experiments in his laboratory

M-Pos188

K_{Ca} CHANNELS FROM UTERINE SMOOTH MUSCLE ARE REGULATED BY A $G\alpha_{o1}$ SUBUNIT. J. Ramos-Franco, L. Toro, J. Codina*, L. Birnbaumer* and E. Stefani. Depts. Molecular Physiology & Biophysics and Cell Biology*, Baylor College of Medicine. Houston, TX. 77030.

Our previous work has suggested that myometrium possesses β-adrenergic receptors coupled to a GTP-binding protein that may directly gate K_{Ca} channels. In order to identify the G protein involved in this functional complex, five different G protein α-subunits were tested. K_{Ca} channels from rat myometrium were reconstituted into planar lipid bilayers. $G\alpha_s$, $G\alpha_{i-2}$, $G\alpha_{i-3}$ subunits were purified from human red blood cells, while $G\alpha_{o1}$ and $G\alpha_{o2}$ subunits were from cow brain. Only one of these α subunits (α_{o1}) mimicked the stimulatory effects of GTPγS on K_{Ca} channels; further, the α_{o1} effects were more pronounced. The α_{o1} action was characterized by: **1)** an increase of the Po (0.08±0.04 to 0.68±0.08, n=6) without a modification of the channel conductance; **2)** a change in the voltage dependence of the channel, the voltage activation curve was shifted toward negative values: from $V_{1/2}$=52±1 mV to -22±2 mV (±SD.) without altering the slope; **3)** an increase in the affinity of the channel for Ca^{2+} by a factor of 4 ($K_{1/2}$=38.03 to 9.36 μM); and **4)** a predominant effect on the closed state of the channel. Dwell time analysis revealed that channel activity was described by 3 and 4 exponential distributions for the open and closed states, respectively. The primary effect of α_{o1} was to decrease the two longest closed times (τ_{c3} 116±49 to 12±4 ms, and τ_{c4} 497±197 to 98±59 ms, n=6,). In addition, the amplitude factor for the shortest closed states rose 2.6 times and for the longest one diminished 2.7 times. These data support the hypothesis that **a)** G proteins activate this channel by shifting their voltage-dependence by altering its Ca^{2+}-sensitivity, and **b)** the nature of the excitatory GTP-binding protein coupled to K_{Ca} channels from uterine smooth muscle is closely related to G_o proteins. In the figure, arrows mark the closed state. Supported by NIH, grant HD-25616 (E.S.).

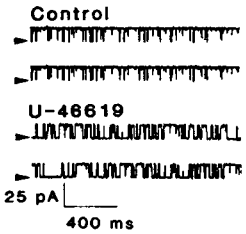


M-Pos189

THROMBOXANE A2 AGONIST INHIBITS K(Ca) CHANNELS FROM THE CORONARY SMOOTH MUSCLE. Scornik, F. S., Stefani, E and Toro, L. Dept. Molecular Physiology & Biophysics. Baylor College of Medicine. Houston, TX 77030.

Thromboxane A2 (TxA2) is a metabolite of the arachidonic acid cascade, primarily released by platelets. This agent is known to be a potent vasoconstrictor. Since K channels are involved in the contraction of vascular smooth muscle, it is possible that the action of TxA2 was exerted via the regulation of these channels. To test this possibility, the effect of U46619, a TxA2 analog, was studied on calcium activated potassium channels, K(Ca), from pig coronary smooth muscle reconstituted in lipid bilayers. Addition of U46619 to the external side of K(Ca) channels, produced a diminution of their open probability (Po) (see figure). Although the effect was dose dependent, the diminution of the Po could be only quantified within concentrations ranging from 50 to 150 nM (15% to 25% reduction of the initial Po, n=6). At doses larger than 150 nM channels were completely inhibited (n=3), but activity could be partially restored by internal GTPγS and Mg^{2+} (one experiment). On the other hand, when the decrease of the Po was 25% the control value addition of 25 μM of Ca^{2+}, to the internal side of the channel, reverted the inhibition by U46619 ($Po_{(control)}$=0.94±0.02; $Po_{(after\ U46619)}$=0.67±0.1; $Po_{(after\ Ca2+)}$=0.92±0.1; n=3). U46619 (100 nM) caused a shift of the voltage activation curve, fitted to a Boltzmann distribution, to more positive potentials ($V_{½}$ changed from -87±6 mV, to -42±5 mV; n=2). No significant changes on the slope factor were observed ($k_{(control)}$= 17±7 mV; $k_{(after\ U46619)}$= 15±4 mV; n=2).

The effect of U46619 seemed to be specific, since the addition of the inactive form of TxA2, Thromboxane B2 (up to 1 μM) was ineffective in lowering the Po of the channel (n=2). Moreover, U46619 itself (100 nM) had no effect when it was added to the internal side of the channel. These results suggest that TxA2 could be a regulatory factor of K(Ca) channels in this preparation, being this regulation one of the mechanisms of TxA2 action as a vasoconstrictor. Arrows mark the closed state of the channel. Supported by AHA-National Center, Grant-in-aid 900963, to Toro, L..



M-Pos190

DIFFERENTIAL REGULATION OF THE EXPRESSION OF VOLTAGE AND CALCIUM ACTIVATED K^+ CHANNELS ON HUMAN SPLENIC B LYMPHOCYTES. *PARTISETI M., *CHOQUET D., #DIU A. et *KORN H. *Neurobiologie Cellulaire, INSERM U261 and #Immunogenetique Cellulaire, Institut Pasteur, 75724, PARIS cedex 15, FRANCE.

K^+ channels are known to play a key role in the control of different stages of lymphocytes mitogenesis. Using the patch-clamp technique, we found that both voltage (IKv, 15-20 pS) and Ca^{2+} activated (IKca, 8 and 29 pS) K^+ channels are present at the level of human B cells. Both types of currents are sensitive to TEA (IC50=10 mM), Verapamil (20 µM) and Charybdotoxin (5-10 nM) and insensitive to Apamin, while 4-AP preferentially blocks IKv. In order to gain information on the relative distribution of these two currents, we compared their amplitudes (measured in whole-cell at +40 mV) on resting and activated cells. For activation, we used PMA or antibodies to Igm (anti-µ). 1) 58% (n=60) resting B cells had only IKv (I≈20 pA), 22% (n=40) had only IKca (I≈36 pA) and 20% (n=40) exhibited both currents. 2) These percentages, as well as the mean current amplitudes were significantly altered after 48 hours of activation: all cells expressed an increased IKca (I≈161 pA with anti-µ, n=44 and I≈1074 pA with PMA, n=30), but only 17% with anti-µ and 50% with PMA displayed IKv (I≈451 pA, n=10 and I≈789 pA, n=15). Given that 1) single channels had comparable conductances on resting and activated cells and 2) similar population were compared since cells proliferate only after 72 hours in culture, our results probably reflect both a true decrease in the proportion of cells expressing IKv, and an increase in the number of KCa channels per cell. They also suggest that subpopulations of human B cells can be distinguished on the basis of wether they carry voltage dependent K^+ channels, or not.

M-Pos191

MODULATION OF Ca^{2+} ACTIVATED K CHANNELS FROM CANINE COLON BY PHOSPHATASE INHIBITORS **A. Carl, J.L. Kenyon, K.M. Sanders. Dept. of Physiology, University Nevada School of Medicine, Reno, NV 89557, USA**

Large conductance 265 pS Ca^{2+}-activated K channels are expressed in myocytes from canine colon. The open probability, p(open), of these channels at resting conditions (-80 mV, 10^{-7}M Ca^{2+}) is very low (p(open) = 10^{-5}-10^{-6}). An increase of intracellular Ca^{2+} and membrane depolarization increases p(open) of these channels and it is likely that they play an important role in membrane repolarization. Therefore, modulation of the activity of these channels may regulate the duration of slow waves and thereby colonic motility.

Many studies have shown that phosphorylation can regulate ion channel activity. We tested the effect of 10 U/ml catalytic subunit protein kinase A (PK-A) on Ca^{2+}-activated K channels in excised membrane patches from freshly dispersed smooth muscle cells of the canine proximal colon. At +50 mV with 10^{-7}M Ca^{2+} and -50 mV with 10^{-6}M Ca^{2+} p(open) of the channels was increased to 230±50 % of control (n=9). This increase was due to a shift in voltage dependent activation by 13.9±5.5 mV (n=3) to more negative potentials. PK-A in the absence of ATP had no effect on channel activity (n=3). These data suggest that Ca^{2+}-dependent K channels are activated by phosphorylation.

Regulation by phosphorylation must be accompanied by dephosphorylation. We tested the effect of two potent inhibitors of protein phosphatases, Calyculin A and Okadaic Acid. Application of 10^{-9} to 10^{-6}M of each inhibitor in the presence of PK-A further increased open probability by up to 150 %. Calyculin A appeared to be less effective in increasing p(open) than Okadaic Acid. Calyculin in the absence of PK-A was ineffective. These data suggest that endogenous phosphatases are found in excised membrane patches and that a balance between phosphorylation and dephosphorylation may provide an important control of colonic motility. (Supported by NIH-DK41315)

M-Pos192

ALTERATIONS IN SODIUM CURRENTS IN CULTURED CAT CARDIOCYTES ARE UNRELATED TO CHANGES IN CELL MORPHOLOGY TE Schackow, JJ La Pres, RS Decker, RE Ten Eick (Intro. by R Novak). Northwestern University, Chicago, IL 60611

We have previously shown that adult cat cardiac ventricular myocytes (VM) obtained from collagenase-perfused hearts and maintained in primary culture on a 2-dimensional surface (laminin-coated glass coverslips) for 10-14 days (2DVM) exhibit sodium currents (I_{Na}) that are strikingly different from those obtained in freshly isolated ventricular myocytes (FVM) (Schackow et al., *Biophys J* 55:295a, 1989). The morphology of FVM is similar to that when *in situ*, being rod-like, whereas 2DVM develop attachment plaques and spread. The question of whether the altered I_{Na} was the result of either culture *per se* or the change in morphology was addressed by examining myocytes maintained in three-dimensional culture (3DVM) provided by an alginate matrix for a comparable period of time. I_{Na} was recorded from cells using a conventional whole-cell-patch technique. The current-voltage relationship in 3DVM was similar to that for 2DVM in that the voltages both for threshold and peak inward current were shifted negative to that for FVM. As a direct result of this, the half-point ($m_{1/2}$) of the conductance-voltage relationship (activation or m_∞ curve) in both 2DVM and 3DVM was shifted approximately 15 mV negative to that for FVM. The peak whole-cell Na conductance normalized to cell capacitance (G_{Na}) was almost doubled in 3DVM relative to FVM, whereas no significant difference was observed between 2DVM and FVM; however, cell capacitance in 3DVM was, on the average, almost 50% smaller than for FVM, while 2DVM and FVM exhibited similar cell capacitances. The steady-state inactivation curve (h_∞), determined by a test pulse to -10 mV preceded by a 1 s conditioning pulse, was also shifted negative in cultured VM relative to FVM, although the magnitude of the shift was greater than for the m_∞ curve. $h_{1/2}$ in both 2DVM and 3DVM was shifted approximately 25 mV negative to that for FVM. The voltage dependence of the fast and slow time constants of inactivation (τ_f and τ_s) in 2DVM and 3DVM was shifted 15-25 mV negative relative to FVM indicating faster I_{Na} inactivation. From these data we conclude that (a) differences in morphology between 2DVM and 3DVM appear to be unrelated to the development of changes in Na currents over time in culture; (b) a nonspecific change in surface charge in cultured VM appears to be an unlikely explanation for the negative shifts in the m_∞ and h_∞ curves since the magnitudes of the shifts are different; and (c) the increased G_{Na} observed uniquely in 3DVM may be the result of diminished cell capacitance rather than increased total Na conductance, implying that membrane area is lost but Na channels are not. TE Schackow is a Howard Hughes Medical Institute Predoctoral Fellow.

M-Pos193

EFFECTS OF CLASS I ANTIARRHYTHMICS ON GUINEA PIG BRAIN AND HEART NA CURRENTS EXPRESSED IN XENOPUS OOCYTES. W.A. Volberg, D.S. Krafte, and A.M. Ezrin. Department of Cardiovascular Pharmacology, Sterling Research Group, Rensselaer, N.Y. 12144.

We have utilized the Xenopus oocyte expression system to study the interaction of Class I antiarrhythmics with brain and heart Na channels. RNA was extracted from the brains of adult guinea pigs using a LiCl/urea procedure and 300 ng of total RNA was injected into individual oocytes. Brain RNA injected ocytes were incubated at 21°C for 2-5 days prior to voltage-clamp recording. Heart RNA injected oocytes were incubated on the first and third days at 30°C with the remainder of time at 21°C. Holding potential was -100 mV in all experiments and 30 ms step depolarizations were given in 10 mV increments from -90 mV to +40 mV. Following test pulses to potentials more positive than -40 mV brain Na currents were observed that were almost completely blocked by 50 nM tetrodotoxin. Peak currents were elicited during voltage steps to -10 mV with the average current being -1270 nA ± 59.6 (mean ± SEM) (n=44). Encainide, quinidine, and lidocaine blocked brain Na current with IC_{50} values of 91 μM, 167 μM, and 945 μM, respectively. During a 10 Hz train of 30 ms depolarizations to -10 mV, lidocaine was the only drug that showed use-dependent block (-40% ± 0.01%), which occurred over the entire concentration range tested (0.3 - 3 mM). At the guinea pig cardiac Na channel, lidocaine blocked currents with an IC_{50} value of 444 μM assessed with the same pulse protocol as above. These data demonstrate a cardiac selectivity to Na channel block.

M-Pos194

HUMAN HEART NA CHANNELS EXPRESSD IN XENOPUS OOCYTES. D.S. Krafte, K.M. Dillon and A.M. Ezrin. Department of Cardiovascular Pharmacology, Sterling Research Group, Rensselaer, NY 12144.

Total RNA was isolated from human right ventricular tissue using a LiCl/Urea extraction procedure. Xenopus laevis oocytes were harvested and injected with 250 ng of total RNA and incubated for 2-4 days. The first and third days of incubations were at 30°C while days 2 and 4 were at 21°C. This protocol increased the levels of expression as assessed by maximal current amplitude and made it possible to quantitatively study the human heart channel. Current amplitudes peaked at -10 mV with an average value of -289 ± 21 nA (n=17). These currents were blocked by tetrodotoxin with an IC_{50} value of 1.3 μM. Steady-state inactivation was assessed using a twin pulse protocol and half maximal inactivation was found to occur at a voltage of -63 ± 1.0 mV (n=10) with a slope factor of 9.3 ± 0.6. Lidocaine at 10 μM produced no tonic block of the Na current (assessed with 30 ms depolarizing test pulses), but gave 18% reductions in peak amplitudes following a 10 Hz train of depolarization. Lidocaine at 100 μM produced 26% tonic block with 75% additional block during a 10 Hz pulse train. The expression of human heart Na channels in the oocyte system should make it possible to study ion channels from different regions of the heart as well as from normal and diseased myocardium.

M-Pos195

BATRACHOTOXIN REDUCES AFFINITY OF LOCAL ANESTHETIC AGENTS FOR CARDIAC Na^+ CHANNELS. K Liberty, J Kelly, P Santucci, MK Myers and JA Wasserstrom, Reingold ECG Center, Department of Medicine (Cardiology), Northwestern University Medical School, Chicago, IL, 60611.

Batrachotoxin (BTX) is a Na^+ channel modifier that is thought to bind to a receptor on nerve Na^+ channel that is distinct from the local anesthetic (LA) drug receptor; however, BTX binding reduces LA binding and blocking potency. The purpose of the present study was to determine if the interaction between these agents is the result of competitive or non-competitive binding at the receptor or of changes in LA binding to Na^+ channels because of BTX-induced alterations of inactivation and/or activation. We examined the effects of BTX on the blocking potency of several LA in cardiac I_{Na} using whole cell voltage clamp techniques. I_{Na} was recorded in enzymatically dissociated guinea pig ventricular myocytes using internal and external $[Na^+]$=5 mM (17°C). In the presence of BTX (100 nM), the inward current was comprised of two components; the first inactivated rapidly and represents unmodified channels whereas the second remained net inward throughout the depolarizing pulse (100 msec) and represents BTX-modified channels. Procaine and lidocaine blocked the first component with K_d = 1.2 mM (n=8) and 0.9 mM (n=5), respectively. In contrast, these agents were far less potent in blocking the steady-state component, with K_d = 15.2 mM and 20.9 mM, respectively. Use-dependent block was present in unmodified channels at 2Hz (10 and 100 msec pulse durations) with each agent but was absent in modified channels. These results demonstrate that 1) BTX modifies cardiac I_{Na} causing a substantial steady-state (non-inactivating) component of I_{Na}; 2) the affinity for LA block of BTX-modified cardiac Na^+ channels is markedly reduced compared to unmodified channels; and 3) BTX-modified channels show no use-dependent block by LA. Our data cannot exclude the possibility that the reduced affinity of LA for BTX-modified Na^+ channels results from a drug binding interaction between BTX and LA. However, the absence of use-dependent block strongly suggests that the reduced potency of LA block results from BTX modification of channel kinetics, namely, a loss of state-dependent binding secondary to alterations in inactivation and/or activation caused by BTX. (Supported by PHS HL-30724).

M-Pos196

PHYSIOLOGICAL CONCENTRATIONS OF TRIIODO-L-THYRONINE INCREASE BURSTING OF CARDIAC Na^+ CHANNELS

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Burst mode gating of single cardiac Na^+ channels, which contributes to a prolongation of the action potential, has been described previously. However, bursts occur in <1% of openings in rabbit ventricle [Grant and Starmer, *Circ. Res.* 1987;60:897-913]. In patch clamp experiments using acutely isolated rabbit ventricular myocytes, physiological concentrations of 3,5,3'-triiodo-L-thyronine (T_3) increased the ratio of long events (LE) to the total number of openings. Long events were defined as openings or bursts with closings ≤250 μsec having a duration ≥5-times the control mean open time (MOT) in the cell-attached configuration. Cells or inside-out patches were perfused at 10°C with (mM) 140 K-aspartate, 10 EGTA, 5 HEPES (pH 7.4), ± T_3. Channels were stimulated at 1 Hz by depolarizing steps from a negative holding potential (typically -130 mV) and recorded at 10 kHz after low pass filtration at 2 KHz. A standard pipette solution of (mM) 280 NaCl, 2 $CaCl_2$, 10 HEPES (pH 7.4), ± T_3 was used in all experiments. In the cell-attached configuration, addition of 5 nM T_3 to the pipette resulted an increase in %LE at all potentials measured (p=.044). The increase had a biphasic voltage-dependence and peaked at -40 mV. At -40 mV, %LE increased >3-fold from 1.0% in controls to 3.2% in T_3. A similar increase in %LE occurred with 50 nM T_3 (p=.029) suggesting saturation at ≤5 nM. There was no evidence of a change in unitary conductance. To investigate the possible involvement of a second messenger, on-cell recordings were made with T_3 added to the bath. Addition of either 50 nM (n=3) or 100 nM T_3 (n=3) to the bath failed to alter the MOT, unitary current, or %LE. Gating also was unaffected by addition of 50 nM T_3 to the cytoplasmic face of inside-out patches (n=4). After showing an increase in %LE in on-cell patches with 5 nM T_3 in the pipette, patch excision to the inside-out configuration caused a further 3.3 ± 0.7-fold increase in %LE (n=6); in one case, 19.9% of openings were long events. Accompanying the increase in bursts was a small decrease in the unitary current and an increase in MOT estimated by a single exponential fit to the open time histogram. These results suggest that T_3 is not membrane permeable during the time scale of the experiments (~20 mins) and that T_3 action requires close proximity to the Na^+ channel. Patch excision facilitates bursting by an unknown mechanism. T_3-induced bursting may be caused by direct binding to the channel, receptor-mediation, or a generalized sarcolemmal outer leaflet disruption. A shift toward prolonged openings may contribute to the propensity for arrhythmias in hyperthyroidism.

M-Pos197

The Different Response of Na^+ Channel to Blocker in Human and Guinea-pig Atrial Myocytes.
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1st Dept. of Medicine and 2nd Dept. of Surgery Tottori University, 1st Dept. of Medicine, Kinki University, Japan.

The drug concentrations to block I_{Na} estimated by using animal model are not always identical with the concentration of agent in clinical use. We initially tried to characterize the Na^+ channel properties of human and guinea-pig atrial myocytes and to elucidate whether the antiarryhthmic agent could induce the same blocking reaction on I_{Na} in both human and guinea-pig atrial myocytes, under conditions of whole cell patch-clamp. Human atrial myocytes (n=6) and guinea-pig atrial myocytes(n=10) were isolated by an enzymatic dissociation. Characterization of I_{Na}: From I-V relationship of Na current, the threshold potential, peak potential, and equilibrium potential were -60± 2.3, -35±3.4, and 2.8±2.2mV in human , and -61± 2.2, -38±3.7, and 3.2±2.0 mV in guinea-pig. From inactivation curve of I_{Na}, V_h and slope factor were -84±4.5mV and 5.0±1.3 in human, and -80± 5.6mV and 5.0±1.2 in guinea-pig. The decay constant of I_{Na} at peak potential was 4.8±1.2ms in human, and 4.7±1.2ms in guinea-pig . Tonic block of I_{Na} by disopyramide: To estimate the equilibrium constant for each channel state, we used the equation described by Bean et al., $1/Kd_{app}=h/Kd_{rest}+(1-h)/Kd_i$, Kd_{app}, Kd_{rest}, and Kd_i were 1.2, 120, and 0.06uM in human (n=3), and 11, 50, and 0.7uM in guinea-pig (n=4). These results suggest that Na channel characteristics in human atrial myocytes have been similar to those in guinea-pig one, but the affinity of receptors to drug molecule might be different between human and guinea-pig atrial myocytes.

M-Pos198

CHARACTERIZATION OF SODIUM CURRENT IN PRIMARY PACEMAKER CELLS ISOLATED FROM THE SINOATRIAL NODE

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It has been suggested that primary pacemaker cells ("P cells") in the center of the sinoatrial node do not exhibit a sodium current (i_{Na}) and that i_{Na} contributes to action potentials only in peripheral regions. Recently, our laboratory has shown that i_{Na} is indeed present in these cells (Berkowitz and Nathan, *Circ.* 82: Suppl., 1990). Now we have begun to characterize this current. The whole-cell configuration of the patch clamp technique was used with single pacemaker cells that were isolated from the rabbit sinoatrial node and cultured for 1-4 days. Most of these cells exhibited *both* i_{Na} and i_h (or i_f, the hyperpolarization-activated current). The bathing solution was chosen to block all other time-dependent currents. It contained (mM): 126 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgCl_2$, 0.3 NaH_2PO_4, 5.5 dextrose, 5.0 HEPES, 20 TEA-Cl, 4.0 4-AP, 4.0 CsCl and 2.0 $NiCl_2$; pH = 7.4 with HCl. The pipette contained (mM): 130 KCl, 5 MgATP, 5 Na_2CrPO_4, 5 EGTA and 5 HEPES; pH = 7.2 with KOH. Under these conditions, 30 μM TTX blocked *all* time-dependent current; therefore, i_{Na} was routinely measured with respect to the steady state current at the end of 50-msec voltage steps (holding potential was -90 mV). In 8 cells, the threshold for i_{Na} was -61 ± 2 mV (mean ± SEM); the peak of the I-V occurred at -19 ± 3 mV; the reversal potential was +48 ± 2 mV; and the maximum amplitude at 26 °C was -2.1 ± 0.8 nA (-89 ± 32 $\mu A/cm^2$). Double pulse experiments were used to determine the inactivation-voltage relationship. The prepulse, 200 msec in duration, was followed by a 2-msec gap at the holding potential (-100 mV) and then a 50-msec test pulse to 0 mV. For 4 cells, h-infinity was 1.0 at -120 ± 10 mV, 0.5 at -75 ± 5 mV and 0 at -38 ± 3 mV. Fits of a Boltzmann equation exhibited a slope factor of 9.2 ± 0.7 mV. The overlap of activation and inactivation ("window current") at potentials between -61 and -38 mV suggests that i_{Na} could contribute to the diastolic depolarization in primary pacemaker cells of the sinoatrial node.
Supported by Texas Advanced Research Program Grant 010674-018.

M-Pos199

CHARACTERIZATION OF EXTRACELLULAR ATP-ACTIVATED CATION CURRENT IN RAT VENTRICULAR MYOCYTES.
A. Christie and A. Scarpa. Dept. of Physiology and Biophysics, CWRU, Cleveland, OH 44106.
(Introduced by B. Lindley)

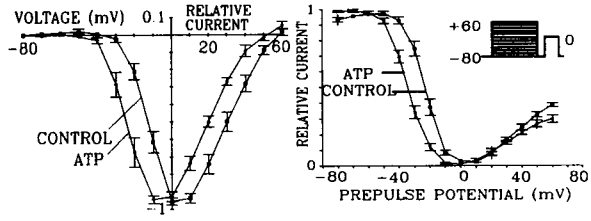
Extracellular ATP-activated currents have been described in a variety of cells including neurons and smooth muscle cells. However, these currents have not been extensively investigated in mammalian heart cells. Using single electrode voltage-clamp techniques, we have characterized ATP-activated whole cell currents in rat ventricular myocytes.

The relative permeability of ATP-activated channels to monovalent and divalent ions was determined from reversal potentials. Replacement of chloride with either nitrate or acetate did not alter the reversal potential indicating that anions do not significantly contribute to the current. Magnesium was not found to contribute to the current but was required for activation of the current. Sodium and Ca^{2+} were found to be permeant ions with Na^+ being more permeable than Ca^{2+}. The current activated by ATP was accompanied by a clear increase in noise. Analysis of the noise indicated that the single-channel current was ~0.1 pA. The current-voltage relationship was linear over the voltage range -100 mV to +70 mV. The currents decayed slowly in the continued presence of ATP and showed prolonged desensitization to subsequent applications of ATP removal of the agonist.
Supported by NIH HL07653 and HL18708.

M-Pos200

EXTRACELLULAR ATP MODULATES CA^{2+} CURRENT IN RAT VENTRICULAR MYOCYTES. A. Christie and A. Scarpa. Dept. Physiology and Biophysics, CWRU, Cleveland, OH 44106. Intro.by A. Kirby

Extracellular ATP has been shown to increase the L-type Ca^{2+} current of single rat heart cells by a cyclic-AMP independent mechanism (Scamps and Vassort, Biophys. J. 57:303a, 1990). Here, we report that extracellular ATP not only increases the Ca^{2+} current but also modulates Ca^{2+} current activation and inactivation. ATP (1-100 1M) increased the L-type Ca^{2+} current in rat ventricular myocytes elicited by depolarization to 0 mV from various holding potentials (-80 to -50 mV). Steady state activation and inactivation curves were shifted to the left ~10 mV at all holding potentials. Inactivation of the Ca^{2+} current, elicited by depolarization to 0 mV from -80 mV, could best be fit by two exponentials (τ_{fast} = 86.8 ± 8.8 ms, τ_{slow} = 13.2 ± 3.5 ms; n = 4). The slow time constant for inactivation was significantly ($p < 0.01$) decresed by ATP (τ_{slow} = 66.6 ± 5.7 ms) while the fast time constant was not effected. These effects were not the result of a Ca^{2+} dependent mechanism since, qualitatively similar results could be obtained using Ba^{2+} as the charge carrier in place of Ca^{2+}. Supported by NIH HL07653 and HL18187.



M-Pos201

PROPAFENONE BLOCKS CALCIUM CURRENT IN GUINEA-PIG VENTRICULAR CELLS.

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The effect of Propafenone (P) on the Ca current (ICa) was studied in isolated cardiac myocytes using the whole-cell configuration of the patch-clamp technique. When depolarizing pulses (150 ms steps from a holding potential of -40 mV to + 10 mV at 0.03 Hz) were applied, P (10^{-6} - 5 x10^{-5} M) produced a dose-dependent inhibition of the ICa which reached steady-state level within 5 min. Half inhibition dose (IC_{50}) for the peak ICa was 4 x 10^{-6} M. P not only reduced the peak magnitude of the ICa but also hastened its decay. The process was well described by a double exponential fit. The fast time constant was similar in the presence and absence of 5 x 10^{-6} M P (5.5 ± 0.1 vs 5.1 ± 0.3 ms respectively), but the slow time constant was significantly shortened by P (41.3 ± 1.6 vs 26.9 ± 1.9 ms, respectively. $P < 0.01$). After repetive stimulation P produced a use-dependent blockade which increased with the frequency of stimulation. In addition, P caused a 7.3 mV hiperpolarizing shift in the steady-state inactivation curve. Under control conditions the time course for recovery from apparent inactivation of ICa can be fitted by a single exponential (Time constant = 502 ms) while in the presence of 5 x 10^{-6} M the recovery process was slowed being better described by a double exponential (Time constants = 1.1 s and 8 s). In conclusion, P produced a dose- and use-dependent decrease of ICa that can explain some of its antiarrhythmic properties.

M-Pos202

ISOPROTERENOL MEDIATED EFFECTS ON ACTIVATION AND INACTIVATION OF WHOLE-CELL L-TYPE CA^{++} CURRENT IN SINGLE CANINE VENTRICULAR MYOCYTES.

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The ß-adrenergic receptor-mediated stimulation of L-type Ca^{++} channel current (I_{CaL}) has been well described, yet its effects on the activation and inactivation relationships (A, I) remain controversial. We studied the effect of Isoproterenol (Iso) on A and I, and on the "window" current (I_{CaW}) defined by their overlap, in enzymatically isolated single canine ventricular myocytes. Iso (2-5 µM) increased the peak I_{CaL} by 58.7±18.9% (mean±SE; n=14 trials), and shifted the peak of the I-V curve negatively by 9.2±2.6 mV. A and I were obtained by fitting the experimental data with the Boltzmann equation:

$$y = \max/1+\exp[(V-V_{1/2})/k] \ ,$$

where y is the Boltzmann distribution, max is the maximum conductance, V is the membrane potential, $V_{1/2}$ is the voltage at half-maximum conductance, and k is the slope constant. These data showed a parallel shift of the A only. The $V_{1/2}$ changed from a control value of -8.5±1.0 to -20.2±2.1 mV in the presence of Iso (n=17 trial; $p<0.05$). There was no significant shift of I; the $V_{1/2}$ changed from a control value of -19.1±1.6 to -22.5±2.7 mV following the addition of Iso ($p>0.05$). Neither k_A nor k_I were changed significantly by Iso. We have previously reported the direct measurement of I_{CaW}, in single canine Purkinje myocytes, in the voltage range of overlap between A and I (Biophys. J. 1989; 55:287a). We report here that we observed I_{CaW} in single canine ventricular myocytes as well (n=17 cells). Compared to control, Iso increased I_{CaW} peak amplitude by 112.4±22.4% (n=8), and shifted the peak of the I_{CaW}-V curve negatively by 10.6±1.1 mV. These results further suggest a possible mechanism for the participation of I_{CaL}, and in particular I_{CaW}, in the modulation of the action potential and regulation of cellular Ca^{++} homeostasis.

M-Pos203

ANGIOTENSIN II RECEPTOR-MEDIATED EFFECTS ON L-TYPE CA^{++} CURRENT IN CANINE VENTRICULAR MYOCYTES: A STUDY USING DuP 753, A NEW, RECEPTOR SPECIFIC, NON-PEPTIDE ANGIOTENSIN II ANTAGONIST.

A. Moscucci, M. Moscucci, M.B. Murphy, and C.T. January (Intro. by M.F. Sheets), Cardiac Electrophysiology Laboratories, The University of Chicago, IL 60637

It has been postulated that the cardiac tissue renin-angiotensin system may play a role in the regulation of cardiac contractility, coronary blood flow, collagen remodeling, and in the development of reperfusion arrhythmias. The recent development of specific, non-peptide, Angiotensin II (AII) antagonists devoid of intrinsic activity may provide a clearer understanding of the receptor-mediated effects of AII in the heart. Accordingly, we report here studies on the effects of AII and of DuP 753, a new, specific AII receptor antagonist, on L- and T-type Ca^{++} currents (I_{Ca}) in enzymatically isolated single canine ventricular myocytes. I_{Ca} was measured during depolarizing steps, by whole-cell patch-clamp technique, with 5 mM Ca^{++} as the charge carrier. In 8 trials, following the addition of AII (100 nM) there was an increase of 23±6% in the peak L-type I_{Ca}, from a control value of 619±48 pA (mean±SE), and the current decay appeared to become faster. The effect of AII was similar at both -80 and -40 mV holding potentials. These effects were maximal by 5 minutes of superfusion of the cells with AII. Addition of DuP 753 (1 µM), in the continued presence of AII, completely reversed the increase in peak I_{Ca}, to 529±41 pA. The antagonist effect of DuP 753 appeared to be reversible upon replacement of AII only solution. DuP 753 (1 µM), in the absence of AII, had no effect on the L-type I_{Ca} (n=2). A small amplitude T-type I_{Ca} was present in these cells, and it appeared to be unaffected by these interventions. These data support the presence of functional AII receptors in canine ventricular myocytes and their role in the modulation of L-type Ca^{++} channel current.

M-Pos204

SLOW INACTIVATION OF L-TYPE CALCIUM CURRENT AND THE MEASUREMENT OF T AND L-TYPE CALCIUM CURRENT IN CANINE PURKINJE MYOCYTES.

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We employed the whole cell patch clamp technique on canine Purkinje myocytes to investigate slow inactivation of L-type calcium current and its relationship to the measurement of both L and T-type calcium current. Studies were conducted at room temperature (26.5-27.5°C) in a solution containing in mM TEACl 140, $CaCl_2$ 10, dextrose 10, 4-AP 0.5 and buffered to pH 7.4 with HEPES 10. Patch pipettes contained in mM TEA 140, Cl 135, dextrose 10, K_4-BAPTA 5, HEPES 10, Mg-ATP 2 and CP 3 (pH 7.4).

We find that slow inactivation of L-type calcium current is present in isolated myocytes as previously reported for Purkinje strands (Kass & Scheuer, 1982, J. Moll. & Cell Card. 14, pp615). The magnitude and kinetics of slow inactivation are voltage dependent becoming larger and faster with increasing depolarization.

One means to measure T-type calcium current is to construct an I-V relationship for the calcium current from a holding potential of -90mV and subtract a similar I-V relation constructed from a holding potential of -40mV. Our results suggest this method gives rise to spuriously large estimates of T-type calcium current because slow inactivation of L-type current reduces L-type calcium current elicited from -40mV but not from -90mV.

Similar difficulties also exist in estimating the L-type calcium current inactivation versus voltage curve. Depending on the protocol employed slow inactivation of L-type calcium current can alter the measured voltage dependence of L-type calcium current inactivation.

Our results suggest that detailed investigation of either L or T-type calcium current must include an analysis of both the voltage dependence and kinetics of slow inactivation of L-type calcium current in order to avoid distortion of other measured calcium current parameters.

Supported by grants HL20558, HL28958 and HL43731.

M-Pos205

IS THE Na^+/K^+ PUMP IN CARDIAC CELLS DIRECTLY REGULATED BY THE ADRENERGIC SYSTEM ?

Frieda V. Bielen, H.G. Glitsch*, F. Verdonck. Intro. by E. Carmeliet (University of Leuven Campus Kortrijk, Belgium; *Ruhr University, Bochum, FRG)

Controversial reports have been published in the literature concerning the effects of adrenergic substances on the Na^+/K^+ pump activity in the heart. To study the effect of alfa- and beta-adrenergic stimulation on the Na^+/K^+ pump current (I_p) in conditions of well-controlled intra-and extracellular ion concentrations we analyzed the effects of isoproterenol (ISO) and phenylephrine (PHE) in single rabbit cardiac Purkinje cells and ventricular myocytes. Cells were superfused with a K^+-free solution. I_p was measured as the current activated by extracellular K^+. Ba^{2+} (2 mM) was added to block K^+ conductances. Solutions could be quickly changed via a multibarrelled pipette. Internal solution contained (in mM): Cs^+ 100; Na^+ 15; Mg^{2+} 5; Aspartate 80; Cl^- 10; ATP 5; creatine phosphate 5; EGTA 10; Hepes 40 (pH 7.2). Currents which are known to be affected by adrenergic agents were measured together with I_p in the same cell to confirm the effectiveness of the drugs. At ISO concentrations (10^{-6} M) which induced stable Cl^+-sensitive currents no effect on I_p could be obtained neither at low nor at high K^+_o. PHE (10^{-6}-10^{-5} M) induced a Ba^{2+}-sensitive shift of the holding current, indicating an effect on I_{K1}. No effect on I_p could be observed. These results indicate that the adrenergic system does not directly regulate the activity of the Na^+/K^+ pump. Earlier reported effects may be caused indirectly by changes of intra- or extracellular activator cation concentrations.

M-Pos206

TWO FUNCTIONALLY DISTINCT Na/K PUMPS IN GUINEA PIG VENTRICULAR MYOCYTES.

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At the last meeting we reported the existence of two distinct DHO-blockable Na/K pumps in guinea pig ventricular myocytes (*Biophys. J.* 57:134a, 1990), having k_d's of roughly 1 and 100 μM. This was also reported by others (Mogul, DJ *et.al., Circ. Res.* 64:1063-1069, 1989). We used this 100-fold difference in binding affinities to investigate the individual properties of each of the two types of Na/K pumps. Results suggested the pump associated with the higher-affinity DHO site also had a higher affinity for potassium (half-activated at 0.4 mM $[K^+]_o$) than the lower-affinity DHO pump (half-activated at 4.3 mM $[K^+]_o$). No difference in intracellular Na^+ affinity was observed; both pump types being half-activated by 9 mM $[Na^+]_i$. Last year we showed that lowering external pH for a short time reduced the total Na/K pump current. We now find that this external pH dependence is entirely a property of the low-affinity DHO blockable pumps. The high-affinity pumps show no immediate (within 5 min.) pH sensitivity in the range of 6.25 to 8.0.

Finally, it is worth pointing out that our results suggest K^+ and glycosides do not compete for the same site on the Na/K pump. Instead, changing external $[K^+]$ alters the fraction of pump current contributed by each of the two pump populations. We believe that this functional diversity plays an important role in normal regulation of Na/K pump function. Supported by grants HL36075, HL20558, HL28958, and HL43731.

M-Pos207

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A CARDIAC CELL LINE.

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We are presently investigating the electrophysiological properties of a stable cell line (MCM1)[1] isolated from a mouse atrial tumor (Behringer et al.,1988, *PNAS* 85:2649-2652). Cells were grown in F10 C medium, containing 15% horse serum and used 2 to 20 days after plating. Such a cell line would provide a useful system for electrophysiological and biochemical studies of cardiac-specific ion channels.

The main currents observed with whole cell voltage clamp technique are: a fast inward sodium current, a delayed outward current with relatively fast onset, and, occasionally an inwardly rectifying potassium current which is increased by bath application of 100-200 μM of acetylcholine. Maximal I_{Na} ranged from 0.1 to 2 nA, while maximal I_K ranged from 0.2 to 0.5 nA.

In normal Tyrode solution, the sodium current begins to activate around -50 mV, peaks near -20 mV and reverses around +65 mV, as expected for a sodium-sensitive channel. In most cells, sodium current is completely insensitive to up to 50 μM TTX. Approximately 10% of the cells tested showed partial sensitivity to micromolar concentrations of TTX. Superfusion with nominally sodium-free solution containing 2 mM Ca^{2+}, completely abolished the inward current at all potentials tested. Other currents are presently under investigation. Therefore, the type of currents as well as the presence of myosin (Behringer et al., 1988) strongly suggest a cardiac origin for this cell line.

Acknowledgements: A.S. is a postdoctoral fellow of CHF. We wish to thank Dr. M. Morton for providing the cell cultures.

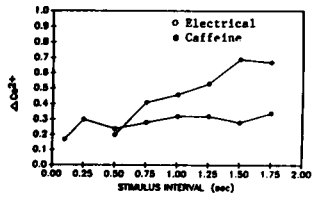
[1] MCM1 cell line obtained from C.L. Gartside and S.D. Hauschka, Department of Biochemistry, University of Washington, Seattle, WA.

M-Pos208

SARCOPLASMIC RETICULUM CALCIUM CYCLING IN FELINE VENTRICULOCYTES.

Beth B. Dinda and Steven R. Houser. Temple University School of Medicine, Department of Physiology, Philadelphia, PA.

It has been hypothesized that sarcoplasmic reticulum (SR) calcium (Ca) cycles within the SR from "uptake pools" which surround the myofibrils to "releaseable pools", i.e. the junctional SR. Feline ventricular myocytes were used to study the putative movement of Ca between these different pools. Indo-1 loaded myocytes were field stimulated at .5Hz until contractions and Ca transients reached a steady state. A premature beat was inserted at a selected time after a normally paced beat. Premature stimuli were introduced via either electrically induced action potentials (AP) or caffeine spritz at .1 - 1.75 sec. Ca release was quantified by the stimulus-induced ΔCa (measured in arbitrary units, see figure): (peak Ca) - (Ca level before release occurred). No AP-induced release occurred at intervals less than 500msec because of electrical refractoriness. Caffeine caused SR Ca release at all premature stimulus intervals, indicating that the Ca is available for release as soon as 100msec after a normally paced beat. Caffeine-induced Ca release remained relatively constant at all intervals, whereas AP-induced release increased as stimulus interval increased. These data indicate that 1) the SR is capable of releasing Ca at very short intervals, 2) the Ca released by caffeine may not be from the same Ca pool as that released by the normal AP.



M-Pos209

ALTERATIONS IN CARDIAC SR CALCIUM RELEASE CHANNEL DURING CHRONIC DOXORUBICIN TREATMENT, by Debra A. Dodd, Robert J. Boucek, Jr., and Sidney Fleischer, Vanderbilt Univ., Nashville, TN.

New Zealand white rabbits were infused intravenously with doxorubicin (1 mg/kg) twice weekly for 12-18 doses (Treated,n=11). Pair-fed controls received normal saline (Control,n=11). Electron microscopy confirmed the presence of dilated sarcoplasmic reticulum (SR) (T 1.24$\pm$0.18 vs. C 0.45$\pm$0.18, p<.001), scored as described by Billingham et al., *Cancer Treat Rep* 1978; 62:865-872. To evaluate possible functional correlates of these morphologic changes, subcellular fractions enriched in SR were isolated from heart ventricles. These SR fractions from T and C, demonstrated no difference in a) total protein yield, b) SR calcium pump protein (CPP) yield estimated by SDS-PAGE, c) calcium stimulated ATPase activity (T 177.2$\pm$11.4 vs. C 200.8$\pm$12.0 nmole/mg·min, p=.11) or d) oxalate-stimulated calcium loading activity (T 115.2$\pm$9.8 vs. C 139.0$\pm$14.6 nmole/mg·min, p=.20). Analysis of ^{3}H-ryanodine (Ry) binding in these same fractions at 10nM Ry (fig. 1) demonstrated a lower level of binding in the treated group (T 0.31$\pm$0.05 vs. C 0.69$\pm$0.04 pmole/mg, p<.0001). Scatchard analysis of binding isotherms (fig. 2) for pooled T and C samples suggests that this difference results from a lower B_{max} in the treated group (T 0.36 vs. C 0.86 pmole/mg), with little change in K_d (T 4.1 vs. C 2.8 nM). These findings suggest that the early morphologic and functional changes in heart seen with doxorubicin cardiomyopathy are mediated by effects of doxorubicin on the calcium release channel, rather than on the CPP.

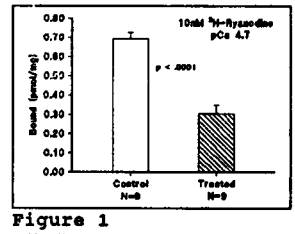


Figure 1

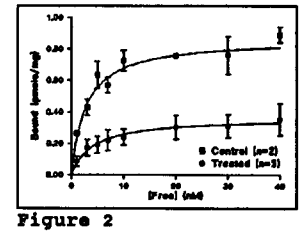


Figure 2

(NIH F32HL08006 to DAD, NIH RO1CA48930 to RJB, and NIH HL32711 to SF)

M-Pos210

RYANODINE DECREASES CALCIUM EFFLUX AND EFFECTS CALCIUM RELEASE FROM THE SARCOPLASMIC RETICULUM IN PORCINE CORONARY ARTERY SMOOTH MUSCLE.

C. Wagner-Mann, O. Hu and M. Sturek Department of Physiology and Dalton Research Center, University of Missouri, Columbia, MO. 65211

Ryanodine's (RY) value as an agent to manipulate Ca release from the sarcoplasmic reticulum (SR) is appreciated from experimental work conducted in skeletal and cardiac muscle. In contrast with RY's known activities and sites of action in striated muscle, its effects in smooth muscle are not as well-established. The hypotheses that at low concentrations ($\leq 10^{-8}$M) RY effects the release of Ca from the SR, while at high concentrations ($\geq 10^{-5}$M) release is blocked were tested using single smooth muscle cells freshly dispersed from porcine right coronary artery and fura-2 microfluorometry. Each cell was depolarized (5 min in 80mM K) to ensure filling of the SR Ca store and then cells were exposed for ten minutes to one of six concentrations of RY ranging from 10^{-9}M to 10^{-4}M. This was followed with a short caffeine (CAF, $5x10^{-3}$M) exposure (1 min) to indirectly evaluate the effects of RY on SR Ca storage/release through any alteration of the effects of CAF on intracellular free Ca (Ca_i). For [RY]$\leq 10^{-6}$M, there were no shifts in the baseline Ca_i nor decreases in amplitude, duration or shape of the CAF-induced Ca_i transient following the RY exposure. At [RY]$\geq 10^{-5}$M, however, the CAF-induced Ca_i peak was reduced by 41%. During the course of the RY exposure a 31% increase in baseline Ca_i was noted. Cells in a Ca-free solution also responded to RY with a 6% increase in Ca_i above baseline. Following CAF exposure Ca_i returned to a level slightly above that reached at the conclusion of the 10 min RY challenge, in contrast to controls and for [RY]$\leq 10^{-6}$M. RY-associated baseline Ca_i increases both in Ca-free and Ca containing media were diminished when, prior to RY challenge, the SR was more depleted by longer (2.5 min) CAF exposure. It was concluded that in porcine smooth muscle: (1) $\leq 10^{-6}$M RY does not measurably effect SR Ca release; and $\geq 10^{-5}$M RY (2) causes SR Ca release, and (3) inhibits Ca extrusion.

Supported by NIH #41033 to M.Sturek and Postdoctoral fellowship form AHA-Missouri Affiliate to C.Wagner-Mann.

M-Pos211

EFFECT OF CATIONS ON THE Ca^{2+}-CHANNEL ACTIVITY IN JUNCTIONAL SARCOPLASMIC RETICULUM VESICLES*.

F. Fernandez-Belda, F. Soler and Juan C. Gomez-Fernandez. Dept. Biochemistry & Molecular Biology, University of Murcia, Spain.

The existence "in vitro" of the Ca^{2+}-induced Ca^{2+} release phenomenon in sarcoplasmic reticulum allows to explore some functional characteristics related to the gating-blockade behavior of the Ca^{2+}-channel. By using a membrane preparation containing feet structures and the labeled Ca^{2+}/filtration technique it was found that the opening of the channel is highly dependent on the size and electric charge of the gating cation. The release of Ca^{2+} as a function of the external free Ca^{2+} concentration shows a bell-shaped dependence. Lower Ca^{2+} concentrations (μM) open the channel while mM concentrations induce a closing response. The apparent dissociation constant for activation is 5 $x10^{-7}$ M. Sr^{2+} is less efficient than Ca^{2+} to stimulate Ca^{2+} release (K_d 3.2 $x10^{-6}$ M) whereas Mg^{2+} is not effective at all. The blockade response appears to be more dependent on the electric charge since it can be obtained by mM concentrations of divalent cations (Ca^{2+}, Sr^{2+}, Mg^{2+}, Mn^{2+}) or μM concentrations of trivalent (La^{3+}, Pr^{3+}, Tb^{3+}) or hexavalent (ruthenium red) cations. The synergistic effect observed by combining high- and low-affinity blocking cations suggests that they interact through the same binding sites. Moreover, the high- and low-affinity cations are noncompetitive blockers of the activating Ca^{2+} suggesting the existence of an inhibitory site different from the activating site. Our data support a gating-pore model to explain the cationic interactions leading to the open and closed states of the Ca^{2+}-channel.

*Supported by CICYT of Spain (PB 87-0704).

M-Pos212

CALCIUM UPTAKE AND RELEASE OF THE SARCOPLASMIC RETICULUM OF SKINNED MUSCLE FIBERS FROM *XENOPUS LAEVIS* AT DIFFERENT ATP AND P_i CONCENTRATIONS.

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Laboratory for Physiology, Free University, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

Calcium uptake and release by the sarcoplasmic reticulum (SR) was studied in fast single fibers of the iliofibularis muscle from Xenopus laevis permeabilized by means of saponin (50 µg/ml, 30 min). Experimental conditions: 1 mM free Mg^{2+}, 10 µM - 5 mM MgATP, 0 or 15 mM P_i, 20 mM PCr, 1 mg/ml CPK, 100 mM TES (pH = 7.1, adjusted with KOH), ionic strength 200 mM (adjusted with K-propionate), temperature 3 °C. The SR was loaded with Ca^{2+} for 5 minutes in a high (20 mM) EGTA solution at pCa=7.0. Ca^{2+} was released by incubating the fiber in a low (0.5 mM) EGTA solution containing 5 mM caffeine. The amount of Ca^{2+} released was derived from the area of the force response. The presence of 15 mM P_i during loading had no effect on the amount of Ca^{2+} released, while 15 mM P_i present during the release caused a reduction of the force area which was in agreement with the depression of maximum isometric force by phosphate.
The amount of Ca^{2+} released increased with the MgATP concentration during loading. It was half maximal at 20 µM MgATP and saturated at higher MgATP concentrations. When the MgATP concentration was varied during the release, the force area normalized for the isometric force at that MgATP concentration, increased gradually from about 0.5 at 10 µM to about 1.0 between 1 and 5 mM MgATP. The delay in force development was practically negligible at 5 mM but was about 5 sec at 10 µM MgATP.
These results indicate that reduction in isometric force to 50% of maximum force during fatigue which is associated with a decrease in the intracellular Ca^{2+} concentration during tetanic contraction (cf. Allen et al., J. Physiol. 415, 433-458, 1989) is not caused by an effect on the SR of an increase in P_i concentration nor by an effect of a reduction in the MgATP concentration (cf. van der Laarse et al., J. Muscle Res. Cell Mot. 11, 77, 1990) on Ca^{2+} uptake. Ca^{2+} release is affected at lower ATP concentrations but not sufficient to account for all of the force reduction during fatigue.

M-Pos213

DIFFERENT CA^{2+} SENSITIVITY OF THE RYANODINE-SENSITIVE CA^{2+} RELEASE CHANNELS OF CARDIAC AND SKELETAL MUSCLE SARCOPLASMIC RETICULUM

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*Cardiovascular Sciences Section, Dept. of Medicine., and ^Dept. of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX. 77030

Single channel properties of canine cardiac (CSR) and mammalian fast twitch skeletal (SSR) muscle sarcoplasmic reticulum Ca^{2+} release channels were compared in planar bilayer experiments (250/50 mM Cs^+ as current carrier). The pharmacology of the two channels were similar. The conductance and the selectivity to mono- and divalent cations were also similar. However, while the Ca^{2+} sensitivity of the SSR channel was biphasic, with activation and subsequent inactivation by micomolar and millimolar Ca^{2+}, respectively, the CSR channel was not inactivated by high $[Ca^{2+}]$ (Fig.). $[^3H]$ryanodine binding to the two channel receptors reflected parallel differences in Ca^{2+} sensitivity in that high $[Ca^{2+}]$ also did not inhibit $[^3H]$ryanodine binding in CSR. The data suggest that there are two Ca^{2+} regulatory mechanisms. The high affinity Ca^{2+} activation mechanism is similar for the SSR and CSR channels. The CSR channel, however, appears to lack the low affinity Ca^{2+} inactivation mechanism. These data may relate to the observed differences in amino acid sequences found for the SSR and CSR channels (Otsu et al, J. Biol. Chem. 265, 13472-13483, 1990). Furthermore, these data directly show that the regulation of Ca^{2+} release in cardiac and skeletal muscle is different. [supported by NIH AR01834 (M. F.), HL13870 (M. L. E.) and AR38970 (E. S.)]

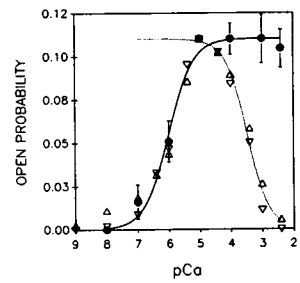


Fig. Normalized opening probability of human and rabbit SSR (open symbols) and dog CSR (filled symbols) as a function of $[Ca^{2+}]$.

M-Pos214

PHOSPHATIDYLINOSITOL-4,5-DIPHOSPHATE (PIP_2)-INDUCED CA^{2+} RELEASE FROM SKELETAL MUSCLE TERMNIAL CISTERNAE: SINGLE CHANNEL AND CA^{2+} FLUX STUDIES

*A. Chu, and ^E. Stefani
*Cardiovascular Sciences Section, Dept. Medicine, and ^Dept. Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX. 77030

Inositol-1,4,5-phosphate (IP_3) has been shown to activate the sarcoplasmic reticulum (SR) ryanodine-sensitive Ca^{2+} release channel in the bilayer. We investigated the effect of the precursor, PIP_2, on the same channel from rabbit terminal cisternae (TC) vesicles in the bilayer, in the presence of physiological Mg^{2+} + ATP. Low $[PIP_2]$ (1 µM) activated the channel, increasing the P_o ~2-12 fold. Open and closed log-time histograms revealed multiple open (~2) and closed (~3) states of the channel. PIP_2 had the following actions: 1)it increased the relative contribution of long open states during bursts (2 ms critical closed time), 2)it increased the fraction of events with long open time constant without greatly affecting the values of the time constants, and 3)it reduced the relative proportion of the long closures. IP_3 inhibited the PIP_2-induced activation of the Ca^{2+} release channel activity, but IP_3 itself did not activate the channel in the presence of Ca^{2+}. It activated the channel only in the presence of very low medium $[Ca^{2+}]$ (with EGTA present), as repoted previously (Isla-Suarez et al, Biophys. J. 54, 737-741, 1988). In Ca^{2+} flux studies, PIP_2-induced Ca^{2+} efflux in medium Ca^{2+} ranging from 10^{-9} to 10^{-3} M. PIP_2 and not IP_3 was more effective in eliciting Ca^{2+} release from rabbit TC. On the other hand, IP_3 and not PIP_2 induced Ca^{2+} release from dog cerebellum microsomes, which are known to contain IP_3-sensitive Ca^{2+} stores. The data suggest that PIP_2 is a specific activator of the SR ryanodine-sensitive Ca^{2+} release channel, and is more potent than IP_3. However, there may be more than one mechanism of action for the inositol polyphosphates on the channel, depending on the level of cytoplasmic $[Ca^{2+}]$. PIP_2 may provide a set point for the activation of the Ca^{2+} release channel, and there is an inositol polyphosphate regulatory site for Ca^{2+} release.

[supported by AHA (Texas Affl.) grant-in-aid (A.C.), HL13870 (M.L.E.) and AR38970 (E.S.)]

M-Pos215

SR CALCIUM UPTAKE IN NORMAL AND DYSTROPHIC MICE.

Margaret E. Kargacin and Gary J. Kargacin.
University of Calgary Health Sciences Center and University of Massachusetts Medical School.

Alterations in skeletal muscle Ca homeostatsis have been associated with muscular dystrophy. In the diseased muscle of mdx mice, which lack the protein dystrophin, higher resting calcium has been noted and a slower return to this higher set point occurs after a contractile stimulus. It is not known whether this is a direct consequence of the absence of dystrophin in affected muscles, a secondary effect, or a consequence of the general diseased state of the muscle. There is evidence suggesting that dystrophin may have a structural role in muscle or may be directly involved in the regulation of calcium influx, consistant with the calcium alterations seen. It is possible however that a secondary alteration in Ca-ATPase activity at the SR may be involved. It is also possible that the SR ATPase may function differently in the muscle to compensate in part for the higher calcium load. Such compensation could account for the fact that dystrophic mice can survive with the disease whereas affected humans cannot. To test the possibility that the Ca-ATPase activity is altered in musclular dystrophy we examined calcium uptake by skeletal muscle vesicles from normal and mdx mice. For both, the Hill coefficient for the dependence of uptake velocity on free Ca^{2+} was similar as was the Ca^{2+} concentration at half maximal pump velocity (~200 nM). These results and our results from previous work suggest, in general, that diseased muscle does not compensate for an increased calcium load by altering SR calcium pump kinetics. Supported by NIH AR39678 and the Alberta Heritage Foundation.

M-Pos216

ADENOSINE MODULATION OF THE SKELETAL MUSCLE Ca^{2+} RELEASE CHANNEL.

Mauricio Díaz-Muñoz, Enrico Stefani and Susan L. Hamilton, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

The activity of the skeletal muscle Ca^{2+} release channel can be modulated by a number of endogenous as well as exogenous compounds. The nucleoside, adenosine, alters both the binding of [^{3}H]-ryanodine and the activity of the channel incorporated into bilayers. Adenosine modulation of channel activity can also be detected in the presence of either ATP or methylxanthines. At low Ca^{2+} concentrations (< 20μm) adenosine increases both the open probability and the mean channel open time of Ca^{2+} release channels incorporated into the bilayer. At higher Ca^{2+} concentrations (>80μM), adenosine has little or no effect on channel activity. However, in the presence of AMP-PCP, adenosine inhibits the channel. Adenosine also has a biphasic effect on [^{3}H]-ryanodine binding. At low Ca^{2+} concentrations, adenosine enhances binding while at high Ca^{2+} concentrations, it inhibits [^{3}H]-ryanodine binding. The ability of the methylxanthines, caffeine, theophylline and IBMX, to potentiate [^{3}H]-ryanodine binding is greater in the presence of adenosine.

Our data suggest that adenosine is involved in the regulation of the Ca^{2+} release channel activity and that its mechanism of action is distinct from that of either ATP or methylxanthines. M. D.-M is a recipient of a Muscular Dystrophy Association Postdoctoral Fellowship.

M-Pos217

EFFECTS OF CAFFEINE AND VOLATILE ANESTHETICS ON CALCIUM RELEASE OF SKELETAL AND CARDIAC SARCOPLASMIC RETICULUM: WHY DO THESE AGENTS DECREASE CONTRACTILITY OF CARDIAC MUSCLE?

S. Tsuyoshi Ohnishi and Masayuki Katsuoka, Philadelphia Biomedical Research Institute, Radnor, PA 19087

Caffeine releases calcium ions from the sarcoplasmic reticulum (SR) of both skeletal and cardiac muscle. Using the skeletal muscle SR, it was demonstrated that caffeine as well as volatile anesthetics (halothane or enflurane) enhance calcium-induced calcium release (J. Biochem.86,1147,1979). These agents are known to induce in vitro contracture in the skeletal muscle of malignant hyperthemia susceptible subjects (both in humans and pigs). We have demonstrated that the calcium channel of pig skeletal SR is abnormal (FEBS Lett.161,103,1983; ABB 247,294, 1987; BBA 897,261,1988).

Volatile anesthetics decrease contractility of the heart (negative inotropic effect), possibly by interacting with the central nervous system, the sarcolemma, SR and myofilaments. Using isolated rat cardiac cells (myocytes) and perfused rat hearts (Langendorff model), it was found that both caffeine and volatile anesthetics (1) induced a momentary increase of calcium transients of the myocytes (as measured by Fura-2) when first applied; (2) eventually decreased the calcium transient of the myocytes; (3) caused a transient increase of contractility of the perfused heart; (4) eventually decreased the contractility of the perfused heart; (5) decreased the total content of calcium (as measured by atomic absorption) of both myocytes and perfused heart. In these experiments, the actions of caffeine and volatile anesthetics were interchangeable. These observations support our hypothesis that cardiac SR may be involved in the observed negative inotropism.

M-Pos218

SILVER ACTIVATION OF THE CALCIUM RELEASE CHANNEL FROM SR Hui Xiong, *Guy Salama, and Jonathan J. Abramson Portland State University, Physics Dept., P.O. Box 751, Portland, OR 97207, and *Dept. of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

We have previously shown that Ag^+ activates rapid Ca^{2+} release from SR vesicles by interacting directly with the Ca^{2+} release mechanism. Here we measure Ag^+ induced Ca^{2+} release rates from SR vesicles as a function of [Ag^+], and observe a multiphasic effect. At low [Ag^+] ([Ag^+] < 10 uM), rapid Ca^{2+} release is completely inhibited by ruthenium red (RR). At slightly higher [Ag^+] (10 uM < [Ag^+] < 20 uM), Ag^+ induced Ca^{2+} release rates are inhibited. While at still higher [Ag^+] ([Ag^+] > 20 uM), Ca^{2+} release rates are further stimulated, but are only partially inhibited by RR. Similar observations were made with SR vesicles fused to a planar BLM. In the presence of 50-100 uM Ca^{2+}, addition of 1 mM Mg^{2+} decreases P_o. Subsequent addition of submicromolar [Ag^+] (~0.2 uM) transiently stimulates channel activity. It is likely that the inactivation phase is caused by binding of Ag^+ to an endogenous SH which closes the channel. This corresponds to the inactivation of Ca^{2+} release observed at 10 uM < [Ag^+] < 20 uM, when monitoring Ca^{2+} fluxes across SR vesicles. Furthermore, we have observed that addition of submicromolar [Ag^+] activates channel activity of the reconstituted 106 kDa Ca^{2+} channel protein, confirming the presence of a critical thiol on the SH activated Ca^{2+} channel protein. Supported by AHA, and ACS.

M-Pos219

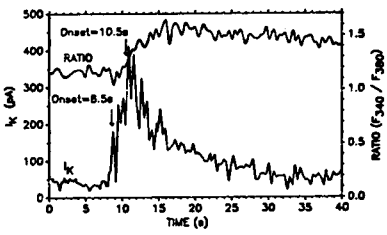
PORPHYRIN INDUCED CALCIUM RELEASE FROM SKELETAL MUSCLE SR VESICLES Jonathan J. Abramson, Scott T. Milne, and *Isaac N. Pessah. Portland State Univ. Physics Dept., P.O. Box 751, Portland, OR 97207, and *Dept. of Pharmacology & Toxicology, Univ. of California, Davis, CA 95616.

Micromolar concentration of both metal containing and metal free porphyrins are shown to induce rapid Ca^{2+} release from SR vesicles via their action on the Ca^{2+} release protein. Tetra(4-N-methylpyridyl)porphine tetraiodide (TMPyP) was the most effective of the porphyrins tested. At 20 uM TMPyP, the Ca^{2+} release rate from actively loaded SR vesicles was half maximally stimulated. Release was strongly inhibited by Mg^{2+} (K_I ~ 220 uM), and ruthenium red (K_I ~ 10 nM), and was stimulated by Ca^{2+} (K_{act} ~ 1.0 um) and by ATP (K_{act} ~ 100 uM). High affinity binding of [^{3}H]-ryanodine was also shown to be stimulated by TMPyP. The iron containing porphyrin, Hemin, was considerably less effective than TMPyP in stimulating Ca^{2+} release, and was also uneffected by Mg^{2+}, ATP, and ruthenium red. When SR vesicles were fused with a planar BLM in the presence of a CsCl gradient, Cs transport through the Ca^{2+} channel was stimulated by addition of TMPyP. The observation that porphyrins directly stimulate the Ca^{2+} release channel, induce Ca^{2+} release from SR vesicles, and stimulate high affinity ryanodine binding may be of physiological significance. Porphyrins are known to be critical components of many membrane bound electron transport reactions, and our previous findings have strongly implicated the oxidation of SH groups in the Ca^{2+} release process. Supported by NIH GM44337 (to J.J.A.), and ES05002 (to I.N.P.).

M-Pos220

SARCOPLASMIC RETICULUM OF CORONARY ARTERY SMOOTH MUSCLE SPONTANEOUSLY RELEASES CALCIUM TO BE EXTRUDED BY THE CELL. L. Stehno-Bittel and M. Sturek, Department of Physiology and Dalton Research Center, University of Missouri, Columbia, MO. 65211

Ca^{2+}-filled sarcoplasmic reticulum (SR) of coronary artery smooth muscle cells (SMC) may spontaneously release Ca^{2+} without elevating the concentration of free Ca^{2+} in the bulk myoplasm (Ca_i) (Stehno-Bittel et al. Am. J. Physiol. 259:H643-H647). We tested the hypothesis that the Ca^{2+} spontaneously released by the SR is extruded by the cell to the extracellular space. SMC were suspended in Ca^{2+} free solution containing membrane impermeant fura-2 to monitor extracellular Ca^{2+} (Ca_o) after one of two pretreatments. First, pretreatment with high K^+ and 2mM Ca_o (to fill the SR Ca^{2+} store) resulted in a significant increase in the fura-2 340/380 fluorescence ratio. Second, pretreatment with 5mM caffeine in 2mM Ca_o (to empty the SR Ca^{2+} store) abolished the rise in Ca_o. The results indicate that when the SR Ca^{2+} store is filled, the cell spontaneously extrudes Ca^{2+} to the extracellular space, but when the SR Ca^{2+} store is emptied Ca^{2+} is not extruded from the cell. Whole cell recordings of Ca^{2+}-activated K^+ current, indicative of Ca^{2+} levels directly under the sarcolemma, were measured simultaneously with intracellular fura-2 measurements of Ca_i in the bulk myoplasm. The caffeine-induced release of Ca^{2+} from the SR, caused an increase in the Ca-activated K^+ current, which preceded the increase in Ca_i by greater than 2 seconds (see Figure, onset of K^+ current 8.5 s versus 10.5 s for onset of Ca^{2+} increase). Simple diffusion rates of Ca^{2+} from the SR to the bulk myoplasm predict that the Ca_i increase should have occurred greater than 100 fold faster than that experimentally measured. We conclude that Ca^{2+} may be released by the SR in a vectorial manner toward the sarcolemma to be extruded from the cell.



M-Pos221

A STUDY OF Ca-INDUCED Ca RELEASE (CICR) FROM THE SARCOPLASMIC RETICULUM (SR) OF BARNACLE MYOFIBRILLAR BUNDLES, USING CAGED CALCIUM.
T.J.Lea & C.C.Ashley
University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK

We have used laser-induced photolysis of caged calcium (nitr-5) to generate a rapid jump in free Ca^{2+} (within 1 ms) in myofibrillar bundles of barnacle muscle fibres, thus overcoming diffusional delays inherent in the use of Ca activating solutions. After equilibration in a muscle solution containing 0.1 mM nitr-5 (initial pCa 6.8-6.6), the myofibrillar bundle (diameter 0.1 mm) was illuminated by a UV laser pulse (25 ns). This produced a phasic contraction with an amplitude of up to P_o and a half rise time of as short as 0.7 s at 12°C. A large part of the contraction could be abolished by ryanodine (0.1 mM) or by 1% Triton, demonstrating its dependence on Ca release from the SR. The size of the Ca^{2+} jump was varied by increasing the pulse energy; both contraction amplitude and rate of development increased with the size of the Ca^{2+} jump (over the post-photolysis pCa range 6.6 - 6.0). The mean half rise time for contractions over 40% P_o was 2.3 s. By comparison, the intact muscle fibres when electrically stimulated at 50 Hz develop tetanus tension with a half rise time of 177 ms at 12°C. Mechanical factors are unlikely to be the cause of the slower responses of the isolated myofibrils, since bundles with inactive SR could be directly activated by larger Ca^{2+} jumps to give faster contractions (mean half rise time: 164 ms). From these results it might be argued that CICR is too slow to account for excitation-contraction coupling in this crustacean muscle, although it is possible that there are unidentified, myoplasmic constituents which make the CICR mechanism more efficient "in situ" than in isolated myofibrillar bundles.

M-Pos222

EFFECTS OF AZUMOLENE ON DOXORUBICIN-INDUCED Ca^{2+} RELEASE FROM SKELETAL AND CARDIAC MUSCLE SARCOPLASMIC RETICULUM. Qing Tian, Arnold M. Katz and Do Han Kim, Department of Medicine, Cardiology Division, University of Connecticut Health Center, Farmington, CT 06030

Doxorubicin can induce Ca^{2+} release from both skeletal (Zorzato et al., J. Biol. Chem. 260, 7349, 1985) and cardiac muscle sarcoplasmic reticulum (SR) (Kim et al., J. Mol. Cell. Cardiol. 21, 433, 1989). The mechanism of doxorubicin-induced Ca^{2+} release from skeletal and cardiac muscle SR was studied by examining the effects of azumolene (a water soluble dantrolene analog) on doxorubicin-mediated Ca^{2+} release and ryanodine binding. Doxorubicin induced a rapid Ca^{2+} release from both skeletal and cardiac SR in similar concentration range ($C_{1/2}$ = 5 - 10 uM). Maximal doxorubicin-induced Ca^{2+} release was seen at 2 and 0.2 uM Ca^{2+} for skeletal and cardiac SR, respectively. Addition of azumolene (~400 uM) led to a partial, but significant inhibition of doxorubicin-induced Ca^{2+} release from both types of SR; skeletal SR had significantly higher sensitivity to azumolene than cardiac SR. In the presence of Ca^{2+}, doxorubicin increased [^{3}H]ryanodine binding to both skeletal and cardiac SR, whereas in the absence of Ca^{2+}, doxorubicin led to significant ryanodine binding to skeletal SR, but not to cardiac SR. In both types of SR, azumolene inhibited doxorubicin activated ryanodine binding, but not Ca^{2+} activated ryanodine binding. Azumolene does not appear to compete with ryanodine on ryanodine binding site(s). Azumolene sensitivity for inhibition of doxorubicin-activated ryanodine binding was much higher in skeletal SR than cardiac SR, consistent with the results for effects of azumolene on Ca^{2+} release. Our results suggest that azumolene inhibits doxorubicin binding by direct competition for doxorubicin receptor(s). Supported by NIH (HL-33026) and AHA-CT. DHK is an Established Investigator of AHA.

M-Pos223

ALKALINIZATION-INDUCED Ca^{2+} RELEASE FROM ISOLATED SARCOPLASMIC RETICULUM.

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Alkalinization-induced Ca^{2+} release from isolated frog or rabbit sarcoplasmic reticulum vesicles consists of two distinct components.

The faster component consists of a direct activation of ruthenium red-sensitive Ca^{2+} release channels in terminal cisternae. In the presence of ATP this release exhibits a maximum near pH 7.5 and declines at still more alkaline pH, suggestive of an alkalinization-induced inactivation process. Alkalinization-induced Ca^{2+} release is Ca^{2+}-dependent and enhanced by elevated extravesicular (i.e., cytoplasmic) free [Ca^{2+}] in the submicromolar range. However, this release is not simply a secondary Ca^{2+}-induced Ca^{2+} release triggered by the other form of release described below. The ability of terminal cisternae Ca^{2+} release channels to respond to alkalinization could be significant for E-C coupling if transient local pH changes in the triadic gap take place.

The slower component of alkalinization-induced Ca^{2+} release involves an increased ruthenium-red insensitive Ca^{2+} efflux through some other pathway distributed throughout the SR. This second form of Ca^{2+} release is also Ca^{2+}-dependent. It appears to contribute significantly to the rapid cessation of Ca^{2+} uptake activity in light SR vesicles at alkaline pH in the absence of phosphate or oxalate. Thus, when intravesicular free Ca^{2+} levels rise, even slightly, to levels that permit high efflux rates, net Ca^{2+} uptake at alkaline pH ceases.

M-Pos224

INTRAVESICULAR CALCIUM TRANSIENT DURING CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM

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In most of the Ca^{2+} release studies either the extravesicular free Ca^{2+} concentration or the total amount of the intravesicular calcium was monitored, and it has not yet been thoroughly determined how the intravesicular free Ca^{2+} concentration ($[Ca^{2+}]_i$) changes during Ca^{2+} release. Tetramethylmurexide (TMX), the Ca^{2+} indicator which is relatively insensitive to Mg^{2+} and virtually insensitive to monovalent cations and pH (Ohnishi, Anal. Biochem. 85, 165, 1978), was accumulated in the SR vesicles during active Ca^{2+} transport. Ca^{2+} release from SR was induced by the release triggering reagents (e.g. polylysine or caffeine) in the presence of an EGTA/Ca buffer (e.g. 20 mM EGTA, 12.9 mM $CaCl_2$). Under these conditions, the extravesicular $[Ca^{2+}]$ remained constant during Ca^{2+} release, and the changes in the TMX signal were primarily attributed to the changes in the $[Ca^{2+}]_i$. Multi-mixing stopped-flow experiments have allowed us to follow first the $[Ca^{2+}]_i$ before the induction of Ca^{2+} release, and then the changes in the $[Ca^{2+}]_i$ occurring after triggering Ca^{2+} release. Immediately after triggering Ca^{2+} release, the $[Ca^{2+}]_i$ rapidly increased to a significantly higher level than that in the resting state. Subsequently, the $[Ca^{2+}]_i$ decreased to a level that was much lower than the resting state $[Ca^{2+}]_i$. The decrease of $[Ca^{2+}]_i$ approximately paralleled ^{45}Ca release, while the rapid $[Ca^{2+}]_i$ increase corresponded with the lag phase that preceded ^{45}Ca release. These results suggest that upon the induction of Ca^{2+} release the internally bound calcium (presumably the calcium bound to calsequestrin) is dissociated, leading to a rapid increase of $[Ca^{2+}]_i$. Subsequently, the dissociated $[Ca^{2+}]_i$ is released across the SR membrane. (Supported by Grants from NIH and MDA).

M-Pos225

THE INVOLVEMENT OF PROTEIN KINASE C, PHORBOL ESTERS AND DIACYLGLYCEROLS IN THE MODULATION OF STRIATED MUSCLE CALCIUM RELEASE.

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We have recently localized protein kinase C (PKC) in the junctional regions of triads isolated from chicken and rabbit skeletal muscle. In this study, we have examined the endogenous substrates of PKC-dependent phosphorylation. Key junctional proteins were phosphorylated including a high Mr protein in the region of the ryanodine receptor. Other phosphorylated proteins include a 85 KDa protein and several lower Mr components. Many of the endogenous protein substrates, including those of SR origin, require the presence of intact triads. Considering the junctional T-tubule localization of PKC, it appears that many endogenous substrates for PKC phosphorylation are of SR origin and are phosphorylated by the T-tubule enzyme. Although PKC enzymatic activity was present in junctional T-tubules and triads, it was not detected in purified junctional SR (Saito R4 fraction) nor did immunoblots with anti-PKC antibodies reveal the presence of the PKC protein in the R4 fraction. We also examined the effects of various PKC activators on calcium release from skeletal and cardiac cells and from isolated junctional SR vesicles. The phorbol esters and diacylglyerols were potent modulators of the calcium release mechanism but many of the observed effects could be explained by actions of phorbol esters ßand DAGs which were independent of PKC activation. This work was supported by grants from the NSF, the California Metabolic Research Foundation, the Consiglio Nazionale delle Ricerche, and the Ministero della Pubblica Istruzione.

M-Pos226

CALMODULIN INTERACTION WITH THE SARCOPLASMIC RETICULUM Ca^{2+}-RELEASE CHANNEL FROM SKELETAL AND CARDIAC MUSCLES.

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Sarcoplasmic reticulum (SR) Ca^{2+}-release channel activity is modulated by activators (uM Ca^{2+}, ATP, nM ryanodine, caffeine), and by inhibitors [Mg^{2+}, uM ryanodine, calmodulin (CaM)] [Meissner et al., (1986) Biochemistry 25:236, 244]. These studies were initiated to define the physiological role of CaM in regulation of the channel protein by characterization of CaM/channel interaction. ^{125}I-labeled wheat germ CaM, modified at Cys 27 with benzophenone-4-maleimide, was crosslinked to heavy SR fractions from cardiac or skeletal muscle. In both cases, the primary crosslinked product (>95%) was a complex of M_r >400,000, corresponding to CaM plus the Ca release channel. Fluorescence anisotropy was then used to probe CaM/channel interactions in SR vesicles. CaM was labeled at Cys 27 with rhodamine-X-maleimide and was titrated with cardiac or skeletal SR vesicles. Binding of CaM to SR vesicles (and thus to the channel) was Ca^{2+}-sensitive at low ionic strength for both cardiac and skeletal SR. However, at physiological ionic strength, CaM bound to skeletal SR (but not cardiac) in the presence of either EGTA or Ca^{2+}, suggesting that CaM could remain bound to the channel protein in skeletal muscle at resting $[Ca^{2+}]$. Titration of CaM into a suspension of cardiac or skeletal SR vesicles in the presence of Ca^{2+} resulted in saturable binding of CaM which was resolvable by Scatchard analysis into 2 classes of sites with K_ds of approximately 1 nM and 30 nM. Binding of CaM to either SR was not significantly affected by addition of ryanodine over the range of 1 nM to 1 uM. However, addition of caffeine over the range of 1 mM to 50 mM inhibited CaM binding to both skeletal and cardiac SR, suggesting differential effects of channel modulators on CaM binding activity. (Supported by AHA/MI, MDA, and the MI Ag. Exp. Station).

M-Pos227

SUBSTATE ANALYSIS OF THE CALCIUM RELEASE CHANNEL FROM SARCOPLASMIC RETICULUM.

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Single channel events of the calcium release channel from skeletal muscle sarcoplasmic reticulum show a variety of subconductance states. The substate pattern found for the purified channel protein (PNAS USA 85:441-445, 1988) has now been confirmed using fusion of terminal cisternae vesicles to black lipid membranes. A particularly designed analysis of substates yields a matrix (M) of transition frequencies between substates. For the calcium release channel, this matrix M is clearly asymmetrical, showing cyclic non-equilibrium conformational flow. The distance from equilibrium is scaled by a dissipation order parameter. The current values of substates fall on integer multiples of a smaller value (i) with reasonable correlation. Transitions between substates occur cooperatively, mostly between levels distant by 2*i, 4*i, and multiples of 4*i. This is consistent with the four-fold symmetry of the calcium release channel (foot structure or junctional channel complex) with a central cavity and four radial canals (Nature 338:167-170, 1989), when these canals are interpreted as allosterically interacting ion pathways. This picture is further supported by the effect of ryanodine binding on the substate transition matrix M, indicating allosteric interaction.

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M-Pos228

ACTION OF VOLATILE ANESTHETICS ON THE SARCOPLASMIC RETICULUM CALCIUM RELEASE CHANNEL OF MALIGNANT HYPERTHERMIA SUSCEPTIBLE MUSCLE. Charles F. Louis, Kristine Zualkernan and James R. Mickelson, Dept. of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108, USA.

Skeletal muscle sarcoplasmic reticulum (SR) from malignant hyperthermia susceptible (MHS) pigs displays several abnormalities in the ryanodine receptor/calcium release channel protein. To determine whether these abnormalities can explain the ability of volatile anesthetics to precipitate MH crisis, we have examined the effect of three volatile anesthetics on the MHS and normal SR calcium release mechanism. At pH 7.0, halothane, isoflurane, and enflurane activated Ca release when calcium release from ^{45}Ca-filled vesicles was induced by 10^{-8} M Ca^{2+} (maximal at approximately 0.5 mM anesthetic in solution). Ca release from ^{45}Ca-filled MHS SR vesicles was significantly greater than from normal SR at a given anesthetic concentration. In contrast, these anesthetics had little effect on Ca release that was induced by transfer to a channel closing medium containing 10 mM EGTA, 10 mM $MgCl_2$ and 10 μM ruthenium red. Thus, this anesthetic-induced Ca release is occurring via the calcium release channel, and can occur at resting levels of myoplasmic Ca^{2+}. For both MHS and normal SR, Ca release induced by 10^{-8} M Ca^{2+} decreases as the pH is decreased. However, below pH 6.6, these anesthetics are unable to induce Ca release from normal SR, while they can still induce significant Ca release from ^{45}Ca-filled MHS SR. These results indicate that volatile anesthetics precipitate MH via their action on the ryanodine receptor/calcium release channel, thus resulting in an elevation of myoplasmic Ca^{2+} concentration. They also indicate that the acidosis that accompanies MH crisis will not prevent this anesthetic induced Ca release, so explaining the contracture of MH skeletal muscle that is characteristic of the MH crisis. (Supported by NIH grant GM-31382).

M-Pos229

PROTEIN KINASE CATALYZED PHOSPHORYLATION OF THE SKELETAL AND CARDIAC MUSCLE SARCOPLASMIC RETICULUM RYANODINE RECEPTOR.

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The possible role that protein kinase catalyzed phosphorylation of the sarcoplasmic reticulum (SR) ryanodine receptor (RyR) plays in the regulation of this Ca^{2+} channel is as yet unclear. To address this issue, the proteins of porcine skeletal and cardiac muscle heavy SR vesicles were phosphorylated with [γ-^{32}P] ATP either under basal conditions (10 mM $MgCl_2$, 1 mM EGTA, 20 mM HEPES at pH 7.0), on the addition of cAMP plus cAMP dependent protein kinase (cAMP-PK), or on the addition of 0.1 mM $CaCl_2$ plus 1 μM calmodulin (CaM). Following electrophoretic fractionation and autoradiography of these proteins, no phosphorylation of either the cardiac or skeletal RyR was detected under basal conditions. On the Coomassie blue stained gels of these samples, the RyR could be observed as a doublet in both types of SR, with the major component (intact RyR) of slightly higher M_r than a proteolytic fragment. The intact cardiac muscle RyR was phosphorylated in the presence of either cAMP-PK or Ca^{2+} plus CaM, with little phosphorylation in the lower M_r proteolytic fragment. In skeletal muscle SR, however, the intact RyR protein was phosphorylated much less than the proteolytic fragment in the presence of either cAMP-PK or Ca^{2+} plus CaM. Significant phosphorylation of the intact cardiac muscle SR RyR indicates a physiological role of this phosphorylation in the regulation of this Ca^{2+} channel. The minimal phosphorylation of the intact skeletal SR RyR argues against such a role. (Supported by the MN Affiliate of A.H.A.)

M-Pos230

[^{3}H]-RYANODINE BINDING IN MALIGNANT HYPERTHERMIA

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Malignant hyperthermia (MH), a metabolic disorder manifested by muscle rigidity, high fever and cardiac stress in response to halothane anesthesia, is associated with abnormalities in the skeletal muscle Ca^{2+} release mechanisms. We have analyzed [^{3}H]-ryanodine binding to sarcoplasmic reticulum membranes from normal and MH pig, dog and human skeletal muscle. In agreement with Mickelson *et al.* (*J. Biol. Chem.* 263:9310-9315, 1989), we find a statistically significant difference in the affinity for [^{3}H]-ryanodine binding between pig MH and control SR membranes (K_d = 5.04 ± 0.13 nM in MH versus K_d = 23.8 ± 9.5 nM for control), in 0.3M KCl, 100 μM Ca^{2+}. [^{3}H]-ryanodine binding to pig SR membranes heterozygous for the MH trait shows an intermediate affinity with K_d = 14.0 ± 2.8 nM. However, no pronounced changes in the binding constants for [^{3}H]-ryanodine were detected with either dog or human MH compared to control SR membranes. The difference in K_d for [^{3}H]-ryanodine binding to pig MH and control SR is attributable to the rate of association with no changes in the rate of dissociation. Adenine nucleotides also increase the rate of association of [^{3}H]-ryanodine, possibly explaining the lack of a detectable difference in K_d's for MH versus control SR membranes in the presence of 1 mM AMP-PCP. Supported by NIH 37028 to SLH.

M-Pos231

INTERACTION OF RUTHENIUM RED WITH CALCIUM AND MAGNESIUM BINDING SITES OF THE SARCOPLASMIC RETICULUM CALCIUM-ATPASE

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Although ruthenium red (RR) is essentially considered as a specific inhibitor of the calcium release channel of the sarcoplasmic reticulum (SR), we found that it interacts with the SR Ca^{2+}-ATPase.

By measuring changes in the RR absorbance spectra, we have studied RR binding to SR vesicles and found three types of RR binding sites on the vesicles. Two of them appear to be located on the Ca^{2+}-ATPase.

We propose that the first pool of sites (9-10 nmoles RR/mg of SR protein, Kd = 3 μM) represents the high affinity Ca^{2+} transport sites of the enzyme for the following reasons : 1) RR binding to these sites was dependent upon the presence of micromolar Ca^{2+} and therefore upon the state (Ca^{2+} bound or Ca^{2+} free) of the Ca^{2+}-ATPase, and 2) RR was found to be a competitive inhibitor of Ca^{2+} on these sites as demonstated by equilibrium $^{45}Ca^{2+}$ binding performed by filtration.

The other pool (11-12 nmoles/mg, Kd = 0.5-1 μM) represents Mg^{2+} sites of the enzyme as demonstated by the three following observations : 1) RR binding to these sites was dependent upon the presence of millimolar Mg^{2+} and unaffected by the presence of micromolar Ca^{2+}, 2) RR was able to inhibit Ca^{2+} or Mg^{2+} binding to low affinity divalent cationic sites of the enzyme and 3) binding of RR to these sites produces a decrease (10-20 %) of the intrinsic fluorescence level of the SR Ca^{2+}-ATPase, probably due to the proximity of a tryptophane(s) and the Mg^{2+} binding sites.

M-Pos232

EFFECTS OF GLOBAL ISCHEMIA AND REPERFUSION ON SR AND CONTRACTILE APPARATUS OF HUMAN SKINNED CARDIAC FIBERS.

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The effects of global ischemia on cardiac sarcoplasmic reticulum (SR) and the contractile apparatus were assessed by studying the SR calcium release activity and the isometric pCa/tension relationship, respectively. For this purpose, chemically skinned atrial fibers from six patients undergoing cardiac surgery by total cardiopulmonary bypass were investigated. Biopsies were collected 5 min. before aortic crossclamping, 10 min. after aortic crossclamping, and 10 min. after aortic clamp removal, for control, ischemic, and reperfused tissue, respectively. Calcium release was measured indirectly by following tension development. Ten minutes of global ischemia caused a three fold increase of the concentration of caffeine needed to activate the SR calcium efflux channel, and a 40% decrease of the rate of calcium release. The rate of Ca-release returned to normal only after reperfusion. Furthermore, ischemia caused a shift to the right of the pCa/tension curve (the calculated Km for calcium increased from 0.69 to 1.38 uM) and changes in the cooperativity index (Hill value decreased from 2.61 to 0.92). Two-dimension electrophoresis of the myofibrillar protein showed a decreased amount of the phosphorylated form of myosin light chain MLC-2. The changes we found on both the SR and contractile apparatus were restored only after reperfusion. These results suggest that global ischemia causes long term changes in the two systems that regulate the contraction-relaxation cycle of cardiac muscle. The decreased calcium release from the SR and the decrease of affinity of the contractile apparatus may explain the impairment of contractility of ischemic heart.

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M-Pos233

DISCRIMINATION OF MULTIPLE STATES OF THE RYANODINE RECEPTOR/Ca^{2+} RELEASE CHANNEL COMPLEX OF SKELETAL SR USING SEVERAL ANTAGONISTS.

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The interaction of ruthenium red (RR), neomycin (NM), and [2,6-dichloro-4-(dimethylamino)phenyl] isopropylamine (FLA 365) with the binding of [^{3}H]Ryanodine ([^{3}H]RY) and RY-induced Ca^{2+} release was studied in detail with junctional SR vesicles (JSR) isolated from fast-twitch skeletal muscle. Double reciprocal plots obtained from equilibrium binding curves (0.5-50nM [^{3}H]RY) in the presence or absence of NM or FLA 365 demonstrated that these compounds inhibit specific high affinity [^{3}H]RY binding in a competitive manner with apparent equilibrium dissociation constants (K_i) of 0.3μM and 1.4μM, respectively. Although RR also appears to compete for the [^{3}H]RY binding site (k_i = 11.3nM), a significant decrease in [^{3}H]RY binding capacity (B_{max}) suggested a more complex mechanism. JSR incubated for 2 hours in the presence or absence of 25nM RR followed by extensive washing in Ca^{2+}-free medium, demonstrated the irreversible nature of RR inhibition since 50% of the B_{max} for [^{3}H]RY was persistently blocked, without significant change in IC_{50} (0.30μM vs. 0.33μM for control and RR pretreated). Heterologous displacement studies of [^{3}H]RY with combinations of antagonists were performed to elucidate the nature of the interactions between inhibitors. RR decreased both the IC_{50} and the extent of maximum inhibition by NM. NM and FLA 365 do not appear to interact in a competitive manner since the IC_{50} for each compound alone is not significantly lowered by the presence of the other. Logit analysis of the NM inhibition of [^{3}H]RY binding indicates NM, but not FLA 365, discriminates multiple affinity states of the receptor complex (logit slope NM=0.6, FLA 365=1.0). Interestingly, the ability to discriminate multiple states with NM decreases in the presence of FLA 365 (logit slope NM=1.0 in the presence of 3μM FLA 365). The effect of NM on the Ca^{2+} dependent activation of ^{3}H-RY binding was also investigated. Half-maximal activation (EC_{50}) of the binding of [^{3}H]RY by Ca^{2+}, in the presence of AMP-PCP, was not influenced by the presence of 0.3μM or 0.6μM NM (EC_{50} = 5-6μM Ca^{2+}, in the presence or absence of NM), suggesting that the mechanism of NM inhibition is not the result of decreased affinity of the activation site for Ca^{2+}. Inhibition of RY-induced Ca^{2+} release from actively loaded JSR by RR, NM, and FLA 365 was determined in the presence of the Ca^{2+} sensitive indicator antipyrylazo III. Supported by NIH ES05002 and American Heart.

M-Pos234

MICROMOLAR RYANODINE IRREVERSIBLY ALTERS THE NATIVE FUNCTION OF THE Ca^{2+} RELEASE CHANNEL OF SKELETAL SR.

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Junctional sarcoplasmic reticulum membrane vesicles (SR) isolated from rabbit skeletal muscle were treated without and with 0.1-500 μM ryanodine (RY) under conditions optimal for receptor binding. Complete removal of bound RY was affected by several washes in Ca^{2+}-free buffer. RY pretreated SR membranes were examined with respect to their capacity to bind [^{3}H]RY and their Ca^{2+} transport properties. Pretreatment of SR with nanomolar RY did not alter the [^{3}H]RY-binding capacity nor the Ca^{2+} transport properties of the washed SR. However, scatchard analysis revealed a marked (~80%) decrease in the capacity of high-affinity [^{3}H]RY binding sites without change in K_D and a complete loss of low-affinity sites with SR pretreated with 10 μM RY. These effects on [^{3}H]RY-binding sites are persistant for 48 hours and deemed to be irreversible. Ca^{2+} release from actively loaded SR pretreated with and washed free of μM RY exhibits a persistent insensitivity to RY and daunomycin. The magnitude of these effects depends on the concentration of RY during pretreatment in the range of 0.1-500 μM. Inclusion of DTT during transient exposure to μM RY significantly protected specific, high-affinity binding sites for [^{3}H]RY. Similar observations were made with SR membranes fused in planar lipid bilayer. Ca^{2+} channel activity monitored in asymmetric CsCl demonstrated that in the presence of 100 μM Ca^{2+} (cis side), nM RY increased the mean open time of the channel while 10 μM RY locked the channel in a subconductance state which was persistent after perfusion of the cis side with RY-free buffer. Addition of DTT (0.5 mM cis) prior to RY prevents the persistent block induced by RY since normal channel activity is restored following perfusion of the cis chamber. This is the first demonstration that μM RY induces a persistent alteration in the function of the Ca^{2+} release channel of skeletal SR and implicates sulfhydryl oxidation in the process. Supported by NIH ES 05002 (INP) and GM 44333 (JJA).

M-Pos235

RUTHENIUM RED SELECTIVELY INHIBITS THE CONTRACTURES INDUCED BY Ins(1,4,5)P_3 IN PERMEABILIZED CHICK ATRIUM.

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We have previously shown that (1-25μM) Ins(1,4,5)P_3 (IP_3) and (1-20mM) caffeine induce a contracture in saponin-permeabilized chick atrial fibers, presumably by releasing calcium from the sarcoplasmic reticulum (SR).

In this study we found that ruthenium red (1-10μM) inhibited the response to IP_3 (IC_{50}= 1.8μM, n_H=2). Exposure to ruthenium red (0.3-10μM) did not inhibit the contracture induced by caffeine but potentiated it to 129±14.5% (m±SD) of control. In addition, ryanodine had a dual effect on the response to IP_3. At low concentrations (0.1-10nM) ryanodine potentiated the response to IP_3, whereas at high concentrations, ryanodine (>0.3μM) inhibited the response to IP_3 in a use-dependent fashion. The effect of ryanodine on the contractures induced by caffeine was solely inhibitory (IC_{50}=34nM, n_H=1).

Our experiments show that high concentrations of ryanodine inhibit both IP_3- and caffeine-mediated calcium release in the same use-dependent way. The dual action of ryanodine in our experiments is consistent with the reported existence of binding sites having high (K_d, 3-10nM) and low (K_d, 0.1-0.3μM) affinity for ryanodine in cardiac SR. Ruthenium red is a useful agent to differentiate IP_3- from caffeine-mediated calcium release, since it selectively blocks the response induced by IP_3 in chick atrial fibers.