M-Pos368

DILTIAZEM EFFECTS ON VOLTAGE DEPENDENT SODIUM AND POTASSIUM CURRENTS. Raymond J. Lipicky, Peter Greif, Knud Knudsen, Pritam Gill-Kumar, and Daniel Grassi; Division of Cardio-Renal Drug Products, Office of Drug Evaluation I, Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, MD 20857.

Using standard step voltage-clamp methodology and the isolated caudal-most stellar nerve of Loligo pealei, the effects of diltiazem on voltage-dependent sodium and potassium channels were studied. Diltiazem was applied both externally in artificial sea water and internally in a perfusate containing KF and K Glutamate. Six concentrations of diltiazem were studied, varying from $3x10^{-5}$ M to $3x10^{-3}$ M in 11 axons, at 3 to 5°C.

A reversible, dose-related decrease in sodium conductance was observed, from 18% at $3x10^{-3}$ M to 97% at $3x10^{-3}$ M. The decrease was not accompanied by any changes in time-to-peak for any step, voltage shift in the peak current, voltage dependence of sodium inactivation, or time constant of inactivation associated with several prepulse magnitudes.

The potassium currents were also reversibly decreased in a dose-dependent fashion, from 4% at $3x10^{-5}$ M to 67% at $3x10^{-3}$ M. Peak and steady-state potassium currents were similarly affected. There was no apparent change in potassium kinetics.

There was no use-dependent effect on sodium currents for a 1 Hertz 60 mv, 10 msec duration pulse.

Increases in pulse duration and/or frequency of pulsing had a modest use-dependent effect.

M-Pos369

FURTHER EVIDENCE FOR THE ROLE OF THE TUBULAR SYSTEM THROUGH THE SCHWANN CELLS AS ION PATHWAY TO THE SQUID GIANT AXON. N.G.Greeff, C.Benoit and Clara Sandri. Physiologisches Institut, Universität Zürich and Station Biologique, Roscoff, France.

The transglial channel system (tgcs) of tubules through the Schwann cells connecting to the extra-cellular and the periaxonal space was found to provide a better pathway for ion flow than the mesaxonal clefts even neglecting their newly observed tight junction vs. the axon (Zwahlen, Sandri & Greeff, J.Neurocytol,17:145,1988). The earlier observation that during gating current experiments the capacity transient acquired a slow tail visible under critical R_s compensation indicated that a growing fraction of the otherwise constant axonal capacity (C_m and hence area) was accessed by a higher R_s (e.g. about 50% of C_m after 40 min, Greeff at al.,Proc Roy Soc Lond B, 215:375,1982).

Suspecting the solution as the cause we now report a quantitative study of the density of tgcs openings towards the axon as obtained from freeze-fractured Schwann cells from fibres incubated for various times t_i in 514 Tris-, 55 Mg^{2+}, 11 Ca chloride. 119 EM fotos with a total area of 6160 μm^2 were analysed. The control average density ($t_i = 0$) was 3.28 openings per μm^2 confirming our earlier estimate (3.3±0.7). At t_i about 10 to 20 min a rapid decrease to about 55% occured followed by a much slower further decrease. This agrees rather well with the electrophysiological observations and confirms the role of the tgcs for ion flow. We also observed and quantified a lower tubular density in the close vicinity (1 μm) of the mesaxonal cleft allowing estimations about the leakiness of the tight junction . (Supported by Swiss National Fund 3.143-0.85).

M-Pos370

MEMBRANE PROPERTIES OF TYPE I SEMICIRCULAR CANAL HAIR CELLS ISOLATED FROM THE ADULT PIGEON. Daniel G. Lang and Manning J. Correia. Departments of Otolaryngology and Physiology and Biophysics, UTMB, Galveston, Texas 77550-2778.

Semicircular canal hair cells were dissociated enzymatically and studied using whole cell patch clamp techniques. Type I and II hair cells were distinguished using the ratio of the minimum width of the neck to the width of the cuticular plate of the cell (Correia, et al., J. Neurophysiol. 62: 924-934, 1989). Under current clamp, the mean resting potential of type I cells (-70±2.6 mV, n=14) was significantly lower than that of type II cells (-57±3.2 mV, n=15, P<0.005). The average V(I) relationships were examined for 5 type I and 7 type II hair cells. For current steps over the ranges of 0 to -40 pA and 0 to +80 pA, the steady-state slope conductances for type I cells were 2.4 and 5.4 nS, respectively, whereas those for type II cells were 0.81 and 4.2 nS, respectively. Under voltage clamp, the outward current at a constant holding potential (V_h) of -30 mV was significantly larger for type I cells (0.56±0.13 nA, n=13) than for type II cells (0.07±0.03 nA, n=11, P<0.005). For type I cells, the reversal potential of the steady-state I(V) curve shifted from -70 to -40 mV when external K^+ was increased from 3.5 to 25 mM. The activation curve of this current was estimated from the instantaneous current change upon returning to V_h after a pulse for cells bathed in normal saline and from the chord conductance calculated from the steady-state I(V) curve for cells bathed in 25 mM K^+ saline. The threshold for activation was about -90 mV with complete activation at about -50 mV. Unlike the delayed rectifier and A current of type II cells (Lang and Correia, J. Neurophysiol. 62: 935-945, 1989), the K^+ current of type I cells activates at more negative potentials and is not inactivated at -30 mV. Thus, type I hair cells posses a distinct type of K^+ current that is active at rest and could regulate the resting potential and conductance of these cells. Supported by ONR and NASA.

M-Pos371

MECHANISM OF NOREPINEPHRINE ENHANCEMENT OF ATP-INDUCED CA^{2+} TRANSIENTS A. Christie, J-S. Zheng, and A. Scarpa. Dept. of Physiol. and Biophys. CWRU Cleveland, OH 44106. Sponsored by S. Davidheiser.

Extracellular ATP induces a transient increase of the intracellular calcium concentration of rat ventricular myocytes. The source of calcium for this response is primarily from the extracellular space and also from sarcoplasmic reticulum calcium stores (Ca^{2+}-induced Ca^{2+} release). Pretreatment of myocytes with NE (0.01-1 μM) shifts the concentration-response curve for the ATP-induced calcium transient to the left. We have investigated this effect of NE. Forskolin (0.25-50 μM) and permeable cAMP analogues also enhance the ATP-induced calcium transient indicating that cAMP formation is critical. This is supported by intracellular cAMP measurements by RIA. Patch-clamp studies indicate that NE does not alter the inward current elicited by ATP but rather it increases the duration of slow action potentials elicited by ATP. We conclude that the effect of NE on ATP-induced calcium transients is partially produced by enhancement of the calcium current during the action potential. Supported by NIH 18708.

M-Pos372

SPATIAL DISTRIBUTION OF TTX-SENSITIVE AND TTX-INSENSITIVE CALCIUM CHANGES IN CEREBELLAR PURKINJE CELLS IN VITRO. V. Lev-Ram, H. Miyakawa, N. Lasser-Ross, and W.N. Ross (Intro. by F. Parker). Dept. of Physiology, N.Y. Medical College, Valhalla, NY 10595.

We measured the spatial distribution of calcium changes related to electrical activity in guinea pig Purkinje neurons injected with fura-2. Calcium changes were detected as changes in 380 nm excited fluorescence using a high speed CCD camera. In TTX Ringer, calcium increases corresponding to both calcium spikes and plateau potentials were detected from all over the dendritic field; the spike related signals were largest over the fine spiny dendrites and were much bigger than the plateau signals. A small but clear potential dependent somatic signal was also detected. In normal Ringer significant calcium changes were recorded corresponding to bursts of fast sodium dependent action potentials and plateau potentials. These signals were strongest in the soma but also extended into the dendritic region.

Supported by the Whitaker Foundation, the NIH, and NSF.

M-Pos373

WHOLE-CELL CLAMP STUDIES OF GIANT *DROSOPHILA* NEURONS DIFFERENTIATED FROM CELL DIVISION-ARRESTED NEUROBLASTS. M.Saito and C.-F.Wu. Dept. of Biology. Univ. of Iowa. Iowa City. IA 52242.

Drosophila is an organism of choice for molecular genetic analysis of ion channels and associated proteins. However, electrophysiological studies of *Drosophila* neurons has been technically difficult because of their small size. It has been shown that cytochalasin B arrests cytokinesis and allows *Drosophila* embryonic neuroblasts to become multinucleated giant cells. These cells display neuronal morphology and are suitable for whole-cell clamp study. In these neurons, we demonstrated the development of Na and Ca inward currents and three kinds of outward currents (A-type, delayed rectifier and Ca-activated) similar to those in other *Drosophila* excitable membranes. Furthermore, it is possible to correlate voltage-clamp and current -clamp data from the same neuron in this culture system. The role of A-type current in the delay of action potential initiation was demonstrated by preconditioning membrane with polarizing currents to control A-type current inactivation and by applying 4-AP which eliminates this outward current. Further experiments are underway to characterize the effects of mutations on different types of currents in these giant neurons.

M-Pos374

BUPIVACAINE BLOCKS Na^+, Ca^{2+}, AND K^+ CURRENTS IN SINGLE HEART CELLS. Sculptoreanu, A., [1]Tétreault, J.-P. and Bkaily, G., Dept. of Physiol. and Biophys. and [1]Dept. of Anesthesia, Fac. of Med., Univ. of Sherbrooke, Sherbrooke, Que. Canada. J1H 5N4.

The ionic mechanisms of the arrhythmogenic effects of bupivacaine have not been described. In this study, we tested the effect of bupivacaine on Na^+, Ca^{2+} and K^+ currents of single heart cells of 3 and 10-day old embryonic chick hearts using the whole-cell voltage clamp technique. In 3 day-old embryonic chick heart cells, bupivacaine blocked the fast transient (ft) TTX- and Mn^{2+}- insensitive Na^+ current without affecting the slow transient type. In old embryonic chick heart cells, bupivacaine completely blocked the TTX sensitive fast Na^+ current and the slow Ca^+ inward current. In both young and adult embryonic heart cells, bupivacaine blocked the delayed outward K^+ current (3 to 9 μg/ml). Finally, the arrythmogenic effects of bupivacaine could be due to its non specific effects on ionic currents of the heart muscle. This work was supported by MRCC (MT-9816) to G. Bkaily who is a scholar of CHF. A. Sculptoreanu is a Ph.D. fellow of CHF.

M-Pos375

ELECTRICAL BURSTING IN EXCITABLE CELL MODEL: A Step Toward Understanding the Neural Network Mechanisms

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How the neurons generate the electrical bursts undoubtedly plays a major role in neuronal information processing. The excitable cell membrane produces and propagates many different types of electrical signals. Identification of the nature of the output signals can provide valuable insight into the cellular mechanisms responsible for generating normal as well as abnormal signals. We present a neuronal model that gives rise to sinusoidal bursting in action potentials and a pancreatic β-cell model that gives rise to square-wave bursting. With bifurcation analyses, we then demonstrate how these cells can generate endogenous chaos.

M-Pos376

A STUDY OF REMYELINATION IN THE TRANSECTED FROG PERIPHERAL NERVE. Chaim T. Rubinstein and Peter Shrager, Dept. of Physiol., Univ. of Rochester, Rochester NY 14642.

We examined the possible role of the nerve cell body in the recovery of function following demyelination. Frog sciatic nerves were demyelinated by lysolecithin. 0-16 days later the nerve was cut near the spinal cord. Experiments were done 6 days after cutting. Distal stumps can survive for up to 10 days at 20-25°C. (Wang, J. Physiol. 368:309). Macrophage-mediated demyelination was followed by Schwann cell proliferation and remyelination. V_m-dependent Na^+ and K^+ currents were recorded from demyelinated internodes of cut fibers. Optically, we found conduction at about 1 m/s through long lengths of early remyelinating axons. Events in transected fibers were identical to those in uncut controls. If trophic substances arise from the neuronal soma they must be transported to the lesion and remain active for up to 6 days. Alternatively, these events may be entirely under local control. Support: NIH NS17965, NMSS RG-1774.

M-Pos377

OPTICAL MEASUREMENT OF CONDUCTION IN DEMYELINATED AXONS AND RESULTS OF K^+ CHANNEL BLOCK. Jin Wu and Peter Shrager (Intro. by R.S. Kass), Dept. of Physiology, University of Rochester, Rochester, NY 14642.

Action potentials were monitored optically in single demyelinated frog axons after staining with the dye RH155. Conduction in fibers with a fully demyelinated internode was generally blocked, but because of the internodal Na^+ channels the safety factor was very close to unity. A very slight improvement in the extracellular layer by bound Schwann cells or even macrophages was in some cases sufficient to restore action potentials at the transition node. The internodal Na^+ channels were able to support conduction once begun at this site, though at a low velocity (1 m/s). The first few turns of new myelin increased the success rate dramatically, with little improvement in velocity. Block of K^+ channels by 4-aminopyridine widens action potentials several-fold and in some cases may restore action potentials at least at the transition point. Support: NIH NS17965, NMSS RG-1774.

M-Pos378

IMAGING THE CA^{2+} TRANSIENT ASSOCIATED WITH HIPPOCAMPAL SLICE EXCITATION. J. S. George, J. C. Fowler and D. M. Ranken. Los Alamos National Laboratory, Life Sciences Division, MS M882, Los Alamos, NM 87545

We have previously reported a cytoplasmic Ca^{2+} transient associated with electrical stimulation of neurons in the rat hippocampal slice preparation, detected using photomultipliers to monitor Fura-2 fluorescence (1). We have recently obtained initial images of the spatial/temporal distribution of this response using an image intensifier optically coupled to a video camera. Experimental conditions were essentially as described. Fluorescence excitation was via overhead condensor, rotated 30° off the optical axis. A sequence of 16 or 32 video frames (33 ms each) were collected, spanning at least 200 ms of the pre- and post-stimulus intervals. Up to 256 sequences (each at 8 bit resolution) were accumulated in computer memory. Bipolar electrical stimulation of the stratum radiatum of CA1 produced a transient increase in fluorescence emission stimulated at 340 nm and a decrease at 380 nm. Because fluorescence changes were small (typically 1% of total) the signal was enhanced by subtracting an average prestimulus image from each image in the sequence, and the resulting sequences obtained at 340 and 380 nm were ratioed. In images, electrical stimulation appeared to produce diffuse activation of CA1, although in some images a pair of bands were apparent.

1. J.S. George and J.C. Fowler, Biophys. J. 53:560a (1988).

M-Pos379

TRANSIENT INWARD CURRENT HAS A CLEAR REVERSAL POTENTIAL IN FELINE VENTRICULAR MYOCYTES. Beth B. Dinda and Steven R. Houser, Dept. of Physiology, Temple Univ. School of Medicine. Phila. Pa.

Spontaneous Sarcoplasmic Reticular (SR) Ca^{2+} release induces a transient inward current (I_{ti}) at negative membrane potentials. Whole cell voltage clamp techniques and Indo-1 were used to study the voltage and Ca^{2+} dependence of I_{ti}. In normal Tyrode (150 mM Na^+) prolonged depolarization from -40 mV caused spontaneous SR Ca^{2+} release. Peak Ca^{2+} during these spontaneous releases had no apparent voltage dependence, however, I_{ti} had a clear reversal potential near +40 mV (n=5). In similar experiments in low (15 mM) Na^+ solution, the magnitude of I_{ti} was reduced and the reversal potential shifted to 0 mV (n=4). These results support the idea that spontaneous SR Ca^{2+} release activates an ion channel and that Na^+ may be the primary charge carrier. (Supported by NIH HL33648 and HL33921).

M-Pos380

ESTIMATION OF THE INTRACELLULAR FREE Mg^{2+} CONCENTRATION IN SINGLE CARDIAC MYOCYTES USING PATCH-CLAMP TECHNIQUES.

Minoru Horie and Chuichi Kawai (Intr. by P.F. Cranefield), 3rd Dept. of Internal Medicine, Kyoto Univ., Kyoto 606, Japan.

In cardiac myocytes, physiological concentrations of intracellular Mg^{2+} cause a unidirectional and voltage-dependent blockade of ATP-sensitive K^+ channels, evident as rectification of unitary currents. The degree of rectification of single channel currents in cell-attached patches on myocytes isolated from guinea pig ventricle, thus provides a measure of the internal Mg^{2+} level. However, in intact cells, these channels are normally inactivated by ATP. Therefore, the cardiac ATP-sensitive channel openers, nicorandil (100 μM) and cromakalim (10 μM), were used so that unitary current-voltage (I-V) relationships could be examined. The patches were then excised into 150 mM K^+ solutions containing various concentrations of free Mg^{2+}. With nominally zero free Mg^{2+} at the cytoplasmic face of the membrane, single channel conductances averaged 39±3 pS (n=12) at 5.4 mM K^+ (33°C). Single channel I-V relations determined in the cell-attached condition were virtually superimposable on those measured subsequently at 0.8 mM Mg^{2+}, which therefore appears to be the level of free Mg^{2+} at the cytoplasmic face of the cell membrane in intact myocytes.

M-Pos381

ALPHA$_1$-ADRENERGIC MODULATION OF A TRANSIENT K^+ CURRENT IN RABBIT ATRIAL MYOCYTES IS VIA A PERTUSSIS TOXIN INSENSITIVE G-PROTEIN AND A DIFFUSIBLE SECOND MESSENGER. D.Fedida, A.P.Braun, R.B.Clark & W.R. Giles. Department of Medical Physiology, University of Calgary, Calgary , Canada, T2N 4N1.

Recently we reported that α_1-agonists reversibly decrease a transient time- and voltage-dependent K^+ current (I_t). At 22°C, whole cell voltage clamp recordings on single myocytes showed that, with non-hydrolysable GTP analogues, GTPγS and Gpp(NH)p, 5mM, in the recording pipette, α_1-stimulation irreversibly decreased I_t, suggesting persistent activation of an α_1-adrenoceptor coupled G-protein. Lower doses (<2mM) or GDPβS (5.0mM) were ineffective. Pretreatment with 0.1μg/ml pertussis toxin (PT) for 8-9 hrs at 36°C did not prevent the α_1-induced decrease in I_t. Cell-attached patch recordings of single I_t channels revealed alteration of the burst properties of I_t channels when α_1 agonists were included in the bath perfusate. This suggests that an intracellular diffusible 2nd messenger was able to modulate patch activity from within the cell. It is unlikely that the effects are mediated via protein kinase C (PKC) as extracellularly applied phorbol-12-myristate 13-acetate ($1.62x10^{-7}$M) and 1-oleoyl-2-acetylglycerol ($6x10^{-5}$M) both increased I_t. The analogue 4α-phorbol which cannot activate PKC was ineffective. Inclusion of $2x10^{-5}$M inositol 1,4,5 trisphosphate in the pipette also failed to prevent the effects of α_1 agonists on I_t. In summary, these results strongly suggest a role for a PT-insensitive GTP-binding protein in the signal transduction mechanism underlying α_1-modulation of I_t in rabbit atrial myocytes. This system leads to the production of an intracellular messenger which does not appear to be IP_3 or to lead to activation of PKC.

M-Pos382

VOLUME REGULATORY CHANGES IN SPHERICAL AGGREGATES OF CULTURED CHICK HEART CELLS

R. Rasmusson, S. Liu, M. Lieberman. Dept. of Cell Biology, Div. of Physiology, Duke Univ. Med. Ctr. Durham N.C. 27710.

Previous work from our laboratory indicated that intracellular calcium changes play a role in controlling cell volume (J. Physiol. 382:105P, 1986). In this study, osmotically induced volume changes in heart cells were measured optically as changes in diameter relative to that obtained under control conditions. Osmolarity of the control salt solution (HEPES/TRIS buffer, 143 mM NaCl, 1 mM $CaCl_2$) was 290 mOsm. Both hypo- and hyper-osmotic (167 and 333 mOsm) solutions contained 36 mM NaCl with the osmolarity adjusted by sucrose addition. Aggregates were exposed to hypo- and hyper-osmotic solutions containing 1 mM Ca^{++} or 0 Ca^{++}+1 mM EGTA for 20 min. Hyper-osmotic conditions did not induce a regulatory volume increase; Ca^{++} did not affect shrinkage. Hypo-osmotic swelling (+Ca^{++}) induced a pronounced regulatory volume decrease (RVD) whereas 0 Ca++ attenuated the RVD. This suggests that cell swelling is necessary for Ca^{++} to play a role in volume regulation.

Supported in part by NIH grants: HL-07063, HL-17670, HL-27105

M-Pos383

STIMULATION OF α-ADRENOCEPTORS IN THE HEART INCREASES Ca^{2+} UPTAKE WITHOUT AN INFLUENCE ON THE SLOW INWARD CURRENT. U. Jahnel, H. Nawrath. Department of Pharmacology, University of Mainz, D-6500 Mainz, FRG.

The effects of phenylephrine (PE) on transmembrane potential and ion fluxes were investigated in left atrial heart muscle from rats. PE caused a concentration-dependent positive inotropic effect, an increase in action potential duration, and a decrease in the resting potential; the effects were antagonized by phentolamine (1 μmol/l) in a competitive manner. In the presence of PE (100 μmol/l), two levels of resting potential were observed when the preparations were, alternately, electrically stimulated or kept at rest (-74±1 mV during activity and -62±4 mV at rest; means ± s.e.m.; n=9). In resting preparations, the depolarization in response to PE was completely eliminated in low Na^+ solution (12 mmol/l) and antagonized by the Na^+ channel blocker tetrodotoxin (10 μmol/l). The PE-induced depolarization persisted in nominally Ca^{2+}-free solution and in the presence of the Ca^{2+} channel blocker (-)-devapamil (1 μmol/l). PE caused a significant increase of $^{22}Na^+$ uptake in resting and of $^{45}Ca^{2+}$ uptake in beating preparations (1 Hz). We conclude that stimulation of α-adrenoceptors by PE activates a Na^+ conductance in rat atrial heart muscle - possibly due to a decrease in potassium currents. The opening of Na^+ channels may increase $[Na]_i^+$ and, secondarily, $[Ca]_i^{2+}$ via the Na^+/Ca^{2+} exchange mechanism, thereby producing the positive inotropic effect.

M-Pos384

WHOLE-CELL RECORDING OF SINGLE CHANNEL K-CURRENTS IN EMBRYONIC VENTRICLE J Satin* & RL DeHaan Dept. of Anatomy & Cell Biol., Emory U., Atlanta, GA 30322

The high input resistance (2-10 G-ohm) and a relatively low K-current density of 7-day embryonic ventricular myocytes permitted recording of single channels from the whole-cell mode of the patch clamp technique. At positive clamp potentials unitary currents are observed with a slope conductance of 34 pS. These unitary channel events were superimposed on a slow, exponentially increasing outward macroscopic current. Thus, at least 2 currents are available during the plateau phase of the embryonic ventricular action potential: a relatively low conductance (≪34 pS) high density channel that produces the smooth whole-cell current, and a 34 pS channel present at low density, perhaps as few as 1 active channel per cell. (Supported by NIH PO1-HL 27385; *Present address Dept. of Cardiol., U of Chicago, Chicago, IL 60637).

M-Pos385

LIDOCAINE REDUCES CALCIUM CURRENT IN GUINEA PIG CARDIAC MYOCYTES.
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University of Maryland, Baltimore MD 21201

A reduction of cardiac calcium current (I_{Ca}) by lidocaine has been described (Ono et.al., Life Sci. 39:1465, 1986, Josephson, J. Mol. Cell. Cardiol. 20:593, 1988), but the mechanism of the effect is unknown. We have recorded I_{Ca} in adult guinea pig ventricular myocytes under whole cell patch clamp, using 30μM TTX extracellularly and 10mM TEA and 20mM CsCl in the pipette to block Na and K currents. I_{Ca} was reduced reversibly by extracellular lidocaine. The lidocaine-sensitive current had a voltage dependence characteristic of L-type calcium channels. I_{Ca} was ten times less sensitive to lidocaine block than I_{Na} (Ono et.al., Arch. Pharm. 339:221, 1989). Lidocaine-dependent reduction of I_{Ca} was similar during trains of depolarizing pulses of 10ms and 50ms duration, given at 0.5Hz from a holding potential of -50mV to 0mV. Lidocaine block of Na channels has been attributed to state-dependent binding. (Hondeghem & Katzung, Biochim. Biophys. Acta 472:373, 1977). These results suggest that lidocaine's action on Ca channels is mediated by a different mechanism.

M-Pos386

HOW DOES [ATP] INFLUENCE THE DURATION OF THE CARDIAC ACTION POTENTIAL ?

Nichols, C.G., Ripoll, C. & Lederer, W.J.
University of Maryland, Baltimore MD 21201

In guinea-pig ventricular cells under current-clamp (at 20 °C, $[ATP]_i$ = 5 mM), the action potential duration at 60 mV (APD_{60}) was 704 ± 48 ms. In order to mimic the effect of K_{ATP} channel activation on the action potential duration, current (I), proportional to the membrane potential (V_m) was injected ($I = G * (E_{ATP} - V_m)$, with E_{ATP} = -80 mV. The conductance factor (G) was increased until the APD_{60} shortened to 50 %. In the mean of 7 experiments, 50 % shortening of APD_{60} occurred at (G) = 1.42 nS. This would require the activation of < 2 % of K_{ATP} channel conductance (G_{max} ≈ 90 nS/cell; Noma & Shibasaki, 1985, J. Physiol. 363, 463-480). This will occur at ≈ 1 mM ATP (with half-maximal channel inhibition at ≈ 100 μM ATP, Hill coefficient = 2) implying that K_{ATP} dependent action potential shortening is likely to occur if ATP falls at all below normal levels (around 5 mM) as may happen regionally, or globally during myocardial ischaemia.

M-Pos387

REPETITIVE ACTIVATION INCREASES INWARD-GOING RECTIFICATION OF I_{K1} IN GUINEA PIG VENTRICULAR MYOCYTES.

Mario Delmar, Jorge Davidenko and Jose Jalife.
SUNY Health Science Center. Syracuse NY 13210.

Subthreshold potentials are thought to be mediated by time-independent, "passive" background currents. Here, we show that the background current-voltage (IV) relation of guinea pig ventricular myocytes is changed significantly by repetitive stimulation, in such a way that cell excitability becomes enhanced. Myocytes were used for whole-cell voltage-clamp experiments. A voltage-clamp ramp (100 mV/sec) to -50 mV was applied from a holding potential (HP) of -100 mV. Subsequently, a train of 50 pulses to +10 mV (duration, 300 ms; interpulse interval, 300 ms) was delivered from HP -85 mV. A new ramp was applied again immediately after the train, and the resulting IV curve compared with that obtained before the train. Pulsing displaced the IV relation to the right, the zero-current point becoming 1-2 mV less negative, and increased the degree of inward-going rectification. These changes were insensitive to tetrodotoxin (30 μM) but disappeared during superfusion with cobalt (2 mM) or verapamil (22 μM). In the presence of cesium (8 mM), pulsing still displaced the IV curve to the right. However, the linear portion of the curve became steeper after the train. Subtraction of the Cs-sensitive current from control revealed that, although the zero-current point remained constant, the IV relation showed a stronger inward-going rectification after pulsing. We conclude that repetitive pulsing improves cell excitability by increasing rectification of the i_{K1} channel. Calcium and Ca-activated, cation-insensitive channels may be involved in these effects.

M-Pos388

VOLTAGE CLAMP CHARACTERIZATION OF RABBIT CARDIAC VENTRICULAR MYOCYTES.

A. Varró, P.P. Nánási and D.A. Lathrop. Dept. of Pediatrics, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45229.
(Intro. by Dr. Lois K. Lane).

Action potentials (APs) and whole-cell ionic currents were measured in single enzymatically isolated rabbit right ventricular myocytes at room temperature. Based upon their AP configuration, the myocytes examined were divided into two groups. In group 1 (n = 15), a prominent phase of initial rapid repolarization (phase 1) occurred following the action potential upstroke. APs recorded in the second group (n = 7) did not exhibit a noticeable phase 1. In group 1, voltage clamp protocols revealed a transient outward current activated upon depolarization from a holding potential of -50 mV to beyond 0 mV. Transient outward currents were not apparent in myocytes from group 2. In both groups, Cd^{+}-sensitive secondary inward currents presumably carried by Ca^{2+} were recorded. This current was activated between -30 & -20 mV and reached a peak within 15 ms following initiation of a command pulse to 0 mV. Inactivation at this voltage was best fit by two time constants (τ_{fast} = 11.5 ± 1.9 ms, τ_{slow} = 103.3 ± 10.9 ms). The I-V relations established at the end of 400 ms long command pulses in both groups displayed a negative slope conductance. Pulses as long as 3 s failed to reveal the presence of a delayed outward rectifier current in either group; *i.e.*, the total membrane currents measured at +50 mV did not differ from one another at 400 ms and 3 s. In Na^{+}-free superfusate, in the range of -70 to -30 mV, no inactivating inward current was discerned. These results suggest that two populations of ventricular myocytes exist in rabbit; neither having a prominent delayed rectifier or a T-type Ca^{2+} current, while one group has a transient outward current and the other not. [Supported by the NIH NHLBI, grant # R01 HL37034]

M-Pos389

EXTRACELLULAR pH ALTERS VENTRICULAR NA/K PUMP V_{max}.

J Gao, RT Mathias, IS Cohen & GJ Baldo. HSC, SUNY Stony Brook, NY.

We investigated the effects of external pH changes between 6.25 & 8.0 on the magnitude of Na/K pump current (I_p) in guinea pig ventricular myocytes. We define I_p as the current blocked by a 1 mM concentration of dihydroouabain (DHO), which blocks about 92% at pH 7.4. The reduction in I_p reaches a minimum at about pH 6.8, where it is 51% of its value at normal pH (7.4). Similarly, the pump is stimulated at alkaline pH, though we did not reach saturation. I_p at pH 8.0 is about 152% of normal. The reduction in I_p at acid pH could be due to: 1) a reduced $[Na]_i$; or decreases in 2) V_{max}; or pump affinity for 3) K^+; or 4) DHO. Changes in $[Na]_i$ in patch-clamped cells are unlikely to be sufficient to account for this effect. Additional experiments demonstrated that the K_m for K^+ and the k_ds for DHO were unaltered at pH 6.8. Thus, acidic external pH may reduce I_p by reducing V_{max}. This reduction in the V_{max} of the Na/K pump at acid pH could play a significant role in the electrophysiologic abnormalities which occur with ischemia.

Grants HL29205, HL20558 & HL28958

M-Pos390

GLYCOSIDE BINDING & IONIC ACTIVATION OF VENTRICULAR NA/K PUMP.

J Gao, RT Mathias, IS Cohen & GJ Baldo. HSC, SUNY Stony Brook, NY.

We studied the Na/K exchange pump in guinea pig ventricular myocytes using the whole cell patch clamp. The affinity for dihydroouabain (DHO) was examined using a 10 mM $[Na^+]$ pipette. DHO had two binding sites with k_d's of roughly 1 and 140 μM. The effects of external $[K^+]$ and internal $[Na^+]$ on the pump current (I_p) were also studied. We estimated the normal resting $[Na^+]_i$ = 5.4 mM and I_p = 0.24 μA/μF, based on the changes in holding current required for various pipette $[Na^+]$s. If the pump ratio is $3Na^+/2K^+$, the normal inward background current is 0.72 μA/μF. Active Na^+ transport at rates different than the passive leak alters $[Na^+]_i$. We corrected for these $[Na^+]_i$ changes (Mathias et al. 1988, Biophys J 55:44a). The uncorrected Na^+ binding curve saturated with a half max of 13 mM; correction reduced this to about 9 mM. The steepness was better fit assuming 3 sites instead of 1. The K_m for K^+ was about 2.5 mM. The DHO affinity and K_m for K^+ differ significantly from those in Purkinje myocytes. This may be of substantial clinical relevance.

HL29205, HL20558 & HL28958

M-Pos391

SIMULTANEOUS MEASUREMENT OF INTRACELLULAR SODIUM ACTIVITY, USING SBFI, AND TWITCH SHORTENING IN GUINEA-PIG VENTRICULAR MYOCYTES.

Simon M. Harrison and *Mark R. Boyett, Department of Physiology, University of Leeds, LS2 9JT, U.K.*

Isolated ventricular myocytes from the guinea-pig were incubated with the acetoxymethyl ester of SBFI (11μM) for up to 2 hr and then resuspended in a Hepes buffered physiological salt solution (1mM Ca, pH 7.4). The cells were illuminated with light at 340 and 380 nm and fluorescence measured at 510 nm. At a stimulation rate of 1 Hz (26°C) intracellular sodium activity (a^i_{Na}) was 7.8 ± 0.9mM (mean ± SEM, n=10). After a 5 min rest period, re-stimulation (1 Hz) led to a rapid recovery of twitch shortening (over 4 to 5 beats) followed by a slower recovery back to control which was associated with a rise of a^i_{Na} of 2.8 ± 0.9 mM (n=3).

The addition of 10^{-5} M strophanthidin (1Hz) led to an increase in both a^i_{Na} (by 3.6 ± 0.7 mM, n=6) and twitch shortening (from 7.7 ± 1.5μm to 13.4 ± 1.4μm, n=6). However, when strophanthidin was washed off, twitch shortening fell with a faster time course than a^i_{Na}. At the same level of twitch shortening (at the maximum width of the hysteresis loop between twitch shortening and a^i_{Na}), a^i_{Na} was 2.4 ± 0.3mM (n=6) higher during washoff than during onset. This effect may be a result of the transient decrease in action potential duration during recovery from strophanthidin exposure. *Supported by the MRC and BHF.*

M-Pos392

β-ADRENERGIC AND MUSCARINIC RECEPTORS IN ADULT RAT VENTRICULAR MYOCYTES.

M. Horackova, J.M. Hao, B. Robinson, M. Wilkinson (Intr. by A.Y.K. Wong) Dept. of Physiology and Biophysics, Medical School, Dalhousie University, Halifax, N.S. Canada.

Cell surface β-adrenergic and muscarinic receptors have been characterized and quantified in intact, isolated adult rat ventricular cardiomyocytes which were previously established as functionally fully compatible with their properties in vivo. The bindings of the hydrophilic radioligands [^{3}H]-CGP and [^{3}H]-NMS were found to be stereospecific, saturable, reversible, of high affinity and demonstrated appropriate drug specificity. Pretreatment with the β-agonist (-)-isoproterenol or with muscarinic agonist carbachol at 37°C resulted in a reduction (down-regulation) in specific bindings of these hydrophilic ligands. The magnitude of these reductions and the rate of recovery were time and concentration dependent. We conclude that our preparation of intact, isolated ventricular cardiomyocytes is ideally suited for the study of cell surface β-adrenergic and muscarinic receptor regulation under physiological and pathological conditions. Supported by grants from MRC Canada (MT-4128) and Nova Scotia Heart Foundation to Dr. M. Horackova.

M-Pos393

REGULATION OF CONTRACTILITY IN ISOLATED RAT AND GUINEA-PIG CARDIOMYOCYTES BY Na^+ GRADIENT.

M. Horackova, Dept. of Physiology and Biophysics, Medical School, Dalhousie University, Halifax, N.S. Canada, B3H 4H7

Action potentials and developed contractions of externally unloaded single ventricular myocytes isolated from adult rat and guinea-pig hearts were recorded by means of an optical system for recording contractile activity during regular stimulation by microelectrodes. Under control conditions the shortenings (twitches) in the rat myocytes were fully inhibited by 0.1μM ryanodine, but they were rather insensitive to 0.2-0.5 μM nifedipine. In contrast the contractions of the isolated guinea-pig ventricular myocytes were greatly suppressed by 0.2-0.5 μM nifedipine (to <30%), while they were only slightly reduced by 1 μM ryanodine. When the Na^+ gradient was decreased by reducing $[Na]_o$ or by elevating $[Na]_i$ in the presence of veratridine, the twitch contractions were increased in both species, and they were followed by tonic components which were ryanodine- and nifedipine-insensitive. It is concluded that Na^+-Ca^{2+} exchange probably does not directly contribute in a quantitative fashion to the activation of contractile activity under normal conditions, but it does when Na^+ gradient is decreased by increase in $[Na]_i$. Supported by grant from MRC Canada.

M-Pos394

EVIDENCE FOR TWO USE-DEPENDENT SODIUM CHANNEL BINDING SITES IN ISOLATED RABBIT ATRIAL MYOCYTES.

M Barber, F Starmer, A Grant. (Intro. by J Moore) Duke Univ Med Ctr, Durham, NC 27710.

Cardiac conduction abnormalities are seen with amitriptyline (AMI) overdosage. Diphenylhydantoin (DPH) has been used clinically to reverse effects induced by AMI but whether this reversal occurs by competition for a single receptor site or allosteric interaction between two separate sites remains unclear. We evaluated effects of AMI (.4μM) and DPH (80μM) on sodium (Na) current in rabbit atrial myocytes. From a holding potential of -130 mV stepping to -30 mV, pulse train stimulation showed use dependent blockade for both drugs. At potentials <-130 mV, DPH showed progressive increase in block while that with AMI was voltage independent. Recovery (τ_r) and uptake (u) constants were measured at varying internal ($_i$) and external ($_o$) pH during control (pH_i =7.3; pH_o=7.4), high external (pH_i=7.3; pH_o=8.0) and high internal (pH_i=8.0; pH_o =7.4) pH. Data are expressed as mean ± SD (*p< .001 from control):

	Control (n=6)		High pH_o (n=6)		High pH_i (n=6)	
	AMI	DPH	AMI	DPH	AMI	DPH
τ_r	13±3	.7±.3	4.2±.1*	.7±.5	13±2	2.6±1.3*
u	1.4±.1	1.2±.4	1.2±.2	1.0±.2	1.3±.1	.7±.2

We conclude that AMI blocked Na channels via a drug-receptor complex sensitive to pH_o only with no evidence of voltage dependence. The DPH-receptor complex showed voltage dependence and sensitivity to pH_i only. Both sites showed use-dependent block.

M-Pos395

PROPERTIES OF THE CHLORIDE CURRENT IN CARDIAC VENTRICULAR MYOCYTES R.D. Harvey and J.R. Hume Department of Physiology, University of Nevada School of Medicine, Reno, NV 89557.

The cardiac chloride (Cl^-) current was studied in isolated guinea-pig and rabbit ventricular myocytes, using the whole cell voltage-clamp technique. This time-independent background current was elicited using the agonists isoproterenol and histamine, and the agonist-induced effects were antagonized by acetylcholine and adenosine. The current could be elicited when intracellular Ca^{2+} was buffered with 5 mM EGTA and extracellular Ca^{2+} was removed. This indicates that Ca^{2+} is not essential for activation of the current. The conductance of the agonist-induced current was reduced upon removal of extracellular Na^+, revealing an unusual and yet unidentified mechanism of regulation. Altering the Na^+ gradient, however, did not affect the reversal potential of the agonist-induced current. The reversal potential did follow the predicted Cl^- equilibrium potential (E_{Cl}), indicating that the current is conducted through a highly selective ion channel. When cells were dialyzed with a physiological intracellular Cl^- concentration (20 mM; E_{Cl}, -50 mV) the current-voltage relationship exhibited outward rectification. When the Cl^- current was elicited with symmetrical Cl^- concentrations inside and out (150 mM; E_{Cl}, 0 mV) the current voltage relationship was linear between -120 and +50 mV, suggesting that the previously described rectification may be attributed to asymmetrical Cl^- gradients.

M-Pos396

REDUCED CALCIUM CURRENT IN EMBRYONIC CHICK HEART WITH PERSISTENT TRUNCUS ARTERIOSUS. Sumi Aiba and Tony L. Creazzo. Department of Anatomy, Medical College of Georgia, Augusta, Georgia, 30912-2000.

Calcium currents were measured using the whole-cell clamp technique in ventricular myocytes from Day 11 embryonic chick hearts with a congenital heart malformation known as persistent truncus arteriosus (PTA). PTA was produced by ablating cardiac neural crest (from mid-otic placode to caudal region of somite 3) at 20-24 hours incubation. Data was obtained from the dissociated ventricles of 8 PTA and 8 sham-operated embryos. The DHP-sensitive, $I_{Ca,L}$, was reduced compared to sham-operated embryos (-1.0±0.15 pA/pF SEM, n=16 cells and -1.6±0.25, n=20, E_{test}= 10mV). Half-inactivation was similar (PTA: V_h=-25mV, n=10 & sham: -21mV, n=5). The response to Bay K 8644 was markedly reduced (PTA: -2.0±1.1 pA/pF, n=3 & sham: -7.5±1.0, n=5, E_{test}=+10 mV). Supported by NIH grant HL 36059.

M-Pos397

SODIUM CURRENT-INDUCED RELEASE OF CALCIUM FROM CARDIAC SARCOPLASMIC RETICULUM. Normand Leblanc, and Joseph R. Hume, University of Nevada School of Medicine, Reno, Nevada, 89557. (Intr. by John Peacock).

We have used the Ca^{2+} indicator, Indo-1, in dialysed guinea-pig ventricular myocytes to investigate whether SR Ca^{2+} release can be elicited by a mechanism independent of Ca^{2+} entry through voltage-gated Ca^{2+} channels. Whole-cell voltage clamped cesium-loaded myocytes were superfused with a solution containing 2.5 mM Ca^{2+} and 5 uM Nisoldipine or 10 uM D 600. From a holding potential of -80 mV, application of 500 ms depolarizing test pulses to -40 mV evoked large (several nA) inward currents that completely inactivated within 15 ms. Following these events, intracellular Ca^{2+} rose quickly to about 320 nM and slowly relaxed towards resting levels. Tetrodotoxin (30 uM) abolished the inward current and the Ca^{2+} transient, suggesting that the inward current reflects activation of fast TTX-sensitive Na^+ channels (I_{Na}), and that it is somehow involved in the Ca^{2+} response. Exposure to Ryanodine (10 uM) or nominally Ca^{2+} free solution abolished the Ca^{2+} transient but had little effect on the amplitude of I_{Na}. These results suggest that the Ca^{2+} transient observed under these conditions results from SR Ca^{2+} release in response to a transient rise in $[Na^+]_i$, which in turn appears to involve a Ca^{2+} entry pathway distinct from voltage-dependent Ca^{2+} channels, possibly reverse mode activity of Na-Ca exchange.

M-Pos398

PROLONGATION OF ACTION POTENTIAL AND DECREASE OF I_{to} IN VENTRICULAR CELLS FROM ADULT RATS WITH GROWTH HORMONE SECRETING TUMORS. X. Xu & P.M. Best. Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801

GH_3 cells were injected subcutaneously into adult, female Wistar-Furth rats to induce growth hormone secreting tumors. In 8 weeks, injected rats doubled body weight and heart size. Action potentials (AP) were recorded from right ventricular cells of both tumor and control rats using whole-cell patch-clamp technique. The resting potential and the AP amplitude were quite similar, -72±2mV & 102±9mV ($\bar{x}$±s.d., n=8) in tumor rats vs. -73±3mV & 98±6mV (n=14) in controls. However, AP duration increased significantly in ventricular cells from tumor rats, the time to 50% repolarization being 22±14 ms in tumor rats compared to 6.6±1.5 ms in controls. The prolongation was mainly due to a decrease of transient outward K current (I_{to}); its normalized conductance decreased from 0.53±0.10 nS/pF (n=17) in controls to 0.32±0.09 nS/pF (n=24) in tumor rats. Longer AP duration increased Ca influx through L-type Ca channels, which may have some importance to the growth and/or to cardiovascular adaptation in the tumor animals. Supported by NIH AR32062, RR-5861 & Ill. Heart Assoc.

M-Pos399

pH MEASUREMENT IN SINGLE CARDIAC MYOCYTES WITH SNARF-1. P.S. Blank*, H.S. Silverman, O.Y. Chung*, M.D. Stern*, R.G. Hansford, E.G. Lakatta, M.C. Capogrossi. *The Johns Hopkins Univ. and Lab. of Cardiovascular Science GRC/NIA/NIH, Baltimore, MD.
SNARF-1, seminapthorhodafluor (Molecular Probes) was used to characterize the intracellular pH in isolated rat cardiac myocytes. The emission spectrum of SNARF-1 contains two well separated emission peaks at 590 and 640nm. This feature allows the indicator to be used in the single excitation, dual emission, ratio mode. Cell suspensions were loaded with 4μM of the acetoxymethyl ester for 10 min. Under these loading conditions, the cells have the following characteristics: 1) the contractile properties are unchanged in the presence of the indicator, 2) the indicator is present primarily in the cytosol (95%-100%) with virtually no partitioning into the mitochondria, 3) the indicator is retained for several hours at room temp (23° C), and 4) steady state pH and transient changes in pH are easily monitored using our time resolved fluorescence microscope system. Consistent intracellular calibration was obtained using nigericin (10-20 μM), FCCP (1μM), and valinomycin (1μM) to abolish trans-sarcolemmal pH gradients.

M-Pos400

ELECTRICAL BREAKDOWN OF CARDIAC CELL MEMBRANE BY VOLT-LEVEL TRANSMEMBRANE POTENTIALS. Leslie Tung and Rory J. O'Neill. Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD 21205

Defibrillation generates high intensity electric fields throughout the heart, which induce large transmembrane potentials (V_m) in individual cardiac cells. When V_m exceeds a critical threshold of about 1 V in a variety of cell types, electrical breakdown (or electroporation) of the surface membrane occurs. We used a modified cell-attached, macro-patch voltage clamp technique on isolated frog ventricular cells to investigate the voltage dependence and time course of membrane breakdown. Following seal formation the pipette potential was increased linearly from 0 to 2 volts in <10 msec, and membrane impedance was monitored. To reduce the effect of action currents due to membrane channel activity, the pipette and bath solutions were intracellular-like. Ca^{2+} was included in the pipette solution, with EGTA in the bath, so that cell contraction could be used to verify membrane breakdown within the macro-patch. Our results suggest that reversible membrane breakdown is initiated at V_m of 510 ± 96 mV (mean ± s.d., N=16). Permanent reductions in membrane impedance occur at V_m of 700 ± 130 mV (N=9) and are step-like with transition times <30 μsec. These results suggest a pathological role of electroporation of the cardiac cell membrane with electrical defibrillatory shock.

M-Pos401

THE INDUCTION OF OVERDRIVE EXCITATION IN ISOLATED VENTRICULAR GUINEA-PIG MYOCYTES. L. Yang and M. Vassalle (Intro. by P. Dreizen). Department of Physiology, SUNY, Health Science Ctr., Brooklyn, N.Y. 11203.

In Tyrode solution, overdrive (90-120 beats/min for 30 sec) induced no changes or a small oscillatory potential (V_{os}). In norepinephrine (NE, 0.1-5 μM), overdrive gradually decreased the resting potential (V_{ex}) (through the underlying current I_{ex}) and induced V_{os} (and the underlying oscillatory current I_{os}). In some cells, V_{os} induced repetitive spontaneous activity. The effects of NE were reduced or abolished by propranolol (0.5-1 μM) and by acetylcholine (0.1-1 μM). Theophylline and caffeine (0.5-1 mM) increased Isi and induced V_{os} and V_{ex} and (under voltage clamp conditions) I_{os} and I_{ex}. Higher concentration of methylxanthines (5-10 mM) abolished I_{os} but not I_{ex}. Finally, in the presence of theophylline, quinacrine (20-40 μM) decreased the amplitude of and prolonged the action potential, markedly decreased the enhanced Isi and abolished V_{os}. Thus, overdrive induces excitation in ventricular myocytes by increasing calcium load and thereby causing V_{os} and V_{ex} especially if calcium influx is increased.
(Supported by NIH grant HL 17451)

M-Pos402

POTASSIUM SENSITIVITY OF DELAYED RECTIFICATION IN THE ISOLATED GUINEA PIG VENTRICULAR CELL: CONTRIBUTION OF EXTRACELLULAR POTASSIUM CONCENTRATION CHANGES. J. P. Arena and R. S. Kass. Department of Physiology, University of Rochester, Rochester, NY 14642. We have investigated the potassium sensitivity of the delayed rectifier (DR) channel in guinea pig ventricular cells using the whole cell arrangement of the patch clamp procedure. The equilibrium potential (V_{eq}) was more positive than that expected for a purely potassium-selective channel but was not affected by substitution of other monovalent and divalent cations or anions. A rapid (tau ≃30 ms) inward current component, associated with DR activation, was present at all voltages where DR deactivates. Its kinetics were not voltage-dependent and had a Q_{10} (≃ 1.2) consistent with free diffusion. After correction for this component, DR records reversed near V_K and the sensitivity of V_{eq} to external potassium agreed well with a K-selective channel. We suggest that extracellular K accumulates in an unstirred layer (t-system or caveolae) during DR activation and that the rapid inward current we observe reflects diffusion of K out of this space upon deactivation of DR.

M-Pos403

BUPIVACAINE BLOCKS I_{to} BUT NOT I_{K1} IN ISOLATED RAT VENTRICULAR MYOCYTES:

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Bupivacaine (Bup) is a local anesthetic with cardiotoxic properties, the mechanisms of which are still poorly understood. In this study the effect of Bup on K^+ currents in rat ventricular myocytes was examined under whole cell clamp in the presence of 10μM TTX and 3mM Co^{2+} to block I_{Na} and I_{Ca}. (±)Bup reduced the peak of the transient outward current (I_{to}) and induced a concentration-dependent acceleration of current inactivation (IC_{50} for reduction of $\int I_{to}dt$ = 20μM). (±)Bup had no effect on the voltage dependence of steady-state inactivation nor the rate of recovery from inactivation. (+)Bup and (-)Bup were equipotent, demonstrating no stereo-selectivity to the block. Increasing the hydrophobicity of the 3º amine on Bup greatly enhanced its potency. Thus octylacaine (Oct, C_8-N) was ~10 times more potent than Bup (C_4-N) and >100 times more potent than mepivacaine (C_1-N). In contrast to their effects on I_{to} neither Bup (300 μM) nor Oct (30 μM) produced any block of the inward rectifier (I_{K1}).

Supported by AHA, Mass.Aff.

M-Pos404

INTRACELLULAR Na^+ ACTIVITY IN PACEMAKER POTENTIAL OF RABBIT SA NODE.

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Recent electrophysiological data have provided the evidences that background currents such as Na^+-Ca^{2+} exchange can modulate significantly cardiac pacemaker activity. In this study, the effects of extracellular Na^+ and Ca^{2+} concentrations on the pacemaker activity were investigated by measuring the intracellular Na^+ activity (a^i_{Na}) with Na^+-selective microelectrode and the results are summarized as follows :

1) In rabbit SA node, a^i_{Na} was 3.2±0.3 mM and mean MDP was -63.3±1.4 mV. 2) A graded decrease of external Na^+ concentration resulted in loss of spontaneous beating, hyperpolarization, and decrease in a^i_{Na}. 3) An increase in extracellular Ca^{2+} concentration in low Na^+ solution augmented the transient decrease of a^i_{Na}, about 3 minutes in low Na^+ solution, until a^i_{Na} started to increase. 4) In low Na^+ solution, which had extracellular Ca^{2+} concentration according to the estimation from the equilibrium state of Na^+-Ca^{2+} exchange, a^i_{Na} was continuously decreased. It was concluded that intracellular Na^+ activity modulated by Na^+-Ca^{2+} exchange could play an important role in the initiation of pacemaker potential.

M-Pos405

COMPUTER MODEL OF SARCOLEMMAL CURRENT AND INTRACELLULAR Ca2+ FLUX IN THE ISOLATED GUINEA PIG MYOCYTE.

Charles Nordin, Albert Einstein College of Medicine, Bronx NY

Since interactions between myocytes may be crucial to the genesis of arrhythmias, a model structurally similar to that of Noble-DiFransesco was constructed for a single myocyte that incorporates more than 50 quantitative parameters and responses determined experimentally. The model was used to examine the generation of early and delayed afterdepolarizations (EAD and DAD). The model shows: 1) apparent voltage sensitive sarcoplasmic reticulum (SR) Ca2+ release and delayed rectifier currents may be due to interactions between Na-Ca flux, Ca2+ induced Ca2+ release and Ca2+ sensitive cation conductance; 2) simple interventions such as raising myoplasmic [Na+] or constant depolarizing current cause oscillatory instability; 3) Na-Ca current is sufficient to cause DAD that generate action potentials if myoplasmic [Ca2+] rises to 1-2 uM (depending on myoplasmic [Na+]); 4) EAD upstrokes are always preceded by rising myoplasmic [Ca2+]: Ca2+ current reactivation is insufficient to cause EAD in normal cells. The model should be useful for examining interactions between cells in heterogeneous syncytia where arrhythmias originate.

M-Pos406

ALPHA$_1$-ADRENERGIC AND MUSCARINIC RECEPTOR STIMULATION DECREASE THE ACTION POTENTIAL DURATION IN GUINEA PIG VENTRICULAR MUSCLE.

R. T. Dirksen, J. Arreola, and S-S. Sheu, Dept. Pharmacology, Univ. Rochester, Rochester, NY 14642.

Alpha$_1$-adrenergic and muscarinic cholinergic agonists have both been demonstrated to increase phosphoinositide turnover and twitch contractions in mammalian ventricular muscle. The effects of the alpha$_1$-adrenergic agonist, methoxamine (MTX), muscarinic receptor agonist, carbachol (CA), and the direct protein kinase C (PKC) activator, phorbol 12,13-dibutyrate (PDBU) on electrically elicited (1 Hz) action potentials from guinea pig papillary muscles were studied using conventional microelectrodes. Each agent produced a dose-dependent and reversible decrease in the action potential duration measured at 90% repolarization (APD_{90}) (MTX: EC_{50}=6.2 µM, R_{max}=8.5 +/- .6%, n=6; CA: EC_{50}=10 µM, R_{max}=4.9 +/- 1.2%, n=5; PDBU: EC_{50}=57 nM, R_{max}=16.3 +/- .8%, n=7). The MTX and CA responses were reversed by prazosin (3 µM) and atropine (1 µM), respectively, but not by 1 µM propranolol. Furthermore, MTX and CA produced an additive effect subsequent to a maximal stimulation with PDBU. In addition, neither agonist-induced decrease in APD_{90} was completely reversed by prior incubation with the PKC inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (50-100 µM). Neither receptor agonist altered the L-type calcium current in whole cell patch clamp experiments. These results suggest that the decrease in the APD_{90} induced by MTX and CA may be through activation of an outward potassium current and may not be mediated solely through PKC.

M-Pos407

INTRACELLULAR Ca^{2+} OSCILLATIONS AS A MECHANISM OF DELAYED AFTERDEPOLARIZATIONS IN GUINEA PIG VENTRICULAR MYOCYTES.

Ru-Chi Shieh and Daniel Williford (Intro. by Jorge Arreolla), Dept. of Pharmacology, Univ. of Rochester, Rochester, NY.

We investigated the mechanism(s) responsible for delayed afterdepolarizations (DADs) in single guinea pig ventricular myocytes. Rapid intracellular Ca^{2+} transients measured with fura 2 and action potentials (APs) were recorded at 23°C from cells stimulated by brief current injection. Stimulation of beta-adrenoceptors with isoproterenol (100-300 nM) resulted in a rise in resting $[Ca^{2+}]_i$ (from 50 to 190 nM) and a marked increase in the amplitude of the transients. This was associated with an increased oscillation of $[Ca^{2+}]_i$ which was present both during the rested and depolarized states, and led to the development of spontaneous "after-transients". Subsequently, the "after-transients" increased in amplitude and there developed associated membrane depolarizations which occurred after full repolarization of the stimulated AP. On some occasions the afterdepolarization reached threshold for initiating a spontaneous AP. Similar results were obtained with the phosphodiesterase inhibitor, milrinone (300 μM). These results suggest that elevation of cAMP results in oscillations in $[Ca^{2+}]_i$ from an intracellular source which may be causally related to the development of DADs. The mechanism of the Ca^{2+} oscillations is currently under study.

M-Pos408

FREQUENCY DEPENDENCE OF THE I_f PACEMAKER CURRENT BLOCKADE. P.P. van Bogaert, (Dept. of Physiology, University of Antwerp RUCA, B-2020 Antwerp, Belgium)

The maximal diastolic depolarization rate (DDRmax) of short cardiac Purkinje fibres stimulated at 0.8, 0.2 and 0.01 Hz declined as a function of UL-FS 49 concentration with an apparent K_d of 8.8×10^{-8}, 2×10^{-7} and 1.01×10^{-6} M. The fully-activated i_f amplitude, measured in voltage-clamp conditions, was reduced to 50% by 4.5×10^{-7} M in preparations pulsed at a low rate (0.04Hz). With a faster pulsing rate (0.8Hz) the 50% reduction of i_f amplitude was obtained with 8×10^{-8} M. The plot of the logarithm of these apparent K_d's against the log of the interstimulus interval is fitted by the expression: Log K_d =0.526*Log ISI-0.1439 with r=0.99. When the blockade by 4×10^{-7} M UL-FS 49 of the i_f current was measured during pulse trains between -30 and -120mV with variable duration, t_τ, of the step to -120mV, a reduction of steady-state block was observed with prolongation of tr. The uptake rate, λ, increased linearly with the duration of t_τ (C.F. Starmer, J. Theor. Biol. 1986, 119: 235-249). The frequency dependent block of i_f as well as the reduction of DDRmax is a consequence of the use dependent block by this drug.

Support: N.F.W.O., Born-Bunge Foundation

M-Pos409

Effect of lytic granules from cytotoxic T lymphocytes (CTL) on guinea pig ventricular myocytes. O Binah, I Rubinstein, S Marom, G Berke *, BF Hoffman **. Rappaport Institute, Technion, Haifa, Israel. * Department of Cell Biology * Wiezmann Inst. Rehovot Israel. ** Department of Pharmacology, Columbia University, NY.

Since CTL contain lytic granules (LG) we tested whether their action on myocytes can be related to overall immunological rejection. Within 5-15 min after exposure to LG, myocytes lose their rod-shape; this contructure is followed by cell death. Prior to morphological changes, action potential (AP) duration (APD) shortens markedly. This is followed by a decrease in AP amplitude (APA) and resting potential (RP) and oscillations in RP. Under control conditions and after 3-6 min exposure to LG, RP, decreased from -80.5±0.6 to -73.0±2.0 mV; APA from 121.4±1.4 to 102.0±6.2 mV; and APD50 from 639.4±53.4 to 195.2±51.2 msec. The decrease in APD is caused by a marked increase in steady state outward current. We then studied the role of $[Ca]_i$ and $[Ca]_o$ in relation to effects of granules: 1.The effects persist in the presence of ryanodine. 2.The granules attenuate $I_{Ca,L}$. 3.In $[Ca]_o$=0, no effect is induced. 4.$CoCl_2$ blocks effects of granules but verapamil does not. We suggest that Ca overload associated with cell destruction is preceded by a Ca influx via a pathway, that differs from the L,Ca channel. These actions of LG may contribute to transplant rejection.

M-Pos410

PACEMAKER CURRENT (I_f) IN EMBRYONIC CHICK VENTRICULAR HEART CELLS R.M. Brochu+, J.R. Clay*, and A. Shrier+. +Dept. of Physiol., McGill, Montreal; and *NIH, Bethesda, MD (Intro. by D.E. Goldman).

We have investigated the pacemaker current in single cells from the ventricles of 5-12 day-old embryonic chick hearts (patch-clamp), and in reaggregates of these cells (two-microelectrode clamp). We had previously attributed a time-dependent current in the -90 to -60 mV range from aggregates to I_{K_2} (Shrier and Clay, Nature, 283:670, 1980). We obtained similar results in this study, although we more systematically investigated currents for V < -100 mV. These results show a biphasic time dependence near E_K (-105 to -95 mV; K_o = 4 mM) and a decaying, outwardly directed time dependence for V < -105 mV, which was blocked by Ba^{+2} (1-5 mM). Results from single cells were similar, except that the inwardly directed component (I_f) was activated in the -120 to -90 mV range rather than -90 to -60 mV. A biphasic response was observed in the -140 to -120 mV range. The outwardly directed component of this response (I_{K_1}inactivation) was blocked by Ba^{+2}. We have concluded from these results that our earlier interpretation (the "I_{K_2} reversal") was due to a mixture of I_f and I_{K_1} kinetics rather than to accumulation-depletion in the extracellular spaces of the aggregate preparation (Difrancesco and Noble, Tran.R. Soc.,307:353, 1985).

M-Pos411

EFFECT OF pH_i ON RESTING TENSION IN CAT VENTRICULAR MUSCLE.

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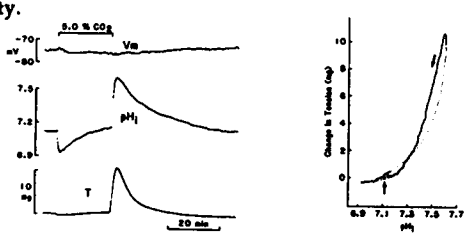
We studied the relationship between pH_i and resting isometric tension (T) in quiescent cat papillary muscle using recessed-tip pH microelectrodes ($t_{90\%} \cong$ 20 sec). A switch from HCO_3/CO_2-free solution (pH_o 7.4, 2.7mM Ca, 24mM Hepes) to solution containing 18.0mM HCO_3/5.0% CO_2 (pH_o 7.4, 2.7mM Ca) elicited a transient decrease in both pH_i and T (Fig. left panel). Return to HCO_3/CO_2-free solution produced opposite effects. The relationship between pH_i and T was nonlinear and displayed modest hysteresis (right panel;↑=application of 5% CO_2; ↓=secondary decline in pH_i following reexposure to HCO_3/CO_2-free solution). A similar nonlinearity and hysteresis occurred in resting muscles exposed to 10mM NH_4Cl (pH_o 7.4). The magnitude of the alkaline contracture elicited by switching from HCO_3/CO_2-buffered to HCO_3/CO_2-free solution was not affected by 5μM ryanodine and it was only slightly reduced (12%) by equilibration in 0.5mM Ca_o^{2+}. Lowering Ca_o^{2+} to 0.5mM did not affect pH_i. Acidosis elicited by application of 5% CO_2 (Fig.) and NH_4Cl (pH_o 7.4) caused a small increase in both Ca_i^{2+} (Indo-1 fluorescence) and cell length of resting ventricular myocytes (cat, guinea pig). In contrast, alkalosis elicited by removal of 5% CO_2 (Fig.) and application of NH_4Cl caused a small decrease in Ca_i^{2+} and a large decrease in cell length. The pH_i-resting T relation appears to be mainly determined by pH_i induced changes in myofilament Ca^{2+} sensitivity.



M-Pos412

A NOVEL VOLTAGE AND TIME-DEPENDENT OUTWARD CURRENT FOUND IN CAT VENTRICULAR MYOCYTES

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In adult cat ventricular myocytes a novel outward current ($I_?$) was elicited using whole-cell voltage clamp method to apply 200 ms conditioning steps (V_c) to -140 mV, followed by 100 ms test steps (V_t) (range -60 to +60 mV), holding at -40 mV. $I_?$ was detected only after ≥ 25 min had elapsed following patching (complete dialysis obtains in < 10 min) with an internal solution containing 30 mM Na^+ and either 5 or 20 mM EGTA. $I_?$ is voltage dependent with an activation threshold ≤ -60 mV. $I_?$ exhibits voltage dependent inactivation-like properties with full availability at $V_c \leq$ -90 mV. The decay phase of the current appears to follow a double exponential time course. $I_?$ can be blocked by 0.2 mM Ba^{2+} but not affected by 0.03 mM TTX. $I_?$ can be partially inhibited by 1 mM 4-aminopyridine (4-AP) and is less sensitive to 10 mM caffeine and 1 mM Cd^{2+}. Prior exposure to dihydroouabain seems to facilitate $I_?$. These findings suggest $I_?$ is not an usual component of I_m such as I_{Na}, I_{Ca}, I_{to}, I_{K1} or I_K because it developed over time in cells with elevated Na^+_i, was not fully blocked by 1 mM 4-AP or caffeine, was completely blocked by 0.2 mM Ba^{2+} and occurred in the presence of 20 mM $EGTA_i$. The ionic character of $I_?$ and its possible physiological significance are as yet undetermined.

M-Pos413

CULTURE ALTERS MORPHOLOGY, BIOCHEMISTRY AND ELECTROPHYSIOLOGY OF ADULT CAT CARDIOCYTES

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Adult cat cardiac ventricular myocytes (VM) were obtained from collagenase-perfused hearts and maintained in culture for 10-14 days either on a two-dimensional surface (2DVM) or in a three-dimensional alginate matrix (3DVM). In culture, 2DVM exhibited cell spreading while 3DVM retained a rod shape similar to freshly isolated VM (FVM). Total protein fractional synthesis rates in 2DVM and 3DVM were low and resembled FVM. Myofibrillar organization appears maintained in 3DVM but not in non-beating 2DVM. Action potentials (AP's) and whole-cell ionic currents were obtained using a conventional whole-cell-patch voltage clamp technique. Quiescent 3DVM elicit AP's that resemble those normally seen in FVM, while 2DVM that are stimulated to beat in culture elicit spontaneous AP's which exhibit both early and delayed afterdepolarizations. I_{K1} in 2DVM and 3DVM was unchanged from that in FVM, but I_{to} found in FVM could not be elicited by depolarization in either 2DVM or 3DVM when holding at -40 mV. These data suggest that cell morphology and cell function may be strongly interdependent. TE Schackow is a Howard Hughes Medical Institute Doctoral Fellow.

M-Pos414

β-ADRENERGIC MODULATION OF SODIUM CURRENTS FROM RABBIT CARDIAC MYOCYTES.

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The effect of isoproterenol on cardiac sodium currents (I_{Na}) was studied in enzymatically dispersed atrial and ventricular myocytes from the rabbit. Using the single pipette patch-clamp technique and low external sodium concentrations (35 mM) we were able to reliably record voltage-clamped sodium currents.

Isoproterenol (1 μM) enhanced I_{Na} at test potentials on the negative limb of the current-voltage relationship but had no effect at more positive potentials. This effect was holding potential dependent such that larger increases were observed at hyperpolarized holding potentials and no effect was observed at holding potentials positive to -70 mV. The isoproterenol-induced increase of I_{Na} could be blocked by propanolol (1 μM) and mimicked by forskolin (1 μM) and dibutyryl cAMP (1 μM) in both ventricular and atrial myocytes. These results suggest that β-adrenergic agonists can modulate cardiac sodium currents and that cAMP can mediate these effects.

Supported by NIH HL 41031 and HL 14388.

M-Pos415

VOLTAGE AND Ca DEPENDENCE OF i_{Ca} INACTIVATION IN PURKINJE MYOCYTES.

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We have examined inactivation of i_{Ca} in acutely dissociated Purkinje myocytes using the whole cell patch clamp technique with a switched voltage clamp. Studies were conducted at room temperature in Tyrode with choline chloride substituted for 75% of the NaCl. 4-AP (0.5mM) was added to block the transient outward current. Patch pipettes contained in mM CsCl 105, KCl 35, EGTA 5, $MgCl_2$ 4, glucose 10, HEPES 10, ATP 2 and CP 3.

Examination of inactivation of i_{Ca} using a 2 pulse protocol (250 ms pre-pulse to V_{pre}, 10ms delay followed by a 250ms test pulse, V_{test} to +10mV, V_{hold}=-50mV) led to a U-shaped relationship between i_{Ca} and V_{pre} indicating that i_{Ca} inactivation may be Ca-dependent. An alternative explanation is that i_{Ca} inactivation is voltage dependent and that the time constant for inactivation becomes slower with depolarization. Experiments to examine this question suggest that at depolarized potentials i_{Ca} inactivation has a rapid initial component (half-decay~50-200msec) followed by a slowly decaying tail (half-decay~0.5-1sec). Additional evidence favouring voltage dependence is the rapid recovery of i_{Ca} from inactivation when a brief (20ms) hyperpolarizing pulse to -120mV is inserted between V_{pre} and V_{test}.

Rapid recovery from inactivation with a brief hyperpolarizing pulse in conjunction with slow inactivation of i_{Ca} at depolarized potentials suggests that in the Purkinje myocyte Ca-mediated inactivation is either not present or that the binding site for Ca-mediated inactivation is steeply voltage dependent.

M-Pos417

PERMEATION OF CS, NA, NH_4 AND RB THROUGH THE DELAYED RECTIFIER IN GUINEA-PIG VENTRICULAR MYOCYTES.

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The permeability of the slow, time-dependent K channel to various monovalent cations was studied with the whole-cell patch clamp in guinea-pig ventricular myocytes. Current through these K channels was isolated kinetically, and by ionic substitution. The analysis was restricted to tail currents activated by a 5 s pulse to +50 mV. It was found that replacing external K with different cations shifted the tail current reversal potential. These shifts were used to determine their relative permeabilities, which were K = Rb > NH_4 = Cs > Na. Cs permeation through these K channels was further investigated by dialyzing the cell with 150 mM Cs. Under these conditions a sizeable time-dependent outward current was seen, similar to that observed with K in the pipette. The reversal potential of this current shifted 46 mV per ten-fold change of $[Cs]_o$. This indicates that there can be significant efflux of Cs through these channels, which needs to be considered when using Cs to isolate other membrane currents.

M-Pos416

Effects of the Protein Kinase Inhibitor H-7 on Diastolic Depolarization and the Pacemaker Current i_f in Canine Cardiac Purkinje Fibers

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The effects of the protein kinase inhibitor H-7 were investigated on the action potential and on the pacemaker current(i_f) of canine cardiac Purkinje fibers. 500μM H-7 can depress the slope of diastolic depolarization (phase 4 of the action potential); and 200μM-500μM H-7 significantly inhibits the effects of the β-agonist isoproterenol (ISO 1μM) on phase 4. In the presence of 4 mM Ba^{2+}, pacemaker current (i_f) was activated by applying hyperpolarizing pulses to the Purkinje fibers. H-7, 100μM-200μM, decreases the amplitude of i_f. A three pulse protocol was employed to demonstrate that this H-7 effect on i_f is due to a negative shift of the i_f activation curve on the voltage axis. ISO (1-5μM) can increase the amplitude of i_f. However, in the presence of 200μM H-7, ISO has no effect. The pacemaker current contributes to diastolic depolarization of the action potential and is vital for cardiac automaticity. Since the kinase inhibitor H-7 reduces i_f and eliminates the effects of ISO (which is known to work through cAMP), these results suggest that the pacemaker current of Purkinje fibers is probably modulated by the cAMP dependent kinase through a protein phosphorylation step.

M-Pos418

EVIDENCE THAT CROMAKALIM AND PINACIDIL ACTIVATE ATP SENSITIVE POTASSIUM CURRENT IN ISOLATED GUINEA PIG VENTRICULAR MYOCYTES.

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Cromakalim (BRL) and pinacidil (PIN) are vasodilators which apparently open potassium channels in vascular smooth muscle. Recent evidence suggests that they activate ATP sensitive K^+ channels in cardiac preparations. We reported that their ability to shorten action potential duration in guinea pig (GP) papillary muscle could be completely reversed by 0.1-10 μM glyburide (GLY), a specific blocker of ATP regulated channels (McCullough and Conder, Soc. Neurosci. Abstr., 14:945, 1988). The effects of external BRL (100 μM), PIN (100 μM), GLY (0.1-10 μM) and intracellular ATP (3-10 mM) on outward potassium current (IK) were studied in GP ventricular myocytes using whole cell patch-clamp techniques. Freshly dissociated cells were internally dialysed with ATP and IK was measured using voltage ramps of 6-8 mV/sec applied from a holding potential of -50 mV. With 3 mM ATP, both BRL and PIN increased IK as previously reported. Addition of GLY to the external solution rapidly reversed the increase in IK, suggesting that the measured IK was responsible for the effects of these compounds on action potential duration. When separate cells were dialysed with various ATP concentrations, the increase in IK caused by BRL and PIN was decreased with increased internal ATP. The relative IK increase measured at +20 mV for BRL was: 10 with 3 mM ATP, 4 with 6 mM ATP and 0 with 10 mM ATP. Similar results were obtained with PIN. These results provide direct evidence that BRL and PIN open ATP sensitive K^+ channels in cardiac cells and suggest competition between these compounds and internal ATP at this site.

M-Pos419

CELLULAR MECHANISM OF CARDIAC TISSUE REFRACTORINESS FOR PREMATURE EXCITATION

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Our purpose was to understand the relative influence of cellular properties on post-repolarization refractoriness (PRR) by comparing the time course of recovery of excitability and responsiveness during premature excitation (S2) in an isolated rabbit ventricular cell. We defined cellular excitability as being inversely proportional to the current threshold (I_{th}). Cellular excitability showed no increase in I_{th} during late phase 3 repolarization or phase 4 diastole but rather a period of supernormal excitability. We defined cellular responsiveness as the maximum upstroke velocity during phase 0 (V_{max}) of a premature action potential. Measurements of V_{max} demonstrated a monophasic time course of recovery with a time constant (tau) equal to 4.1 mSec. The time course of recovery of V_{max} closely paralleled our measurements of tissue excitability in the papillary muscle. Elevated $[K^+]_o$ (8 mM) produced a PRR with a I_{th} for premature excitation approximately 10% above the diastolic threshold and increased the tau for V_{max} by four-fold (25.5 mSec). These results show that the decreases in tissue excitability during phase 3 repolarization are not consistent with the hypothesis that there is a corresponding decrease in excitability at the cellular level. They do suggest that it is a decreased ability of each cell within the tissue to generate a sufficient net inward current for action potential initiation.

M-Pos420

SPECIFIC BLOCK OF A COMPONENT OF DELAYED RECTIFIER K^+ CURRENT BY A BENZENESULFONAMIDE, E-4031, IN GUINEA PIG VENTRICULAR MYOCYTES.

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E-4031 (N-(4-((1-(2-(6-methyl-2-pyridinyl) ethyl)-4-piperidinyl)carbonyl)-phenyl)-methanesulfonamide, "E"), a new Class III antiarrhythmic agent lengthens cardiac action potentials by blocking a small component (I_{Kr}) of total outward K^+ current that is distinctly different than the larger, classical delayed rectifier K^+ current (I_{Ks}). Like I_{Ks}, the magnitude of I_{Kr} was dependent on temperature (Q_{10}=2.4). However, unlike I_{Ks}, I_{Kr} was not increased by isoproterenol (1 μM, N=9). I_{Kr} was blocked in a conc-dependent manner by E (IC_{50} = 490 nM), with complete block at 5 μM. At 5 μM, E had no effect on I_{Ks}, inward rectifier K^+ current (N=10), or I_{Ca} (N=11). Lack of effect on I_{Ks} by E was confirmed using bullfrog atrial cells. Thus, in guinea pig ventricular cells there are two delayed rectifier K^+ currents, each contributing significantly to phase 3 repolarization of the action potential.

M-Pos421

TWO TYPES OF DELAYED RECTIFIER K^+ CURRENTS IN GUINEA PIG VENTRICULAR MYOCYTES.

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To determine if delayed rectifier K^+ current (I_{KDR}) in guinea pig myocytes results from activation of a single current, an envelope of tails test was performed. Pulses were varied from 10 ms to 5 s. Holding potential was -40 mV; test potential +40 mV. In 10 cells the avg ratio of tail current to time-dependent I_{KDR} ($\Delta I_{tail}/\Delta I_{KDR}$) was > 1 for pulse durations < 50 ms, and decreased as duration was lengthened; 0.43 for 5 s pulses. Exposure of cells to the antiarrhythmic agent, E-4031 (5 μM), had no effect on the magnitude of I_{KDR} at +40 mV, but decreased I_{tail} and the $\Delta I_{tail}/\Delta I_{KDR}$ ratio became constant (0.38; N=14; predicted=0.4), independent of pulse duration. At < +40 mV, E-4031 blocked a rapidly activating component (I_{Kr}) of time-dependent I_{KDR}, leaving a slowly activating K^+ current (I_{Ks}). I_{Kr} activated at approx -40 mV, peaked at 0 mV (0.8 pA/pF), had a negative slope conductance at > 0 mV. Half-activation (V 1/2) of I_{Kr} occurred at -21.5 mV; slope factor (SF) = 7.5. I_{Ks} activated at -10 mV, with V 1/2 = 0 mV; SF = 12.2.

M-Pos422

INFLUENCE OF ANOXIA ON K^+ AND CA^{++} CURRENTS IN ISOLATED CARDIOCYTES OF GUINEA PIG

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Whole cell currents in single cardiocytes of guinea pig were measured under anoxia (pO_2<0.5 torr). After variable periods of delay (2-32 min), time independent outward currents appeared which saturated after 20 to 70 seconds in the range of 4 nA at 0 mV. The net current voltage relationship between -100 and +20 mV intersected the zero current level at the resting potential of the cells (-82 to -90 mV). Within the first minute after the first extra outward current had appeared, reoxygenation led in more than 90% of the cells to a complete disappearance of the outward current after two seconds. Ca currents were not affected at the time of the appearance of extra outward currents.

Conclusion: Anoxia-induced outward current is mainly carried by K^+ ions through K_{ATP} channels which open at ATP concentrations below 1 mM (Noma and Shibasaki, J. Physiol. 363, 463-480 (1985)). Such a decrease in ATP concentration may appear at the time of sufficient glycolytic impairment due to an adenine nucleotide disturbance. The normal Ca channel function is not affected by this degree of ATP depletion.

M-Pos423

EFFECT OF ISOPROTERENOL AND Ca^{2+} ON Na-K PUMP CURRENT IN ISOLATED CARDIAC MYOCYTES.

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The mechanisms underlying catecholamine-induced Na-K pump stimulation in heart have been investigated by studying the effect of isoproterenol (Iso) on the Na-K pump current in rabbit ventricular myocytes. Cells were superfused at 37 °C in a fast-flow chamber with Hepes-buffered solutions containing 0.5 mM Ba^{2+}. Na-K pump activity was determined by measuring the K^+-induced transient outward current following 3-6 min incubation in zero $[K^+]$. The decaying component of these strophanthidin-sensitive transients could be fitted by a double exponential, with time constants, τ, of 7.0 ± 0.6 and 56 ± 6 s (mean ± SEM, n=11). In presence of 2 mM Ca^{2+}, Iso markedly increased the rate of relaxation of the slow component (τ= 31 ± 2 s) as well as the amplitude of the transients (by a factor of 1.33 ± 0.15). In absence of Ca^{2+} (replaced with Cd^{2+} and Mg^{2+}), Iso caused a similar increase of current amplitude but failed to affect significantly the time constants. The results suggest that: 1) Ca^{2+} is required for Na-K pump stimulation by β-agonists, and 2) Iso can enhance Na^+ influx during pump inhibition.

M-Pos424

REGULATION OF SCALLOP MYOSIN BY MUTANT LIGHT CHAINS? E.B. Goodwin*, L.A. Leinwand# and A.G. Szent-Gyorgyi*. *Brandeis Univ., Biology, Waltham, MA 02254. #Albert Einstein College of Medicine, Microbiology & Immunology, Bronx, NY 10461.

Scallop adductor myosin is regulated by the direct binding of Ca^{2+} to the myosin molecule. To investigate what regions of the R-LC are involved in heavy chain binding, Ca^{2+} binding the Ca^{2+} sensitivity, mutations of the scallop R-LC were made and analyzed for altered functions. The R-LCs were expressed in E. coli using the pkk223-3 (Pharmacia) expression vector. The expressed wild type R-LC restored Ca^{2+} binding and Ca^{2+} sensitivity. A point mutation in domain I (Asp_{39}-Ala) resulted in a R-LC which bound weaker to the heavy chain, restored the regulatory Ca^{2+} binding site but not Ca^{2+} sensitivity of the Mg ATPase. A mutation which altered the last 11 residues of the COO-terminus restored the Ca^{2+} regulatory site but not Ca^{2+} sensitivity. Several other point mutations did not alter light chain function. The results indicate that an intact domain I and the COO-terminus are required for Ca^{2+} sensitivity but not for Ca^{2+} binding.

M-Pos425

PRIMARY STRUCTURE OF MYOSIN HEAVY CHAINS FROM SCALLOP MUSCLES. L. Nyitray*, E. Goodwin*, L. Leinwand+ and A.G. Szent-Gyorgyi*. *Brandeis Univ., Dept. of Biology, Waltham, MA 02254. +Einstein College of Medicine, Bronx, NY 10461 (Introduced by C. Cohen).

Myosin heavy chain (MHC) clones, isolated from adductor and catch muscle cDNA libraries encoding 80% of the MHC sequence (residues 353-2030) and the entire 3' untranslated region, were sequenced. The two MHC sequences are identical in a 550 residue overlap (1080-1636). In general the scallop sequence has a similar overall structure as other MHCs. The primary sequence includes the region involved in the heavy chain portion of the Ca^{2+}-binding regulatory fragment (see Kwon et al., these abstracts). The MHC fragment starts from L-812 or V-817 as determined by protein sequencing. It shows no apparent EF-hand structure which can contribute directly to the specific Ca^{2+}-binding site. This segment has 67%, 55%, 54% and 51% homologies with Drosophila, Nematode, chicken smooth and rat skeletal muscle myosins, respectively.

Supported by NIH AR 15963 and MDA.

M-Pos426

THE TROPONIN C CENTRAL HELIX MODULATES REGULATION Z.Dobrowolski & S.E. Hitchcock-DeGregori (Intro. by S.Malamed) UMDNJ-Robert Wood Johnson Med. Sch. Piscataway, NJ. 08854

A central helix connecting the two Ca^{2+} binding domains is conserved in calmodulin and TnC. In avian TnC the sequence of the linker is $_{87}$KEDAKGKSEEE$_{97}$. To learn its function we made a series of mutants changing its length (Dobrowolski et al. 1989. Biophys.J. 55, 274a). The mutant proteins, produced in E. coli, were similar to wildtype in certain functions: Ca^{2+} binding to low and high affinity sites and affinity for TnI. However, some mutants differed in regulation of the acto-S1 and acto-myosin ATPase. Two deletion mutants, dKGK and dKEDAKGK, partially released inhibition by TM-TnI-TnT in the absence of Ca^{2+}, i.e. they were defective in "turning off" the thin filament. In contrast, dKG and dEDA were less effective than wildtype in releasing inhibition by TM-TnI-TnT in the presence of Ca^{2+}, i.e. in "turning on" the thin filament. Deletion or duplication of SEEE did not affect regulatory function. We suggest that changes in central helix can affect transmission of structural changes in TnC to other thin filament components. See also Z. Sheng et al., this meeting. Supported by NIH-GM36326, MDA, AHA-NJ Affiliate.

M-Pos427

EFFECTS OF MUTATIONS IN THE CENTRAL HELIX OF TROPONIN C ON ITS BIOLOGICAL ACTIVITY. Z. Sheng,[+] J-M. Francois,[+] S.E. Hitchcock-DeGregori,[*] and J.D. Potter[+]. [+]U. of Miami Med. Sch., Miami, FL 33101, [*]UMDNJ-R. Wood Johnson Med. Sch., Piscataway, NJ 08854.

Five mutants[*] of the central helix of chicken troponin C (CTnC), dEDA, dKG, dKGK, dSEEE, (deletion of residues 88-90, 91-92, 91-93, 93-97, respectively) and In+7 (insertion of EELAKSE at 95) were tested for their effects on TnC depleted rabbit skeletal muscle skinned fibers. By comparison with rabbit TnC (RTnC), wild-type TnC (WTnC), CTnC and all mutants except dKG equally restored force development and Ca^{2+} sensitivity. In contrast, ~4 times more dKG than RTnC was required to reach 50% force restoration (4.7 μM for dKG vs 1.1, 1.6, and 1.8 μM for RTnC, WTnC and dKGK, respectively). Also, the pCa_{50} for dKG activation of force was decreased (pCa_{50} for dKG = 5.34 vs 5.6 for RTnC, WTnC and dKGK). Thus most of the TnC mutants that we studied have not significantly altered biological activity. However, the 2 residue deletion in the central helix (dKG) significantly affected TnC activity. This deletion causes a 160° rotation in the α helix vs 60° for dKGK and dEDA, 40° in dSEEE and 20° in In+7. Thus, the change in orientation of the 2 domains may be a major parameter affecting TnC activity. Gly 92 does not appear crucial for TnC activity in this assay. Supported by the MDA and NIH. [*](Also see abs. by Dobrowolski & Hitchcock)

M-Pos428

PURIFICATION OF MYOSIN LIGHT CHAIN PHOSPHATASE FROM SMOOTH MUSCLE ACTOMYOSIN.

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Smooth muscle actomyosin ATPase activity is regulated by the phosphorylation of 20kDa myosin light chain. Although the kinase responsible for the phosphorylation is identified as myosin light chain kinase, the phosphatase which is responsible for the dephosphorylation in vivo is not identified yet. We have attempted to purify myosin light chain phosphatase from smooth muscle actomyosin fraction. The soluble phosphatases were removed by washing actomyosin exhaustively and the phosphatase which tightly bound to actomyosin was extracted by high $MgCl_2$. This was specific to $MgCl_2$ and high KCl did not extarct the phosphatase. The phosphatase was further purified using DEAE-Sephacel and thiophosphorylated light chain affinity column, and the properties of the phosphatase was studied.

(Supported by NIH, Syntex and AHA)

M-Pos429

PHOTOMODIFICATION OF SMOOTH MUSCLE MYOSIN LIGHT CHAIN KINASE WITH VANADATE

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The enzyme activity of myosin light chain kinase (MLCK), a key regulatory enzyme of smooth muscle contraction, is regulated by Ca^{2+}/calmodulin. Recently the location of the inhibitory region and calmodulin binding region of MLCK was identified, however, the interaction between the catalytic site and the regulatory site of this enzyme is still obscure. We employed the vanadate induced photooxidation to approach this problem. Vanadate (Vi) is known as a analogue of phosphate. Recently Yount et al [Biochemistry 1988,27, 8408-8415] reported that UV irradiation to the myosin,ADP,Vi complex generate the specific cleavage at Ser residue due to the photooxidation of Ser residue. When MLCK was irradiated with long wave UV in the presence of ADP and Vi, MLCK (130kDa) was cleaved into dominantly 100kDa and 30kDa peptides. This cleavage was significantly inhibited in the presence of ATP. Monoclonal antibody which recognizes 25kDa C terminus tryptic fragment did not bind to 100kDa fragment. This suggests that the cleavage site is 30kDa from C-terminus. Since putative ATP binding site of MLCK is thought to be in the middle of the molecule, our results may indicate that ATP binding site is close to regulatory site in the three dimensional structure. (supported by NIH,syntex and AHA)

M-Pos430

PURIFICATION AND CHARACTERIZATION OF CALMODULIN DEPENDENT PROTEIN KINASE (CALDESMON KINASE) FROM SMOOTH MUSCLE.

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It was shown previously by Walsh and co-workers that Ca^{2+}/calmodulin dependent protein kinase activity co-purified with smooth muscle caldesmon. It was also shown that the phosphorylation of caldesmon by endogenous kinase reverses the inhibitory activity of caldesmon towards actomyosin ATPase. However, it remains obscure whether or not the kinase activity is derived from caldesmon or a contaminating kinase. We succeeded in separating calmodulin dependent kinase from caldesmon by using high $MgCl_2$ buffer. The kinase was further purified using calmodulin affinity column and CM 5 PW column. The purified kinase had a similar molecular weight as brain calmodulin dependent multifunctional kinase and was autophosphorylated. Other properties of the kinase were characterized. (Supported by NIH, Syntex, and AHA).

M-Pos431

PHOSPHORYLATION OF SMOOTH MUSCLE MYOSIN LIGHT CHAIN KINASE BY SMOOTH MUSCLE CALMODULIN DEPENDENT MULTI-FUNCTIONAL KINASE.

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Myosin light chain kinase (MLCK) is a Ca^{2+}/calmodulin dependent enzyme which serves as a key regulatory protein for smooth muscle contraction. It has been reported that cAMP dependent kinase and protein kinase C can phosphorylate MLCK and the phosphorylation results in the decrease in the affinity to calmodulin. In this study we found that MLCK was phosphorylated by smooth muscle calmodulin dependent multi-functional kinase. Maximum extent of phosphorylation obtained was 1.5 mol phosphate/mol and the phosphorylation sites were determined by isolating the phospho-peptides. The major phospho-peptide was identified as L-S-S*-M-A-M-I-S-G-M-S-G-R (Sequence 510-522) and Ser 512 was phosphorylated. The minor phospho-peptide was A-S*-G-S-S-P-T-S-P-I-N-A-D-K (sequence 524-537) and Ser 525 was phosphorylated. (Supported by NIH, Syntex, and AHA).

M-Pos432

INHIBITION OF FORCE DEVELOPMENT BY TRIFLUOPERAZINE (TFP) ON SKINNED SMOOTH MUSCLE AND SKELETAL AND CARDIAC MUSCLES

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TFP is a known inhibitor of calmodulin, and acts by binding to two hydrophobic domains on the central helix. We have previously shown that calmodulin can substitute for troponin C in skeletal fast-twitch fiber for the regulation of force development. Presently, we further study the mechanism of the inhibition by TFP on calmodulin-loaded fiber. The dose-response curve was measured. Force development was fully inhibited at 100μM. In contrast, the TFP inhibition of unextracted rabbit psoas fibers and hamster right ventricular trabeculae occurred at 500μM. By gels, we find that, unlike TnC, calmodulin is released on binding TFP. Since the TFP inhibition in unextracted fibers occurs presumably by acting on TnC, K_d is lower for skeletal and cardiac TnCs than that for calmodulin. TFP inhibition of force of guinea-pig taenia coli was similar to calmodulin-loaded psoas fiber. Since calmodulin regulates in smooth muscle, by activating myosin kinase, our results indicate that the mechanism of calmodulin in psoas fiber is similar to its normal action in smooth muscle. The data also suggest that calmodulin-loaded skinned fiber is a useful model for the mechanism of calmodulin. *[Funded by NIH]*

M-Pos433

CALMODULIN AND RECOMBINANT MUTANT REGULATION OF FORCE OF SKINNED MUSCLE FIBERS

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Calmodulin (CaM) and troponin C (TnC) are homologous proteins, and we have previously shown that CaM can substitute for TnC in the skinned fiber for force development. The global structure of both CaM and TnC is dumbbell shaped. But, for CaM, central helix is shorter, and the arrangement of the N-and C-terminus lobes is off by 60° compared to that in TnC. To directly examine the possibility of the influence of the global structure on regulation in fibers, we have utilized three recombinant mutants of CaM in which: 1) Glu-84 (CaMΔ84), 2) also Glu-83 (CaM Δ83-84), and 3) also, Glu-82 & Ser-81 (CaMΔ81-84) were deleted from the central helix (mutants supplied by A.Persichini; Persichini et al JBC 264,8052,1989). Deletion of a single residue causes a relative rotation of the two lobes by 100° and shortens the central helix by 1.5Å. Presently we find that developed force was normal with the first two mutants, but was markedly inhibited (to $0.3P_o$) with the third mutant. By gels, the uptake by the fiber was the same for all mutants. The results provide the first direct indication that length of the central helix (i.e. distance between the lobes) is critical for function. The data suggest that communication between Ca^{2+}-binding sites in the two lobes is essential for regulation. *[NIH Funds]*

M-Pos434

EFFECTS OF C-PROTEIN EXTRACTION ON CA2+ ACTIVATED TENSION IN SINGLE SKINNED CARDIAC AND SKELETAL MUSCLE FIBRES.

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C-protein, a substantial component of thick filament, has been associated with changes in twitch relaxation rate in intact cardiac muscle and has been shown to bind actin and to alter myosin ATPase activity in vitro. In the present study partial extraction (50-60%) of C-protein from skinned rat myocytes and rabbit psoas fibers caused: (1) a left-shift of the tension-pCa relation with no change in tension at maximal activation and (2) a decrease in the slope of the tension-pCa relation. Readdition of purified C-protein reversed these effects. The modulation of tension does not appear to be mediated by TnC since C-protein extraction from 40-60% TnC deficient myocytes produced effects similar in size to those in TnC replete cells. Stretching sarcomeres to lengths over 3.5 μm, to minimize C-protein-actin interactions, reduced the magnitude of effects due to C-protein removal. These data are consistent with a model in which C-protein acts by either binding to actin or interacting with myosin to reduce the probability of actin-myosin association.

M-Pos435

REPLACEMENT OF SKELETAL TNC WITH CARDIAC TNC DOES NOT ALTER THE LENGTH DEPENDENCE OF TENSION DEVELOPMENT IN SKINNED SKELETAL MUSCLE FIBERS.

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The Ca^{2+} sensitivity of isometric tension was measured at sarcomere lengths (SL) of 2.4 and 1.9 μm in skinned fibers from rabbit psoas muscles. To test the hypothesis that the type of TnC present would alter the length dependence of Ca^{2+} sensitivity, similar measurements were made in skinned fibers in which up to 85% of the endogenous sTnC was replaced with cTnC. In control fibers the pCa_{50} of isometric tension decreased by 0.15 pCa units when SL was shortened. In cTnC recombined fibers, there was no significant change in the length dependent shift of PCa_{50} if recombination was greater than 85% complete. For recombinations less than 85%, the shift in pCa_{50} upon reduction of SL was greater than control. We conclude that the type of TnC present within a muscle does not affect the length dependence of Ca^{2+} sensitivity of isometric tension.

M-Pos436

EFFECT OF CAFFEINE AND pH ON Ca BINDING TO ISOLATED TROPONIN C.

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The Ca sensitivity of skinned fibres is increased by caffeine and decreased by low pH. To investigate whether these effects are related to direct actions on the Ca affinity of troponin C (TnC), we measured the Ca binding to isolated TnC (rabbit skeletal and bovine cardiac), labelled with DANZ and IAANS, respectively. The fluorescence of these labels increases when Ca binds to the regulatory site(s) (Johnson et al., J. Biol. Chem. 253, 6451, & 255, 9635). The solutions were similar to those used for skinned muscles (1 mM Mg^{2+}, ionic strength 0.2 M, 25°C).

At pH 7.0, the pCa for 50% increase in fluorescence (pCa50) was 5.55 ± 0.02 (S.E, n=4) for skeletal TnC and 5.26 ± 0.07 (n=4) for cardiac TnC. Reducing the pH to 6.2 decreased the pCa50 for skeletal TnC by 0.34 ± 0.02 (cf. El-Saleh & Solaro, J. Biol. Chem. 263, 3274) and for cardiac TnC by 0.50 ± 0.07. However, 20 mM caffeine did not significantly affect the pCa50 for either type of TnC at pH 7.0. Thus the effect of pH on the Ca sensitivity of skinned fibres may be due, at least in part, to a direct action on the Ca affinity of TnC, whereas the effect of caffeine cannot be explained by this mechanism.

Supported by the BHF, SERC and Ciba-Geigy plc.

M-Pos437

FUNCTIONAL PROPERTIES OF A 26K-FRAGMENT OF TROPONIN T FROM FAST SKELETAL MUSCLE OF RABBIT.

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The amino terminal region of troponin T (TnT) is widely thought to be involved in the cooperative activation of regulated actomyosin. A truncated TnT (26K-TnT) missing 45 N-terminal residues (Ohtsuki et al. J. Biochem. 95:1337-1342) was used to probe the functions of this region. 26K-TnT was purified using a new procedure including gel filtration, anion and cation exchange chromatography. The identity of the protein was confirmed by amino acid composition. Formation of ternary complex of 26K-TnT, TnC and TnI was demonstrated by gel filtration. Both reconstituted Tn containing TnT and that containing 26K-TnT conferred Ca^{2+}-sensitivity on the MgATPase of myosin S-1-actin-tropomyosin. Substitution of 26K-TnT for TnT in Tn had very little effect on Ca^{2+}-sensitivity and degree of cooperativity of the regulated actoS-1 ATPase (pCa_{50} = 5.83, Hill n = 1.67 ± 0.11 with TnT; pCa_{50} = 5.89, n = 1.51 ± 0.16 with 26K-TnT). The results are tentatively interpreted as suggesting that residues 1-45 of TnT are not crucial for cooperativity in Ca^{2+} activation of actomyosin. (Supported by NIH grants NS07097, NS08384 and HL31962).

M-Pos438

PROXIMITY RELATIONSHIP BETWEEN TROPONIN C AND TROPONIN I IN TROPONIN•TROPOMYOSIN COMPLEX.

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Using fluorescence resonance energy transfer, we have determined the distance between Cys 98 of troponin C (TnC) and Cys 133 of troponin I (TnI) in the troponin•tropomyosin (Tn•Tm) complex from rabbit skeletal muscle. The fluorescent probe N-(iodoacetyl)-N'-(5-sulfo-1-naphtyl)ethylenediamine (IAEDANS) was selectively attached to either Cys 98 of TnC or Cys 133 of TnI as energy donor. Another probe 5-(iodoacetamido)eosin (IAE) was labeled to either Cys 133 of TnI or Cys 98 of TnC as energy acceptor. To avoid uncomplexed fluorescently labeled troponin, the Tn•Tm complex was prepared with excess Tm. At pH 7.5, the donor-acceptor distance in the calcium-free complex was 43.4 A. Addition of Ca^{2+} to the Tn•Tm complex resulted in a large increase in the distance to 54.8 A. The results indicated that binding of Ca^{2+} to Tn•Tm induces the two regions modified by the probes on TnC and TnI to move away from each other. These results differ from those obtained with regulated actin (See neighboring poster). (Supported in part by NIH NS08384, HL31962, HL07090, NS07097).

M-Pos439

INTERSUBUNIT DISTANCE OF TROPONIN IN REGULATED ACTIN.

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The distance between Cys 98 of troponin C (TnC) and Cys 133 of troponin I (TnI) in regulated actin (actin•tropomyosin•troponin) from rabbit skeletal muscle has been measured by fluorescence resonance energy transfer. TnC was labeled with N'-(iodoacetyl)-N'-(5-sulfo-1-naphthyl)ethylenediamine (IAEDANS) at Cys 98 as energy donor. TnI was labeled with 5-(iodoacetamido)eosin (IAE) at Cys 133 as energy acceptor. Quenching of donor fluorescence was monitored to determine the efficiency of the resonance energy transfer. In the presence of EGTA at pH 7.5, the donor-acceptor separation (R) in the protein complex was 53 A based upon $\kappa^2 = 2/3$. When TnC was saturated with Ca^{2+}, the distance decreased to 49 A. The results suggested that the two specific sites in TnC and TnI move toward each other in response to Ca^{2+} binding to TnC in regulated actin. This move may be important in Ca^{2+} regulation because of the interaction of the region of TnI with actin (Tao, et al., 1987, 51, 27a). (Supported in part by NIH NS08384, HL31962, HL07090, NS07097).

M-Pos440

EFFECTS OF PARTIAL TNC EXTRACTION AND EXCHANGE OF CARDIAC FOR SKELETAL TNC ON LENGTH DEPENDENT Ca^{+2} SENSITIVITY IN SKELETAL MUSCLE FIBERS

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The effects of altered length on force-Ca relations was determined in glycerinated rabbit psoas fibers in which the endogenous TNC was either partially extracted (force decreased to about 40% of maximum) or in which skeletal TNC was extracted and the fibers reconstituted with cardiac TNC from rabbit ventricular tissue. Reconstitution of fibers with C-TNC resulted in better than 75% recovery of maximum force. Bathing solutions contained (in mM) K^+ (130), EGTA (10), CPK (15), ATP (4) and MOPS (10-25); pH 7.0, 200 mM ionic strength, temp. 22°C. The effects of partial TNC extraction were determined at sarcomere lengths of 2.3, 2.7, 3.1 and 3.4 μm. At each length partial TNC extraction caused decreased sensitivity and slope of force-Ca relations, while the effect of length was altered little. When C-TNC was exchanged for S-TNC Ca sensitivity was measured at 2.7 and 3.1 μm sarcomere length. These preliminary experiments suggest that substitution of C-TNC for S-TNC in skeletal fibers causes decreased sensitivity and slope, and increased length dependence of force-Ca relations. The results suggest that the properties of TNC may be important in determining the effect of length on calcium sensitivity. (Supported by NIH grant HL31962.)

M-Pos441

REACTION OF SOME FLUORESCENCE PROBES WITH CARDIAC TROPONIN C.

Ying-Ming Liou[1], Franklin Fuchs[1], and Zenon Grabarek[2]. Dept. of Physiology, Univ. of Pittsburgh School of Medicine[1] and Dept. of Muscle Research, Boston Biomedical Research Institute[2].

The two cysteine residues of bovine cardiac troponin C (cTnC) are located in non-functional Ca^{2+} binding site I (Cys-35) and at the N-terminal end of the central helix (Cys-84) close to the regulatory Ca^{2+} binding site (site II). The reaction of cTnC with DTNB can be described by a single exponential equation, with Ca^{2+} binding at site II bringing about a two-fold increase in reaction rate (Fuchs, et al, J. Biol. Chem., in press). The anionic fluorescence probes IAANS and MIANS display similar reaction kinetics. However, the highly lipophilic maleimide derivatives of methylcoumarin, dimethoxystilbene, and pyrene exhibit more complex kinetic behavior. The reaction without Ca^{2+} is monoexponential . With Ca^{2+} bound to site II there is a biphasic reaction attributable to an increased reactivity of Cys-84 and a decreased reactivity of Cys-35. This effect of Ca^{2+} on the reactivity of the lipophilic probes provides a means for relatively selective labeling of Cys-35 and Cys-84 with suitable donor-acceptor pairs for fluorescence energy transfer studies of conformational changes in the regulatory domain of cTnC. Supported by grants from NIH and AHA.

M-Pos442

EFFECTS OF PHOSPHORYLATION OF TROPONIN I ON FLUORESCENCE RESONANCE ENERGY TRANSFER DISTANCES IN THE TROPONIN I•TROPONIN C COMPLEX.

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Fluorescence resonance energy transfer was used to estimate molecular distances in protein complexes. One was formed between cardiac troponin C and troponin I (CTnC•CTnI) and the other between skeletal troponin C and cardiac troponin I (STnC•CTnI). The single Trp of CTnI served as energy donor in both complexes. CTnC was labeled with IAANS at Cys 35 and 84 and STnC with either DNZ at Met 25 or IAEDANS at Cys 98 as energy acceptors. Upon addition of Mg^{2+} and Ca^{2+}, the distance (R) in the cardiac complex increased from 28.8 to 30.2 and 32.0 Å, respectively. Phosphorylation of CTnI resulted in an increase of the distance to 33.7 Å. R in the phosphorylated complex was 30.1 Å in Mg^{2+} and 31.1 Å in Ca^{2+}. These results suggest that the phosphorylation resulted in a larger separation between the two proteins and that, in response to Ca^{2+} activation, two specific regions of CTnC and CTnI move toward each other. The results obtained with STnC•CTnI will be discussed. (Supported in part by NIH AR 25193).

M-Pos443

CHARACTERIZATION OF A NOVEL Mr=11000 SMOOTH MUSCLE Ca^{2+} BINDING PROTEIN.

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A new low molecular weight calcium binding protein designated as SMCaBP-11, has been isolated in a homogeneous form from chicken gizzard. Molecular weight studies by both sedimentation equilibrium in 6 M guanidine hydrochloride and 15% polyacrylamide SDS gels indicated the subunit molecular weight to be 11000, and since a molecular weight of 21000 was obtained in native solvents, the protein exists as a dimer in benign medium. The amino acid composition of this protein is similar but distinct from other known low molecular weight Ca^{2+}-binding proteins. Ca^{2+} binding assays using Arsenazo III (Sigma) indicated the protein to bind 2 mol of Ca^{2+}/subunit. Upon binding calcium the protein underwent a conformational change as revealed by UV difference spectroscopy and circular dichroism studies. When the protein was excited at 280 nm, the tyrosine fluorescence emission maximum was centered at 306 nm. Ca^{2+} addition resulted in a nearly 15 percent decrease in intrinsic fluorescence intensity. Fluorescence titration with Ca^{2+} exhibited two classes of calcium binding sites with K_d values of 0.2 μM and 80 μM in agreement with UV difference spectral data.

M-Pos444

TROPONIN C-TROPONIN I INTERACTIONS AND THE CONTROL OF MUSCLE CONTRACTION. R. Scott Fredricksen and Charles A. Swenson, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

Rabbit skeletal troponin C was specifically cleaved at the site in the interconnecting helix which binds to the troponin I inhibitory peptide. Cleavage at cysteine 98 was accomplished with 2-nitro-5-thiocyano-benzoic acid and the two fragments were separated by gel exclusion chromatography. The binding of the fragments to troponin I and the ability of the fragments to exert calcium control in the regulated actomyosin ATPase have been studied. Calcium control was achieved in the regulated actomyosin ATPase system when troponin was reconstituted with both troponin C fragments and with just the N-terminal domain, residues 1-97, suggesting that the interactions essential to control lie within this fragment. The association constants for the binding of the fragments to troponin I showed only a small calcium dependence (x10) in contrast to troponin C binding to troponin I (x500). The data suggest that allosteric interactions transmitted through troponin I are part of the control switch. (Supported by the American Heart Association).

M-Pos445

Ca^{2+}-BINDING BY REGULATORY FRAGMENT REQUIRES MOLLUSCAN ESSENTIAL LIGHT CHAIN. H. Kwon*, E.B. Goodwin, L. Nyitray, E. Berliner*, and E. O'Neall-Hennessey, F.D. Melandri and A.G. Szent-Gyorgyi. Brandeis Univ., Dept. of Biology and *Biophysics, Waltham, MA 02254.

Regulatory fragment (RF) comprising regulatory light chain (RLC), essential light chain (ELC) and 10-12 kDa heavy chain fragment (HCF) was obtained from scallop papain S1 with clostripain. HCF starts from either L-812 or V-817 based on the homology with other myosins (see Nyitray et al., these abstracts), indicating the binding sites for LCs are within V-817 and C-terminus of papain S1. RF binds Ca^{2+} with the same affinity and specificity as intact myosin. HCF, free of LCs, can be isolated in Gu-HCl. RF was reconstituted with addition of stochiometric amounts of LCs to HCF. The renatured RF regains Ca^{2+}-binding quantitatively. Hybrid RF containing both types of Mercenaria LCs binds Ca^{2+}, while hybrid RF containing rabbit skeletal A2LC and scallop RLC does not. The results demonstrate that ELC from Ca^{2+} regulated myosin is required for specific Ca^{2+} binding.

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M-Pos446

FORCE, SHORTENING, AND Ca^{2+}-TROPONIN C AFFINITY IN SKELETAL MUSCLE. Franklin Fuchs and Yi-Peng Wang. Dept. Physiology, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261.

Isotopic measurements indicate that force generation promotes increased Ca^{2+} binding to cardiac troponin C (TNC) (Hoffman and Fuchs, 1987) but not to skeletal TNC (Fuchs, 1985). However, studies of skinned rabbit psoas fibers containing substituted fluorescent TNC analogs suggest that force generation causes enhanced binding of Ca^{2+} to skeletal TNC (Guth and Potter, 1987). We have re-examined this question using a double isotope technique to measure Ca^{2+} binding to skinned rabbit psoas fibers. Ca^{2+} binding was measured during steady state force generation at sarcomere length 2.5-2.7um and after force was reduced by either 1) adding vanadate (Vi) at the same sarcomere length or 2) allowing the fibers to shorten to sarcomere length 1.5-1.7um. With Vi-induced relaxation there was a small reduction in binding to Ca^{2+} regulatory sites, of smaller magnitude than seen with cardiac muscle. Reduction in sarcomere length had no significant effect on Ca^{2+}-TNC affinity. These results point to quantitative differences between skeletal and cardiac muscle with respect to cooperative effects of cross-bridge activity on Ca^{2+}-TNC affinity.

M-Pos447

RELATIONSHIP BETWEEN Ca^{2+} AND Sr^{2+} BINDING TO FURA-2, INDO-1 AND FLUO-3, AND ISOMETRIC FORCE AND ATPASE IN SKINNED MUSCLE FIBERS. W. Glenn L. Kerrick, John R. Sama, Steve M. Tjoe-Fat, and Phyllis E. Hoar, Dept. of Physiol. & Biophys., Univ. of Miami, Miami, FL. 33101

The use of Ca^{2+} probes for the determination of Ca^{2+} and Sr^{2+} activation of skinned muscle cells was investigated. Since Fura-2 and Indo-1 became nearly saturated with Ca^{2+} before the cells were activated, they are poor indicators for the Ca^{2+} activation of all skinned muscle cell types. By comparison Fluo-3 is a very good indicator since it bound Ca^{2+} over the same range at which Ca^{2+} activates the fibers. Fluo-3 is also a good indicator for the Sr^{2+} activation of fast-twitch muscle because it binds Sr^{2+} over the same range at which Sr^{2+} activates the fibers. In the case of slow-twitch and cardiac muscle, both Indo-1 and Fura-2 are good indicators of Sr^{2+} activation for the same reason. It was also found that Indo-1 bound strongly to skinned fibers, whereas Fura-2 bound only slightly and no binding of Fluo-3 was detected. These studies show that the most useful of the fluorescent indicators for studying the Ca^{2+} activation of striated muscle contraction is Fluo-3 since it binds Ca^{2+} over the same concentration range that force and ATPase is activated and it does not bind to the intracellular proteins associated with skinned fibers. Supported by grants from NIH (AR37447), American Heart Association, Muscular Dystrophy Association, and the State of Florida.

M-Pos448

EFFECTS OF CALDESMON ON Ca^{2+}-INSENSITIVE TENSION IN PARTIALLY DE-REGULATED SKINNED SKELETAL MUSCLE FIBERS. R.L. Moss and M.P. Walsh*, Dept of Physiol, Univ of Wis, Madison and *Dept of Medical Biochem, Univ of Calgary, Alberta.

A possible regulatory role for caldesmon, a protein associated with the thin filaments of smooth muscle, was investigated using rabbit skinned psoas fibers activated in the absence of Ca^{2+} by removal of whole troponin (Tn) complexes. Tn was removed as described previously (J Gen Physiol 87:761). The de-regulated fibers developed isometric tensions of 0.3-0.7 P_o in relax solution in which free [Ca^{2+}] was 10^{-9} M. Addition of 1.5 mg caldesmon per ml relax solution caused a small (<20%) increase in Ca^{2+}-insensitive tension; addition of 3.0-5.0 mg caldesmon per ml relax solution inhibited Ca^{2+}-insensitive tension by as much as 90%. The inhibitory effect of caldesmon was reversed by bathing the fibers in caldesmon-free relax solution. These results suggest that under some conditions caldesmon can regulate the level of activation of muscle.

M-Pos449

DETERMINATION OF UNIQUE PROTEIN KINASE C PHOSPHORYLATION SITES IN MAMMALIAN CALDESMON: A COMPARISON WITH CALDESMON PHOSPHORYLATED IN INTACT MUSCLE. Leonard P. Adam and David R. Hathaway. Krannert Institute of Cardiology, Ind. Univ. Sch. Med., Indpls., IN 46223

We have shown that caldesmon (CALD) is phosphorylated in porcine carotid arterial muscle (PCA) in response to agonists that induce sustained tension. To investigate this problem further, mammalian CALD was phosphorylated in vitro with protein kinase C and cleaved with the cysteine-reactive agent, NTCB. All phosphate was found to reside in a 22 kDa fragment. Phosphorylated CALD was digested and phosphopeptides were purified by iron-chelate affinity chromatography followed by reverse phase HPLC. Three phosphopeptides were identified: GSS*LKIEE; AEFLNKS*VQK; and, NLWEKQS*VDKVTSP. By comparison to the published sequence for gizzard CALD deduced from cDNA (Bryan et al, JBC 264:13873, 1989) all three peptides were identified in the carboxyl end of the molecule. We purified phospho-CALD from PCA preloaded with $^{32}PO_4$ and stimulated for 15 minutes with KCl. Approximately one-half of the phosphate was found in the 22 kDa-NTCB fragment (carboxyl terminus) and one-half in the larger, amino terminal fragment (130 kDa). The stoichiometry of phosphorylation was found to be 1.6 mol PO_4/mol CALD suggesting 2 sites. Thus, CALD kinase is unique and is not protein kinase C. Furthermore, CALD is phosphorylated in its native state on both carboxyl and amino terminal fragments as defined by NTCB cleavage.

M-Pos450

REGULATION OF LOBSTER TAIL MUSCLE. A.Miegel and Y.Maéda. EMBL at DESY, D-2000 Hamburg 52, FRG.

As an example of an invertebrate muscle which is regulated by a troponin(Tn)-tropomyosin(Tm) system, lobster tail muscle has been studied and all the Tn subunits, Tm and myosin light chains (LC's) have been isolated, purified and partially characterized.

Quantitative SDS-PAGE shows that lobster Tn consists of I, C and T with a molar ratio of 1:1:1. Each subunit is identified according to its effect on the acto-S1 ATPase; Tn-I alone inhibits the ATPase, which inhibition is removed on adding C. Only after adding T, is the ATPase Ca^{2+} sensitive. The $pCa_{1/2}$ of the ATPase is about 6.0 (60mM KCl, 3mM free Mg^{2+}, 1mM Mg-ATP, 13mM HEPES, pH=7.2 at 17.5 °C).

The heterogeneity of purified preparations after ion-exchange chromatography has been studied on 1D-SDS- and 2D-PAGE gels; (1) Tm shows single spot at *Mr.* of 38kD; (2) the Tn-T fraction contains 4 or 5 isomers which differ in IEP, sharing a *Mr.* of 52kD; (3) the Tn-I fraction contains 5 isomers, all showing similar IEP, differing in *Mr.* in the range 28 to 31kD; (4) two Tn-C fractions showing a range of *Mr.* from 18.5 to 19 kD. C1 gives rise to single spot while the more acidic C2 shows 2 spots.

Lobster myosin consists of heavy chains, LC1 (22kD) and LC2 (17.5kD) with a molar ratio of 1:1:1. The myosin preparation shows weak Ca^{2+} sensitivity, the $pCa_{1/2}$ is 5.2 to 5.0, which is corresponding to [Ca^{2+}] an order of magnitude higher than that of the Tn-Tm system. Both LC's have been purified, although the functions are not yet known.

It is tentatively concluded that this muscle has both myosin- and actin-linked regulatory systems, the latter being predominant under physiological conditions.

Supported by MDA.

M-Pos451

THE INHIBITION OF SKELETAL MUSCLE CONTRACTILITY BY A CALMODULIN-BINDING PEPTIDE. +J.D. Potter, +Z. Sheng, +J-M. Francois, *J.T. Penniston, and +Z. Lou. +Dept. of Pharmacology, U. of Miami, Miami, FL 33101. Dept. of Biochem. & Mol. Biol., Mayo Clinic, Rochester, MN 55905.

A synthetic calmodulin-binding peptide, C28R2, corresponding to the calmodulin-binding domain of the human erythrocyte Ca^{2+} pump was tested on rabbit skeletal muscle skinned fibers and was found to inhibit force development. This inhibition was Ca^{2+} dependent. The concentration of peptide required to block 50% of the force was ~100 μM. This inhibition was fully reversible by washing the fibers in relaxing solution. If the fibers were incubated with the peptide in the absence of Ca^{2+} and then transferred to contracting solution without peptide, no effect was observed. Taken together, these results suggest that the peptide interferes with the TnC-TnI interaction in a Ca^{2+} dependent manner. In the absence of Ca^{2+}, TnC probably does not show the required conformation for the binding of the peptide. The homology of the peptide with a TnC and actin binding site on rabbit skeletal TnI probably accounts for its inhibitory properties. The inhibitory effect of the peptide is probably mediated through binding to a homologous region on calmodulin and TnC.

M-Pos452

THE Ca^{2+} BINDING PROPERTIES OF ISOFORM 2 OF BARNACLE TROPONIN C. [+]J-M. Francois, [+]A. Mandveno, [*]C. C. Ashley and [+]J.D. Potter. [+]Dept. of Pharm.,U. of Miami Med. Sch., Miami, FL 33101. [*]Dept. of Physiol., U. of Oxford, England OX1 3PT

Isoform 2 of barnacle troponin C ($BTnC_2$) containing a single cysteine residue (Collins, J.H., et al., 1989 Biophys J. 55, 589a) has been labeled using the thiol specific fluorescent probe: acrylodan (Prendergast, F.G., 1983, J. Biol. Chem. 258, 7541). Upon binding of Ca^{2+}, the fluorescence emission spectrum of acrylodan $BTnC_2$ undergoes a blue shift of about 30 nm whereas the fluorescence quantum yield is hardly modified. Addition of Mg^{2+} to the protein does not alter the fluorescence emission spectrum seen in the presence or absence of Ca^{2+}. The affinity of Ca^{2+} for acrylodan $BTnC_2$ has been calculated by measuring (in the presence and absence of Mg^{2+}) the fluorescence emission changes upon titration with Ca^{2+} ($K_{Ca} \sim 10^5\ M^{-1}$, $K_{Mg} \sim 2 \times 10^2\ M^{-1}$). Direct calcium-binding experiments using the fluorescent calcium indicators (Fluo-3 and Rhod-2) also show that $BTnC_2$ has 2-3 Ca^{2+}-specific type sites ($K_{Ca} \sim 10^5\ M^{-1}$). Thus, this protein does not contain any Ca^{2+}-Mg^{2+} type sites. The spectral and metal binding properties of the labeled $BTnC_2$ will make it useful for studying the Ca^{2+} binding to troponin in intact barnacle muscle fibers.

M-Pos453

CD STUDIES OF THE SECONDARY STRUCTURE OF A MUTANT TROPONIN C (TnC4882). N. Gusev, Z. Grabarek, and J. Gergely, Dept. of Muscle Res., Boston Biomed. Res. Inst., Boston MA 02114

We have studied the effects of Ca^{2+} and temperature on the secondary structure of the oxidized (ox-TnC4882) and of the reduced and carboxyamidomethylated (CM-TnC4882) form of a mutant rabbit skeletal troponin C in which Cys98, Gln48 and Gln82 are replaced with Leu, Cys and Cys, respectively (cf. Grabarek et al. this Meeting). In the absence of Ca^{2+} the $[\Theta]_{222}$ of both forms of the mutant is 15-20% higher than that of sTnC. Upon Ca^{2+}-binding $[\Theta]_{222}$ increases to the same value for all three proteins. Ca^{2+}-titration reveals at least two transitions: the first at pCa 7.3 for sTnC and at pCa 7.5-7.8 for the mutants, the second (25% of the total) at pCa 5.2-5.9 for all three proteins. On increasing the temperature there is a gradual decrease in $[\Theta]_{222}$ in the range of 25-45 ^{o}C, followed by a cooperative transition for sTnC and CM-TnC4882. No cooperative transition occurs up to 75 ^{o}C for ox-TnC4882. These results suggest that substitution of Leu for Cys-98 leads to some stabilization of the C-terminal domain reflected in the increase in Ca^{2+}-affinity of sites III and IV, while formation of the disulfide bridge C48-C82 stabilizes the N-terminal domain against thermal unfolding. (Supported by NIH, MDA, AHA)

M-Pos454

CHARACTERIZATION OF ZERO-LENGTH CROSSLINKS BETWEEN TROPONIN I AND TROPONIN C.
T. Kobayashi, *Z. Grabarek, *J. Gergely and J.H. Collins. Dept. Biol. Chem., Univ. of Maryland School of Medicine, Baltimore, MD 21201, and Dept. Muscle Res. Boston Biomedical Research Institute, Boston, MA 02114.

We have used zero-length crosslinking with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to study interaction between the Ca-binding component (TnC) and the inhibitory component (TnI) of rabbit skeletal muscle troponin. Previous data (Leszyk et al., Biochemistry, in press) showed that the inhibitory segment (residues 96-116) of TnI crosslinks with the N-terminal domain of TnC but not with the C-terminal domain, which was also thought to interact. In the present study we have blocked intramolecular crosslinking in TnC by acetylating Lys side chains prior to EDC activation of its carboxyl groups and crosslinking with TnI. The resulting AcTnCxTnI was cleaved with CNBr and proteases, then crosslinked peptides were purified by HPLC and sequenced. Results indicate that interactions between TnC and TnI are quite extensive, involving both the N-terminal and C-terminal domains of TnC and several regions of TnI. (Supported by grants from the NSF and NIH.)

M-Pos455

FLUORESCENCE ENERGY TRANSFER STUDIES ON A SYNTHETIC PEPTIDE BOUND TO TNC P. C. Leavis, E. Gowell, Z. Grabarek, J. Gergely and T. Tao, Dept. Muscle Research, Boston Biomedical Research Inst., Boston, MA 02114

The synthetic peptide G-K-F-K-R-P-P-L-R-R-V-A, which corresponds to residues 104-115 of rabbit skeletal TnI has been shown to bind to actin and TnC and to inhibit actomyosin ATPase activity(Van Eyk and Hodges, J Biol Chem, 263; 1726, 1988). In order to further study the structures of peptide complexes with TnC, we have synthesized the same peptide with the addition of a Cys residue labeled with the chromophore 4-dimethylaminophenylazophenyl-4'-maleimide (DABMI) at its N-terminus. The modified peptide forms a Ca^{2+}-dependent complex with TnC as indicated by gel electrophoresis under nondenaturing conditions and can be crosslinked to TnC using the zero-length procedure (cf Grabarek et al. Biophys. J. 55;589a, 1989). Steady state and lifetime energy transfer studies carried out in complexes in which TnC is labeled with the fluorescent donor IAEDANS at Cys 98 indicate that a dark complex is formed between donor and acceptor. This is consistent with a donor-acceptor distance less than 2 nm. (Supported by grants from NIH and MDA)

M-Pos456

COOPERATIVE STRUCTURAL EFFECTS OCCURRING UPON CALDESMON-ACTIN INTERACTION ARE MEDIATED BY CALMODULIN G.M. Strasburg, Michigan State University; B.A. Levine, University of Oxford; E. Audemard and D. Mornet, Universite Montpellier.

Limited proteolysis, chemical modification, and NMR techniques were used to characterize binding of caldesmon (CaD) to actin and calmodulin (CaM). CaD treated with thrombin yielded a 35 kD fragment which was digested further to produce a 15 kD peptide. CaM binds to the 35 kD fragment, but not to the 15 kD peptide. Titration of CaM with spin-labeled 35 kD indicates that CaM binds to the Cys-containing domain, located downstream from the 15 kD region of the the 35 kD fragment. A peptide corresponding to actin residues 16-41 binds to the 15 kD fragment; titration of CaM + 35 kD with the actin 16-41 peptide resulted in cooperative binding of CaM to 35 kD. A peptide corresponding to residues 1-18 of actin binds to the 35 kD fragment, enhancing the 16-41 peptide's binding to 35 kD. These results suggest that part of the smooth muscle regulatory mechanism involves Ca^{2+} + CaM enhancement of CaD/actin interaction.

M-Pos457

PROPERTIES OF THIN FILAMENTS DEFICIENT IN TROPONIN C SUGGEST COOPERATIVE INTERACTIONS BETWEEN TROPONIN-TROPOMYOSIN COMPLEXES. L. Tobacman and F. Fan, The University of Iowa College of Medicine, Iowa City, Iowa 52242. Both thin filaments (TF) and TF deficient in troponin C have been reconstituted from cardiac actin, tropomyosin, and mixtures of whole troponin (labeled on troponin C with IAANS) and troponin T-troponin I. Troponin and troponin T-troponin I competed for binding to the same TF sites. Replacement of troponin IAANS with troponin T-troponin I (in the absence of myosin) disproportionately diminished the Ca^{2+}-induced increase in fluorescence intensity (20%) observed for actin-tropomyosin-troponin IAANS. TF containing 40% troponin IAANS and 60% troponin T-troponin I had negligible change in fluorescence intensity upon $CaCl_2$ addition. Replacement of troponin with troponin T-troponin I inhibited by 75% the TF activated MgATPase rate of myosin. These results suggest that TF troponin molecules interact, even in the absence of myosin. They also suggest that interactions between inhibited actin-tropomyosin-troponin units along the TF are particularly strong.

M-Pos458

A FLUORESCENT PEPTIDE SUBSTRATE FOR MYOSIN LIGHT CHAIN KINASE. Bonita F. Bowman and James T. Stull, Dept. of Physiology, University of Texas Southwestern Medical Center at Dallas, Texas 75235

The peptide, KKRAARACSNVFS, contains the primary sequence determinants for substrates of myosin light chain kinase. This peptide was synthesized and labelled with acrylodan at the cysteine residue. The peak emission of this fluorescent peptide occurred at 530 nm, and the peak excitation wavelength at 364 nm. The fluorescence lifetime, as determined by the Lorentzian distributional model, was 1.43 nsec. The steady-state polarization was 0.1487. When the peptide was incubated with rabbit skeletal muscle MLCK, Ca^{2+}/calmodulin and [γ-^{32}P]ATP, there was a decrease in the emission intensity at 540 nm proportional to the extent of phosphorylation. Calculations indicated a 14% decrease in emission intensity with 1 mol phosphate incorporated per mol peptide. This peptide will be useful for investigating the kinetic mechanism of myosin light chain kinase. Supported in part by HL23990 and HL06296.

M-Pos459

CHARACTERIZATION OF CALDESMON BINDING TO MYOSIN. M.E. Hemric. Department of Biochemistry, East Carolina University, Greenville, North Carolina 27858-4354. We have previously shown that while caldesmon inhibits the actin activated ATPase of all skeletal and smooth muscle myosin subfragments, caldesmon inhibits or enhances subfragment*ATP binding to actin-tropomyosin depending on the myosin source and subfragment type. We demonstrated that this varied effect on binding is due to a direct binding of myosin to caldesmon (Hemric, M.E. and Chalovich, J.M. (1989) J. Biol. Chem. 263, 1878-1885). We now show that caldesmon binding to smooth myosin saturates at a stoichiometry of 1 to 1. The constant for smooth myosin binding to caldesmon is 1.2 E^5 M^{-1} and decreases with increasing ionic strength. The caldesmon binding domain lies in both the subfragment-2 and subfragment-1 regions of myosin. Smooth and skeletal myosin subfragments, as well as calcium-calmodulin, competitively displace caldesmon from smooth myosin, whereas tropomyosin has no effect on myosin binding. Also, the purified myosin-binding fragment of caldesmon binds to myosin with a stoichiometry and affinity similar to that of intact caldesmon. The strength and specificity of this interaction indicate a possible functional role in smooth muscle.

M-Pos460

QUENCHING OF INTRINSIC FLUORESCENCE OF BOVINE CARDIAC AND RABBIT SKELETAL TROPONIN C BY ACRYLAMIDE. Ronglih Liao. Graduate Program in Biophysical Sciences, University of Alabama at Birmingham, Birmingham, AL 35294

The environments of the tyrosyl residues in troponin C (TnC) from bovine cardiac and rabbit skeletal muscle have been investigated by acrylamide quenching of intrinsic steady-state fluorescence. The Stern-Volmer plots for cardiac TnC (CTnC) exhibited an upward curvature indicative of the existence of static quenching. The most striking result was that the reactions of acrylamide quenching of CTnC was relatively insensitive to Ca^{2+} and pH. The quenching data have been fitted to a modified Stern-Volmer equation. The quenching parameters indicate that all three tyrosines can be quenched by acrylamide with various degree of accessibility. Of the three tyrosyl side chains, two were more accessible to the quencher and the third was less accessible. The results obtained from STnC suggested that there was only one class of fluorophores. However, the quenching of STnC was sensitive to the presence of Ca^{2+} and pH. (Supported in part by NIH AR25193).

M-Pos461

Absorption and Fluorescence Spectroscopic Studies of the Ca^{2+} Dependent Lipid Binding Proteins P36, P1 and P2.

G. Marriott, N. Johnsson and K. Weber. (Introduced by W. R. Kirk)
Max Planck Institut für Biophysikalische Chemie, Göttingen. BRD.

We have investigated the ultraviolet absorption and steady-state and time-resolved fluorescence properties of the unique tryptophan residue in the Ca^{2+} free and complexed forms of P36 and P2, and the two tryptophan residues of P1. Upon Ca^{2+} binding the indole absorption and emission of P36 and P1 are blue-shifted. Specifically, with excitation of these proteins at 295 nm or longer, we observe the maximum emission wavelength shift from 322 nm to 312 nm. These emissions obey Teale's rule. We propose an interpretation of the unusually blue-shifted absorption and emission spectra based on evaluations of the spectroscopic properties of indole in model solvent systems and tryptophan emission in single tryptophan proteins. We have also investigated the properties of the Ca^{2+} binding site in relation to the tryptophan residue of P36 by means of the sensitised luminescence of bound Tb^{3+}.

M-Pos462

THE EFFECT OF INORGANIC PHOSPHATE AND ACIDIC PH ON CA-BINDING BY TROPONIN C IN SKINNED MUSCLE PREPARATIONS OF BEEF HEART. T. Takayasu and R. J. Solaro, Dept. of Physiology and Biophysics, University of Illinois, College of Medicine, Chicago, IL 60680.

Increases in Pi are known to reduce force in heart muscle and also possibly to shift the relation between pCa and force to the right. We tested whether these effects are associated with a change in Ca-bound to troponin C (TNC) in detergent extracted fibers using procedures detailed in Pan and Solaro, J. Biol. Chem. 262:7839,1987. Measurements were made at pH 7.0 or pH 6.5 in 0.1 mM or 20 mM Pi in 5 mM MgATP, 2 mM Mg^{++}, 12 mM CP, I=0.16M, 22°C. At pH 7.0, 0.1 mM Pi the affinity (K_1) of the regulatory binding site was 2×10^5 M^{-1}. At 20 mM Pi, K_1 was 1×10^5 M^{-1}. With a reduction in pH to 6.5, K_1 fell to 6×10^4 M^{-1} at both 0.1 and 20 mM Pi. In all cases the stoichiometry of Ca-binding to the regulatory site was one mol/mol TNC. The affinity and stoichiometry of Ca-binding to the higher affinity, sites on TNC was unaffected by pH and Pi. Our results indicate that Ca-binding to myofilament TNC is reduced with acidic pH but unaffected by Pi and therefore by the population of weakly and strongly attached cross-bridges.

M-Pos463

FUNCTION OF ALTERNATIVELY SPLICED EXONS IN α-TROPOMYOSIN. Y.-J. Cho and S.E. Hitchcock-DeGregori. Dept. of Anatomy, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854

Smooth and striated muscle α-tropomyosins (TM) differ from each other as a consequence of alternative splicing of exon 2 (res. 39-80) and exon 9 (res. 258-284). To understand the function of the alternatively spliced exons, we constructed two chimeras from rat striated and smooth α-TM cDNAs (gift of B. Nadal-Ginard) in which the striated TM has a smooth exon 2 (chimera 2) or a smooth exon 9 (chimera 9). The cDNAs were expressed in E. coli to produce fusion (f) and nonfusion (nf) TM (Hitchcock-DeGregori and Heald, (1987) JBC, 262, 9730) for comparison with recombinant smooth and striated TMs. nf-smooth TM bound well to actin, in contrast to nf-striated TM which bound poorly. Of the two chimeras, only chimera 9 bound well. When troponin-linked regulation of the acto-S1 ATPase was measured with fusion TMs, f-striated TM showed higher activation of the ATPase than f-smooth TM in the presence and absence of Ca^{2+}. Fusion chimera 2 was comparable to f-striated; f-chimera 9 to f-smooth. Our results suggest that exon 9 of α-TM is responsible for the functional differences between striated and smooth α-TM. Supported by NIH-HL 35726 and AHA.

M-Pos464

STABILITY OF α-TROPOMYOSINS EXPRESSED IN E. COLI.

Y. Ishii, S.S. Lehrer, & S.E. Hitchcock-DeGregori*. Boston Biomed. Res. Inst., Boston, MA. and *UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ.

The sequence of the striated and smooth α–tropomyosin (Tm) differs by alternative splicing of 2 exons at # 39-80 (exon 2) and # 258-284 (exon 9). The thermal unfolding profiles of striated and smooth Tm and the 2 striated-smooth chimeras expressed as fusion (f) and non-fusion (nf) forms in E. coli. (Hitchcock-DeGregori & Heald (1987) JBC 262 9730) were studied by circular dichroism and fluorescence. Nf-smooth Tm unfolded at 33° without a pretransition; nf-striated Tm unfolded at 45° with a pretransition, similarly to the unfolding of native striated Tm. The thermal unfolding profile of nf-striated Tm with smooth exon 9 was similar to nf-smooth Tm while the unfolding of nf-striated Tm with smooth exon 2 was intermediate between nf-smooth and nf-striated Tm (38°). The fusion peptide (which was non-helical and folded below 65°) stabilized the N-terminal half of f-striated Tm as shown by a new major transition at 57° in addition to the transition at 45°. Similar stabilization was observed for f-Tm with striated exon 2 but only slightly for f-Tm with smooth exon 2. The stability of the lower temperature transition of these f-Tm's was consistent with that of the transition of nf-Tm's. Thus the substitution of exons and modification of the N-terminus affected the conformation of Tm by operating over long distances along the coiled-coil molecule, and the stability of smooth Tm was primarily determined by exon 9. Supported by AHA.

M-Pos466

THE B- AND C-HELICES IN THE N-TERMINAL DOMAIN OF TROPONIN-C INTERACTS WITH TROPONIN-I.

Z. Wang, J. Wang, S. Sarkar, J. Gergely & T. Tao. Dept. of Muscle Research, Boston Biomed. Res. Inst., Boston, MA 02114 & Dept. of Anatomy & Cellular Biology, Tufts Univ. Sch. of Med., Boston, MA 02111.

We have used *in-vitro* mutagenesis to synthesize a mutant rabbit skeletal troponin-C with a single Cys at residue 57 in the C-helix (TnC57). TnC57 labeled with benzophenone maleimide photocrosslinks with troponin-I (TnI) in the ternary troponin complex, but not with troponin-T (TnT). The fluorescence lifetime of 1,5-IAEDANS labeled TnC57 in the presence of Ca^{2+} was 11.8 ns in the absence of TnI and 18.0 ns in its presence. Further addition of TnT did not affect the lifetime. Removal of Ca^{2+} caused the lifetime of the labeled TnC57 to increase when uncomplexed, and decrease when complexed with TnI and TnT. Qualitatively similar results were obtained when a mutant TnC containing a single Cys residue at position 45 in the B-helix was studied. Our results show that the region in the N-terminal domain of TnC comprising helices B and C interacts with TnI but not with TnT; they are consistent with previous zero-length crosslinking studies (Leszyk et al., Biochemistry, in press), and with a model for Ca^{2+}-regulation proposed by Herzberg et al. (J. Biol. Chem., 261, 2638-2644, 1986).

M-Pos465

EFFECT OF TROPONIN-T BINDING TO THE OVERLAP SITE OF αα-TROPOMYOSIN - MODULATION BY PHOSPHORYLATION.

D.H. Heeley, P. Dubord and L.B. Smillie, MRC of Canada Group in Protein Structure and Function, Dept. of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

We have investigated the effects of two phosphorylation events (residue 1 of Tn-T and residue 283 of αα-TM) in viscosity and ATPase experiments. Preparation of the various forms was by F.P.L.C. Fragments T1 of Tn-T (residues 1-158), an effective inducer of nonphosphorylated αα-TM polymerization, inhibited ATPase activity (0 → 60 μM actin, 0 → 17 μM TM, 0.3 μM S1, i.s=30 mM, pH=7.00, T=25°C) by approximately 50%. While a phosphate at acetyl serine 1 had no measurable effect, phosphorylation at serine 283 markedly lowered the level of fragment enhanced polymerization and also the inhibition (10-15%). These observations were confirmed using mixtures of whole Tn(s) and TM(s) and were reversible upon phosphatase treatment. Therefore, the attachment of Tn-T (or T1) to the TM overlap is related to the effects seen in the ATPase assay. The results suggest a modulatory role for Tn-T and are discussed in connection with the developmentally occurring changes in phosphorylated TM levels, and the Ca^{2+} sensitive cooperativity existing between sites I and II for Tn-T attachment on TM.

M-Pos467

WITHDRAWN

M-Pos468

THE LENGTH OF SMOOTH MUSCLE THIN FILAMENTS. J.S. Drew and R.A. Murphy. Dept of Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22908.

The thin to thick filament length ratio in striated muscle is 0.625 (thin filaments, 1.0 μm; thick filaments 1.6 μm). Smooth muscle thick filaments are 2.2 μm long in rabbit vein (Ashton et al., 1975, J. Mol. Bio. 98:17). Smooth muscle thin filaments would measure 1.38 μm if the thin to thick filament length ratio were the same in striated and smooth muscle. We isolated native thin filaments from swine stomach and measured individual filament lengths from electronmicrographs. The filament length distribution was narrow and unimodal, in sharp contrast to distributions observed when filaments are fragmented and allowed to reanneal (Murphy et al., 1988, J. Cell Bio. 106: 1947). Mean filament length was 1.34 +/- 0.08 μm. This is the same as the predicted length of 1.38 μm. This suggests that the ratios of thin to thick filament length are similar in striated and smooth muscle and further that the contractile mechanisms are comparable in the two types of tissue. Supported by NIH grant 5P01 HL19242 and an NSF predoctoral fellowship to JSD.

M-Pos469

EFFECT OF MUCOSAL INFLAMMATION ON COLONIC SMOOTH MUSCLE CONTRACTION. Y. Xie, WJ Snape Jr., SN Reddy, VE Eysselin, F Cominelli. Harbor-UCLA Medical Center and The Inflammatory Bowel Disease Center, Torrance, CA 90502 U.S.A.

The aim was to examine the mechanism of the decreased colonic smooth muscle contraction in colitis. Mucosal inflammation was induced in New Zealand White rabbits using intrarectal formalin and intravenous immune complexes. The circular muscle (CM) was removed from the inflamed colonic segment. Isometric contraction to bethanechol (10^{-8}M to 10^{-4}M) was decreased ($p<0.01$) in colitis muscle compared to muscle from normal animals. The effect of intracellular Ca^{2+} on contraction was evaluated in the two sets of tissues using saponin permeabilized thin strips (200um). The tissues were exposed to standard contracting solutions containing ATP (5mM), calmodulin (2.5uM), and increased concentrations of Ca^{2+} (10^{-8} - 10^{-5}M). The maximal force of contraction at Ca^{2+} ($3x10^{-7}$M) was decreased by 60% in CM from colitis ($p<0.04$) (n=6). Force-velocity measurements using quick release showed that the Vmax of muscle from colitis was decreased (range 0.04 - 0.01 Lo/sec) compared with normal muscle (0.05 - 0.1 Lo/sec) ($P<0.05$). These studies suggest that 1) colonic CM from animals with colitis develop decreased isometric tension 2) the rate of crossbridge formation is slowed.

M-Pos470

FUNCTIONAL ANALYSIS OF CALDESMON USING MONOCLONAL ANTIBODIES. G.C. Scott-Woo, Y. Araki, M. Ikebe. Dept. Physiology and Biophysics, Case Western Reserve University

The inhibition of the actomyosin Mg^{2+}-ATPase by caldesmon (CaD) is reversibly regulated by calmodulin (CaM)-dependent phosphorylation. We are trying to elucidate the inhibitory mechanism of CaD and thirteen anti-CaD producing cell lines have been established. Epitopes for these antibodies have been mapped to the CaD primary structure using chymotryptic and NTCB digestion mapping techniques. The effect of these antibodies on phosphorylation of CaD by CaD kinase has been studied. One antibody which recognizes the myosin-binding domain and two which recognize the actin-/CaM-binding domain showed more than 50% inhibition of maximal phosphorylation of exogenously added heat-treated CaD with little (<30%) or no effect on phosphorylation of smooth muscle myosin 20,000 dalton light chain. (Supported by AHFMR (GSW), NIH, Syntex, AHA (MI))

M-Pos471

LENGTH DEPENDENCE OF MYOSIN PHOSPHORYLATION TRANSIENTS IN THE SWINE CAROTID MEDIA. Chi-Ming Hai, Brown University, Providence, RI 02912

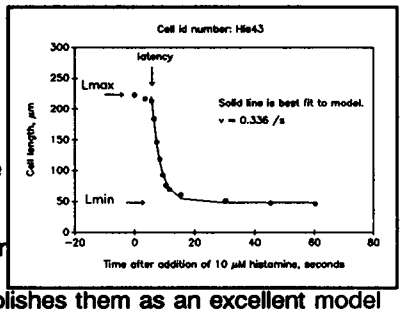
We measured myosin phosphorylation (MP) transients induced by K^+-depolarization at optimal length (L_o) for contraction, $1.5L_o$, and slack length. In comparison to tissues at L_o, MP transient at $1.5L_o$ has a lower peak but similar steady-state values. In Ca^{2+}-depleted tissues in which the initial peak in MP was almost eliminated, steady-state levels of MP at L_o and $1.5L_o$ were similar but the increase in MP was faster at $1.5L_o$, as would be predicted from the shorter diffusion distance in tissues at L_o. These data suggested that intracellular Ca^{2+} release was reduced but Ca^{2+} influx remained unchanged at $1.5L_o$. Unlike tissues at $1.5L_o$, MP transient at slack length was lower than that at L_o at all measured times (0 - 30 min). MP levels in slack tissues were further lowered during a second contraction at slack length. In contrast, time courses of MP induced by 10 uM Ca^{2+} in chemically skinned tissues at L_o and slack length were similar. These data suggested that both intracellular Ca^{2+} release and Ca^+ influx were partially inhibited in tissues at slack length.

M-Pos472

SHORTENING VELOCITY OF INTACT ARTERIAL SMOOTH MUSCLE CELLS: FASTER THAN MUSCLE STRIPS, AND INCREASED AT LOW [Ca^{2+}].

T.J. Dougherty and S.P. Driska, Physiology Dept., Temple Medical School, Philadelphia, PA 19140.

Unloaded shortening of freshly isolated hog carotid artery smooth muscle cells was studied at 37°C after histamine stimulation in solutions of varying [Ca^{2+}]. Cell lengths (L) were measured from videotape and were fit well by the model L = Lmin + (Lmax-Lmin) exp (-V (time-latency)), where latency is the time from histamine addition to beginning of contraction, and V is an exponential rate constant. Cells shortened rapidly (V as high as 0.4 s^{-1}) and substantially (Lmin/Lmax=0.25). In 1.6 mM Ca, mean V was 0.177 s^{-1}; elevation of Ca to 5 or 10 mM did not increase V, but lowering Ca to 0.5 or 0.16 mM significantly increased V to 0.288 s^{-1}. This is about 4× more than the highest unloaded shortening velocity we have ever measured in strips of this tissue by the slack test. The extent and rapidity of shortening, as well as the superior calcium tolerance of these cells establishes them as an excellent model for studies of the contractile system of smooth muscle.



Supported by NIH HL24881 & BRSG S07 RR05417, and AHA, Southeastern Pennsylvania Affiliate.

M-Pos473

SINGLE CHANNEL MEASUREMENTS OF THE Ca^{2+}-GATED RYANODINE-SENSITIVE Ca^{2+} RELEASE CHANNEL OF VASCULAR SMOOTH MUSCLE.

A. Herrmann-Frank, E. Darling and G. Meissner. Dept. of Biochemistry, Univ. of North Carolina, Chapel Hill, NC 27599-7260.

The CHAPS-solubilized Ca^{2+}-gated Ca^{2+} release channel of canine and porcine aorta sarcoplasmic reticulum (SR) was sedimented according to the method of Lai et al. (Nature,331,315,1988). The 30S protein fraction, incorporated into lipid bilayers (PE:PS=1:1) in the presence of 250mM KCl, 50μM free Ca^{2+}, pH 7.0 at 22°C, exerted a slope conductance of ~400 pS. Perfusion of the trans chamber, i.e. the luminal side of the channel, with a 100mM $CaCl_2$ buffer reduced the conductance by up to 30% at negative voltages (-40 mV) with E_{rev}=15 mV, corresponding to P_{Ca}/P_K=3.3. The channel was activated by μM Ca^{2+} and mM ATP applied to the cytoplasmic (cis) side of the channel and was inhibited by mM Mg^{2+} cis. Ryanodine blocked the channel completely at concentrations ≥500μM. Supported by DFG (FRG) and NIH grants.

M-Pos474

PORCINE CAROTID ARTERY CREATINE KINASE KINETICS USING NMR SATURATION TRANSFER.

J.F.Clark, P.F.Dillon. Depts. of Physiology and Radiology, Michigan State University, East Lansing MI 48824.

Saturation transfer (ST) experiments were performed on perfused porcine carotid arteries to determine the kinetics of creatine kinase (CK). ST was performed on arteries perfused with physiological salt solution containing 0.1 mM Pi. ^{31}P NMR spectra were taken for all kinetic measurements. Conventional ST (CST) measured molecular exchange between ATP and phosphocreatine due to CK and assumed a 2-site molecular exchange. Results indicate k_f = 0.29 ± 0.08 s^{-1} and k_r = 0.14 ± 0.04 s^{-1} (n=6, SE). The net flux ratio, which was not significantly different from one, (1.05 ± 0.20) indicates a reaction at equilibrium. The ATPase in the stimulated arteries exposed to 40 mM creatine was determined, assuming a 3-site molecular exchange, using multisite ST (MST). MST results did not detect observable ATPase activity in the porcine carotid artery under these conditions. The lack of observable ATPase activity obviates the need for MST when measuring CK kinetics in porcine arteries under similar conditions. These results indicate that further CST CK experiments on porcine carotid arteries can assume a 2-site exchange.

M-Pos475

IONIC CURRENTS IN SINGLE MYOCYTE OF GUINEA PIG URETER.

J. L. Sui, S. Y. Wang and C. Y. Kao. Department of Pharmacology, SUNY Downstate Med Ctr, Brooklyn, NY 11203

Freshly dispersed ureteral myocyte have been clamped in the whole-cell mode. From a HP of -50 mV, I_{Ca} starts at -30 mV. In 3mM Ca^{2+}, the current is maximal at 10 mV and reverses at 56 mV; in 30 mM Ca^{2+}, it is maximal at 30 mV and reverses at 67 mV. It is blocked by nifedipine (2 μM) and increased by BAY-K 8644 (2 μM). Half-activation is at 3 mV in 3 mM Ca^{2+} and 26 mV in 30 mM Ca^{2+}. BAY-K doubles the conductance at 0 to 30 mV. At 22°C, maximal I_{Ca} is attained at 19 ms. Inactivation has two components, with τ of 134 and 1380 ms. Half-inactivation occurs at -16 mV in 3 mM Ca^{2+} and 1 mV in 30 mM Ca^{2+}. No fast I_{Na} is seen even at HP of -80 mV.

The outward current is carried by K^+. A first part with a gradual onset and 100 ms long shows voltage-dependence, outward rectification and sensitivity to $[Ca^{2+}]_o$. It declines to a less rectifying part with prominent irregular fluctuations. The slowly inactivating I_{Ca} and fluctuating g_K could account for the complex spike activity of ureteral myocyte. (Supported by NIH grant DK 39731).

M-Pos476

THE MYOSIN-PRODUCT COMPLEX IN RESTING AND RELAXING SMOOTH MUSCLE. T.M. Butler, M.J. Siegman, S.U. Mooers and S.R. Narayan. Department of Physiology, Jefferson Medical College, Philadelphia, PA 19107.

In resting permeabilized rabbit portal vein which has been exposed to [^{3}H]ATP and [γ-^{32}P]ATP, followed by a chase in an unlabeled relaxing solution, the ratio of bound [^{3}H]ADP to bound [^{32}P]Pi is close to unity, and both are released at about the same rate. This suggests that myosin exists with both ADP and Pi bound under resting conditions. In contrast, there is a 30% excess of bound Pi over ADP in a muscle during relaxation from an isometric contraction. Under these conditions, while force output is slowly decreasing, both light chain phosphorylation and ATPase activity have decreased to near-resting values. The time course of relaxation is similar to the time course of Pi release from both the relaxing and the resting muscle. We propose that during relaxation, the dephosphorylated crossbridges which are bearing force have Pi but not ADP bound, and that detachment of the crossbridge (and thus force decay) is limited by Pi release from myosin which occurs at the same rate as in the resting muscle. (Supp. by HL15835 to the Pennsylvania Muscle Institute.)

M-Pos478

THE WARMING-INDUCED CONTRACTION OF AORTIC SMOOTH MUSCLE IS INDEPENDENT OF EXTRACELLULAR AND INTRACELLULAR CALCIUM. John Pawlowski, Kathleen G. Morgan. Harvard Medical School, Boston, MA 02215

Circular strips from ferret aorta were used to investigate the warming-induced contraction of smooth muscle. On warming from 21^{0} ±1 to 37^{0}±1, the muscle produced an average force at steady state of .029 ± .013x10^{5} N/m^{2} (n=8). Strips placed in Ca-free Krebs solution (plus 2 mM EGTA) pre-stimulated with 10 mM caffeine showed a force on warming of .035 ± .021 x 10^{5} N/m^{2} (n=9). Using the photoprotein aequorin, intracellular Ca concentrations averaged 235.9±21.8 nM in normal Krebs and 210.9±24.1nM in Ca-free solution at 21^{0} and did not change significantly at 37^{0} (233.3±32.6nM and 221.3± 32.6 nM respectively (n=7). Mechanical determinations of stiffness using small sinusoidal length oscillations showed an increase in stiffness on warming in both Ca-containing (.087±.013x10^{5}N/m^{2}) and Ca-free (.089±.018x10^{5}N/m^{2}) Krebs solution (n=9). We conclude that the apparent increase in the number of attached crossbridges occurs in a functionally Ca-independent manner. (Support: HL31704 and an AHA EI to KGM, GM 07592 to JP)

M-Pos477

ACETYLCHOLINE CAN ELEVATE $[Ca^{2+}]_i$ IN VOLTAGE-CLAMPED SMOOTH MUSCLE CELLS VIA RELEASE FROM INTERNAL STORES.

P.L Becker, T. Itoh, J.V. Walsh, J.J. Singer & F. S. Fay. Dept of Physiology, Univ. Mass. Med. Center, Worcester, MA.

Acetylcholine (Ach) is an important mediator of gastric smooth muscle contraction. The ability of Ach to elevate the $[Ca^{2+}]$ was studied by monitoring $[Ca^{2+}]$ of voltage clamped smooth muscle cells loaded with fura-2. When the cell's V_m was clamped at -100 mV, the local application of 10^{-4}M Ach elevated the $[Ca^{2+}]$ by 207 nM (±29, n=11), peaking in 2-6 secs but returned to near rest levels despite continued presence of Ach. No change in the membrane current was detected. Responsiveness to Ach recovered in ~ 1 min. This demonstrates that Ach can gate the release of Ca^{2+} from internal stores independent of an effect on V_m or membrane currents. Brief application of IP_3 to saponin skinned cells in a 100 nM Ca^{2+} (200 µM EGTA),5 µM fura-2 solution produced sustained releases of calcium, suggesting that desensitization of whole cells to Ach reflects inhibition of 2nd messenger generation rather than depletion of the calcium store. Support: NIH (HL-14523, AM07807, DK31620), NSF (DCB8511674), MDA.

M-Pos479

EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON THE CALCIUM-FORCE RELATIONSHIP IN SMOOTH MUSCLE OF FERRET AORTA. E Suematsu and KG Morgan. Department of Medicine. Harvard Medical School. Boston, MA 02115.

The mechanism of relaxation by Atrial Natriuretic Peptide (ANP) in smooth muscle is generally recognized to involve elevated intracellular cGMP levels, but little is known about the further processes. We examined the effect of ANP on the contraction and relaxation of smooth muscle in ferret aorta by measuring the isometric tension and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) simultaneously using the aequorin technique. ANP(10^{-7}M) inhibited the contraction induced by 24 mM K^{+} (1.23±0.14g to 0.73±0.15g), and reduced $[Ca^{2+}]_i$ (346±15nM to 282±15nM). ANP also decreased the resting tone and calcium level. The calcium-force curve constructed by plotting the calibrated aequorin light signal against the resulting force was not shifted by ANP. This was in contrast to forskolin which was previously reported to shift the curve to right. Thus we conclude that ANP induces the relaxation of vascular smooth muscle not by a direct effect on the contractile apparatus but solely by the decrease in $[Ca^{2+}]_i$. Support: Mass. Heart & NIH HL31704

M-Pos480

CHANGES IN $[Ca^{2+}]_i$ FOLLOWING RECEPTOR-MEDIATED ACTIVATION OF HYPERTROPHIC VASCULAR SMOOTH MUSCLE. P Papageorgiou and KG Morgan, Harvard Med. Sch. Boston, MA

Fura-2-loaded single smooth muscle cells and aequorin-loaded rings from normal rat aortae had a resting $[Ca^{2+}]_i$ of 222±15 nM. Angiotensin (AII) produced a contraction and a $[Ca^{2+}]$ spike 445%±92% of resting $[Ca^{2+}]_i$, whereas the phenylephrine (PE)-induced contraction was accompanied by little or no Ca^{2+} response. In contrast, hypertrophic rat aortic muscle, shown to be polyploid by ethidium-dimer fluorescence, had an increased resting $[Ca^{2+}]_i$ of 332±27 nM, showed a smaller spike to AII (261%±39% of resting $[Ca^{2+}]_i$), and a significant spike to PE (170% ± 20% of resting $[Ca^{2+}]_i$). Although forces produced by AII and PE were not different between normal and hypertrophic muscle, the latter displayed increased sensitivity to PE (ED_{50} 1.3±0.18 x 10^{-8}M, vs 2.5±0.3 x 10^{-8}M) and decreased sensitivity to AII (ED_{50} 7.3±1.3 x 10^{-9}M vs. 3.6±0.4 x 10^{-9}M). The data suggest that Ca^{2+} signaling pathways of pharmacomechanical coupling have been altered in the hypertrophic/polyploid vascular smooth muscle. [Support: NIH HL 31704 & 42293, Mass. Heart]

M-Pos481

EFFECTS OF INTRACELLULAR CYCLIC GMP ON VASCULAR SMOOTH MUSCLE STUDIED WITH A PHOTOLABILE PROBE L.H. Clapp, G. Allerton-Ross & A.M. Gurney. Dept. of Pharmacology, UMDS, St. Thomas's Hospital, London, UK.

Nitrovasodilators and EDRF are thought to relax blood vessels through cGMP. We have studied the actions of nitroprusside and cGMP on rabbit pulmonary arterial muscle, using a photolabile precursor, caged cGMP (Nerbonne *et al*, Nature 310: 74-76, 1984). Voltage-activated K^+ currents were studied in isolated cells using whole-cell recording, while isometric tension was recorded from muscle strips. Caged cGMP was applied either in the bath (strips) or in the patch pipette, and a light flash released free cGMP. In the absence of caged cGMP, single flashes relaxed muscle strips precontracted with norepinephrine (NE) and altered K^+ current in single cells. Relaxations were blocked either by hemoglobin (<1μM), known to inhibit guanylate cyclase, or by reducing light intensity. Under these conditions, flashes produced rapid relaxations with caged cGMP (1-100μM), which were always larger in tissues precontracted with NE rather than with high K^+. In isolated cells, both light flashes and nitroprusside induced spontaneous outward currents and enhanced steady-state K^+ current, although an initial decrease was sometimes observed. The decrease in current amplitude could reflect a fall in intracellular Ca^{2+}, since K^+ currents in these cells are highly Ca^{2+} sensitive. This could result from changes in Ca^{2+} handling, which our preliminary studies suggest are important for the flash-induced relaxations. Furthermore, increases in steady-state and spontaneous currents could directly contribute to relaxation.

M-Pos482

EFFECT OF SMOOTH MUSCLE PHOSPHATASES I-IV (SMP I-IV) ON CONTRACTION AND ATPASE IN SKINNED SMOOTH MSUCLE FIBERS. Phyllis E. Hoar, Mary D. Pato*, & W. Glenn L. Kerrick, Dept. of Physiol. & Biophysics, Univ. of Miami, Miami, FL. 33101 and *Dept. of Biochemistry, Univ. of Saskatchewan, Saskatoon, Sask. S7N 0W0, Canada.

SMP-II and SMP-IV were found to increase force and fiber ATPase of chicken gizzard fiber bundles already at steady state tension in submaximal $[Ca^{2+}]$, an effect which was partially reversible upon return of the fibers to the control submaximal $[Ca^{2+}]$ solution. In contrast SMP-I and SMP-III had no effect on force, but decreased the fiber ATPase somewhat. The force results for SMP-IV were in agreement with our previously published study (Hoar, et al., J. Biol. Chem. 260:8760, 1985), but the increase in ATPase would be unexpected if due mainly to the actomyosin ATPase, since more cross-bridges would be in the non- or slowly-cycling latch state. However the presence of SMP-II and SMP-IV could possibly increase the "pseudo" ATPase associated with the phosphorylation-dephosphorylation cycle of myosin light chain-20. Similar effects on submaximal force were observed if the fibers were pretreated with the phosphatases in relaxing solution before exposure to the submaximal Ca^{2+} solution. Supported by NIH grant AR37447, the American Heart Assn. and Florida Affiliate, and Medical Research Council of Canada.

M-Pos483

TWO-DIMENSIONAL PHOSPHOPEPTIDE MAPPING OF THE 20,000-DALTON MYOSIN LIGHT CHAIN IN PHORBOL DIBUTYRATE-CONTRACTED ARTERY. K. Bárány, A. Rokolya and M. Bárány. College of Medicine, Univ. Illinois, Chicago, IL 60612.

The incorporation of ^{32}P-phosphate into LC of a 1-hr PDBu-treated artery was slightly increased as compared to that of a resting muscle. 2D phosphopeptide mapping showed the usual MLCK-phosphorylated peptides (Arch. Biochem. Biophys. 226, 583, 1988) and two PK-C-phosphorylated peptides containing SER-P and THR-P, respectively. This is the first observation for PK-C-phosphorylated THR of LC in intact muscle. PDBu elicited slow tonic contraction; upon addition of K^+ to the 1-hr PDBu-contracted artery the force immediately doubled and LC phosphorylation greatly increased. 2D phosphopeptide mapping of LC from the PDBu/K^+-contracted muscle showed a relative increase in the MLCK-phosphorylated residues and a decrease in PK-C phosphorylated residues as compared to the muscle contracted by PDBu alone. It appears that in phasic contraction the MLCK-induced phosphorylation is dominant, whereas during the tonic PDBu-contraction the PK-C-induced phosphorylation takes place. (Supported by AHA and NIH, AR 34602).

M-Pos484

CLONING OF THE 17,000 DALTON LIGHT CHAIN ISOFORMS FROM VASCULAR SMOOTH MUSCLE. Debra J. Helper, Joseph A. Lash and David R. Hathaway. Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN 46223.

In an earlier study, we identified two isoforms of the 17,000 dalton light chains (LC_{17}) in vascular smooth muscle. These were identified as LC_{17A} (acidic) and LC_{17B} (basic) based upon migration via isoelectric focusing. Moreover, we demonstrated that the V_{max} of actin-activated Mg^{2+} ATPase activity of VSM myosin was proportional to the ratio of the isoforms. Both isoforms have been cloned and sequenced from a bovine vascular smooth muscle cDNA library. The cDNA for LC_{17B} differs from that of LC_{17A} as a result of a 45-nucleotide insert near the encoding 3' end of the cDNA. The insert codes for 9 unique amino acids that constitute the carboxyl terminus: LC_{17A}, ELVRMVLNG; LC_{17B}, AFVRHILSG. An oligonucleotide probe corresponding to the unique LC_{17B} sequence was prepared and used to probe several tissues. These data were compared to conventional 2-dimensional electrophoresis of myosin. Human platelets and porcine stomach were found to contain LC_{17A} exclusively while bovine aorta contained both LC_{17A} and LC_{17B}. Cultured bovine endothelial cells and vascular myocytes contained LC_{17B}. Our results suggest that LC_{17} isoforms cannot be conveniently classified as "muscle" and "non-muscle" since expression appears to be tissue specific.

M-Pos485

PHOSPHORYLATION OF VASCULAR SMOOTH MUSCLE MYOSIN BY PROTEIN KINASE C AND PROTEIN KINASE M. Joseph A. Lash and David R. Hathaway, Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN 46223.

We have reported that protein kinase C (PK_C) can selectively phosphorylate the 204 kDa heavy chain (HC) isoform of vascular smooth muscle (VSM) myosin, although to variable stoichiometry. Using highly purified PK_C, we found very little incorporation into the 204 kDa HC (ie. < 0.1 mol PO_4/mole HC) although up to 3 mole PO_4 was incorporated into the 20,000 dalton light chain subunits (LC_{20}). Cleavage of PK_C with calpain II generated a 51 kDa fragment that was active in the absence of Ca^{2+} and phospholipids (PK_M). PK_M rapidly and specifically phosphorylated the 204 kDa VSM myosin HC as well as LC_{20}. α-chymotrypsin liberated the phosphopeptide by producing limited digestion of the carboxyl terminus of the 204 kDa HC isoform. This was confirmed by comparing peptide sequences to sequence deduced from cDNA's encoding most of the light meromyosin domain of the 200 and 204 kDa isoforms cloned from a VSM cDNA library. The 204 kDa VSM myosin HC cDNA was found to contain a 39 nucleotide insert resulting in a reading frame shift thereby encoding a unique 43 amino acid peptide constituting the carboxyl terminus. Potential PK_C phosphorylation sites in this peptide were identified. The 204 kDa VSM myosin HC possesses a unique carboxyl terminus and phosphorylation sites for PK_C. However, it is a proteolytic fragment of PK_C, PK_M, that catalyzes this phosphorylation.

M-Pos486

MOLECULAR CLONING OF GIZZARD MYOSIN 20kDa LIGHT CHAIN
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It is well known that myosin 20kDa light chain(LC_{20}) plays an important role in the regulation of smooth muscle contractile activity. To analize the details of the molecular mechanism of smooth muscle contraction, we isolated a cDNA clone corresponding to smooth muscle LC_{20} from chicken gizzard lambda gt11 library.

The nucleotide sequence of the isolated cDNA(approximately 1.0 kb) was determind and it was found that the open reading frame of this clone started from serine-2 residue of the known amino acid sequence of gizzard LC_{20}. The amino acid sequence deduced from cDNA sequence confirmed the chemically determined amino acid sequence of chicken gizzard LC_{20}.

The nucleotide sequence which encoded chicken smooth muscle LC_{20} shows very high homology(>85%) to the human vascular smooth muscle LC_{20}.

(Supported by NIH, Syntex, and AHA.)

M-Pos487

DIFFERENTIAL EFFECT OF W7 ON Ca-INDUCED CONTRACTION AND MYOSIN PHOSPHORYLATION IN TRACHEAL SMOOTH MUSCLE. W.T. Gerthoffer, and J. Mangini. Dept. Pharmacology, Univ. Nevada School of Medicine, Reno, NV 89557.

We investigated the effects of the calmodulin antagonist, W7, on myosin phosphorylation induced by muscarinic stimulation of tracheal muscle. Muscles, depleted of external Ca^{2+}, were incubated with 100 μM W7 for 20 min in Ca^{2+}-free solution and then stimulated with 1 μM carbachol in Ca^{2+}-free solution. W7 reduced subsequent Ca^{2+}-induced (0-2.4 mM) contractions to 37% ± 9% of control. In contrast, W7 had no effect on myosin phosphorylation. Ca^{2+}-induced contraction of K^+-depolarized muscles was significantly less sensitive to W7 (70% ± 8% control). Myosin phosphorylation was significantly higher than control at 0.1 and 1.6 mM external Ca^{2+} in the presence of W7. There may be a Ca^{2+}-calmodulin dependent system regulating contraction which is independent of calmodulin-regulated myosin light chain kinase. Muscarinic stimulation may also activate a calmodulin-independent myosin light chain kinase in airway muscle. (Supported by NIH grants HL35805 and DK41315)

M-Pos488

EFFECTS OF ALTERATION OF EXTRACELLULAR CALCIUM ON ACh-INDUCED CHANGES IN INTRACELLULAR CALCIUM. C. C. Shieh, M. Petrini, T. M. Dwyer and Jerry M. Farley, Dept. Pharm. & Tox., Dept. Med. and Dept. Physiol., Univ. of MS. Med. Ctr., Jackson, MS 39216.

Acetylcholine (ACh)-induced alterations in intracellular calcium concentration ($[Ca^{2+}]_i$) in swine tracheal smooth muscle were measured using Fura-2. The effects of removal of extracellular calcium ($[Ca^{2+}]_e$) were determined. A five minute exposure to ACh (0.1 μM) induced a peak increase in $[Ca^{2+}]_i$ of ~140 nM which declined to a steady state of ~120 nM. The resting $[Ca^{2+}]_i$ is 200±30 nM. Removal of $[Ca^{2+}]_e$ caused the resting $[Ca^{2+}]_i$ to decrease by ~20%. The increase in $[Ca^{2+}]_i$ induced by ACh (0.1 μM) after a 10 minute exposure to Ca^{2+}-free solution reached a peak of ~120 nM which then rapidly declined to resting levels of $[Ca^{2+}]_i$. Washout of ACh with Ca^{2+}-free buffer further reduced basal $[Ca^{2+}]_i$. If the ACh is washed out using solution containing Ca^{2+}, $[Ca^{2+}]_i$ returned to control levels however a subsequent response to ACh was enhanced ~40% when compared with the control response. We conclude that the $[Ca^{2+}]_i$ used by ACh are labile and can be decreased by removal of extracellular Ca^{2+} or increased by rapid replacement after depletion. The initial transient response to ACh is dependent on internal stores of calcium. (Sppt: by MS Lung Assoc. and NIDA 05094)

M-Pos489

ION TRANSPORT PROCESSES UNDERLYING SLOW WAVES OF VERTEBRATE INTESTINE.
Lloyd Barr, John Neill and C. Ladd Prosser, Dept. of Physiol. and Biophys. U of I, Urbana.

A system of ion transport processes probably generates the electrical slow waves of vertebrate intestines. In cat circular layer, slow waves might be as large as 35 mV and range from 10 to 20 cycles/minute. Slow waves in vivo trigger action potentials which result largely from a DHP sensitive calcium current and calcium dependent potassium current. We first studied the steady state of ion transport processes, concentrations, membrane potential and cell volume. Three dimensional plots provide steady state values of physiological variables (voltage etc.) as functions of membrane parameters (conductances etc.). System oscillations are complex and very sensitive to Ca current, Ca pump current and Ca dependent potassium current.

M-Pos490

EVIDENCE FOR PRESENCE AND ROLE OF CHLORIDE CHANNEL FLUX IN SMOOTH MUSCLE. Danuta H. Malinowska. Dept. of Physiology & Biophysics, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0576.

Cl^- channels in vascular and non-vascular smooth muscle may be involved in fast depolarization and/or in modulation of action potential duration. Cl^- uptake was measured in plasma membrane vesicles from porcine gastric smooth muscle using the Cl^- sensitive dye, 6-methoxy-N-(3-sulfopropyl)quinolinium, SPQ. Cl^- channel flux was identified by sensitivity of Cl^- uptake to the Cl^- channel blockers diphenylamine-2-carboxylate (DPC) and its analogues. Vesicles, loaded with 50 uM SPQ by freeze-thaw were washed, suspended in 300 mM sucrose, 10 mM Pipes/Tris, pH 7.4 and challenged with 20 mM KCl. Extent of Cl^- uptake was calibrated by Triton-X 100 lysis. Cl^- uptake was rapid and was abolished by 200 μM DPC or 100 μM H131, an analogue of DPC. Furosemide (10^{-4} M) and H_2DIDS (10^{-4} M) had no effect. In rat aorta smooth muscle primary cell cultures, DPC-sensitive Cl^- uptake was also demonstrated. The vasoconstrictor, angiotensin II caused increased Cl^- uptake which was DPC sensitive. These findings support the view that Cl^- channel flux is present, is regulated and thus plays a role in smooth muscle function. H131 was a gift from Hoechst. Supported by DK38808 and AHA SW 89-24.

M-Pos491

SPEED OF SHORTENING IN PREGNANT AND NON-PREGNANT RAT UTERUS.
J.L. Smart and F.J. Julian, Dept. of Anesthesia Research Brigham & Women's Hospital, Boston, MA 02131

Previous studies of force-velocity relationships of pregnant (P) and non-pregnant (NP) rat uterine longitudinal smooth muscle revealed a reduced unloaded speed of shortening (SOS) in P rats (1). In those experiments load clamps were initiated from forces corresponding to 65-75% of maximal force (Fmax). In order to further characterize the pregnancy-associated decrease in SOS we examined the time course of SOS for very light loads in P and NP rat uterus. Strips of longitudinal muscle were dissected from NP rats and P rats late in gestation, mounted between a servo motor and force transducer, and stimulated electrically for 10s. At various times (250ms to 10s) during activation, force clamps were imposed to the level of resting tension using the quick release method. SOS was determined from the interval between 50 and 75 ms after the step. A pattern of an early peak followed by a decline to lower plateau levels was observed for SOS in P and NP rat uterus. Time of peak SOS occurred earlier in strips from NP (0.91±0.10s, n=5) than P (1.21±0.04s, n=11) rat uterus. However, SOS, in muscle lengths/s, at time of peak was similar: 0.825±0.055 (P) and 0.839±0.027 (NP). Slower onset of force generation and faster decay to plateau levels are also observed in P rats. This may explain the decrease in SOS previously observed when releases were made at 65-75% Fmax. These results suggest that when comparing force-velocity relationships of different physiological states in the same tissue it is important to consider the time course of SOS as well as the force from which release is initiated. Supported by NIH HD07008 (JLS) and HL35032 (FJJ).
1) Smart and Julian, Biophys J. 51:337a, 1987.

M-Pos492

THE ASCENDING LIMB OF THE ACTIVE LENGTH VS. TENSION RELATIONSHIP (L:T) IN SINGLE SMOOTH MUSCLE CELLS (SMC's). D. Harris* and D. Warshaw, Physiol & Biophys, U. of Vermont, Burlington, VT. (Intro by J. Peterson) The active L:T may provide information about changes in contractile protein interactions as muscle length changes. To characterize the ascending limb of the active L:T in toad stomach SMC's, cells were attached to a force measurement system, stimulated, and isometric force and stiffness determined at the cell's initial length and shorter. Cells generated maximum force (Fmax) at their isolated rest length (Lcell). At shorter lengths, active tension fell by 1.7Fmax/Lcell. Stretching the relaxed cells up to 1.4Lcell shifted the subsequent active L:T by the amount of the stretch, but did not change the shape or the slope of the L:T. Stiffness analysis showed that at short lengths, force declined as a result of reduced numbers of attached crossbridges. Thus stretching relaxed smooth muscle cells may not alter contractile filament overlap but once activated, shortening reduces the number of attached crossbridges.(Support: NIH AR34872, AHA)

M-Pos493

SLOWING DURING AN ISOTONIC SHORTENING RESPONSE IN SINGLE SMOOTH MUSCLE CELLS (SMC's) IS NOT CAUSED BY REDUCED NUMBERS OF ATTACHED CROSSBRIDGES. D. Harris* and D. Warshaw, Physiol & Biophys, U. of Vermont, Burlington, VT. (Intro by G. Webb) The progressive decline in shortening velocity seen during an isotonic shortening in SMC's may be caused by a continuous reduction in numbers of attached crossbridges. If so, isometric force generating capacity and cell stiffness should decrease during isotonic shortening. SMC's from the toad stomach were attached to a measurement system which controlled either cell length (Lcell) or force. To test if shortening under load reduced subsequent isometric force generating capacity, cells were subjected to a 0.1Lcell/s isovelocity shortening to 0.93Lcell. No effect on isometric force was observed above that caused by a step reduction in length even though isotonic shortening over this length range caused dramatic slowing. Cell stiffness measured during isotonic shortenings did not decline sufficiently to explain the observed slowing on the basis of reduced numbers of attached crossbridges. Thus shortening deactivation and/or reduced cooperativity among contractile proteins could not be the sole cause of slowing during isotonic shortening. (support:NIH AR34872,AHA)

M-Pos494

STRUCTURAL STUDIES ON TURKEY GIZZARD CALDESMON. J.H. Collins,*M.-C. Harricane, J. Leszyk,*C. Cavadere, and #D.Mornet. Dept. Biol. Chem., Univ. Maryland School of Medicine, Baltimore, MD 21201, *CNRS/INSERM U.249, Univ. de Montpellier I, 34033 Montpellier, France, and #INSERM U.300, Fac. de Pharmacie, 34060 Montpellier, France.

We determined the complete sequence of CaD35, a 274-residue, C-terminal thrombic fragment which binds actin, tropomyosin and calmodulin. Residues 1-96 comprise CaD15, an actin-binding subfragment similar to the tropomyosin-binding segment of troponin T. We have raised specific antibodies against CaD35 which strongly recognize CaD15, and when these are added to actin-CaD or actin-CaD15 complexes, we can prepare electron micrographs of bundled filaments in which the antibodies are located. We observe transverse striations at 40 nm intervals, in agreement with a previous study (Lehman et al., J. Muscle Res. Cell Motil. 10, 101-112, 1989) of a 40 kDa chymotryptic fragment. Our results provide further evidence that a CaD fragment, even one as small as CaD15, can induce a torsion which weakens the flexibility of actin filaments and assembles them into bundles. These bundles are, however, more compact when intact CaD or CaD35 is used in place of CaD15.

M-Pos495

POSSIBLE STRUCTURAL DOMAINS OF CALDESMON FROM CHICKEN GIZZARD. Philip Graceffa & Agnes Jancso, Boston Biomed. Res. Inst., Boston.

Caldesmon (CaD) is a long, thin, flexible monomeric molecule with 756 amino acids. While Chou-Fasman analysis predicts an α-helix content of about 80% (1), it has been reported to be only 10% (2). Therefore we reinvestigated the CD spectra of CaD and its fragments from thrombic cleavage at residue 483 (3). CaD has about 40% α-helix content at 20° and the N- and C-terminal fragments have about 50% and 20%, respectively. The thermal melting of the α-helical structure of CaD occurs reversibly and in a gradual, linear fashion without any steep, cooperative transitions. This accounts for the high thermal stability of CaD. The melting of the N-terminal fragment is more steep than that of the whole molecule and is only partially reversible. The C-terminal fragment melts more gradually than the intact molecule and the unfolding is completely reversible. These results suggest that the two fragments represent separate structural domains, with the C-terminal part having less periodic structure with greater thermal stability. (1)Bryan et al. JBC (1989). (2) Lynch et al. JBC (1987). (3) Leszyk et al. BBRC (1989). Supported by NIH AR-30917.

M-Pos496

SMOOTH MUSCLE MYOSIN BINDS THE N-TERMINAL REGION OF CALDESMON. C.-L. Albert Wang, Adelaida Carlos and Renne C. Lu, Dept. of Muscle Research, Boston Biomedical Research Inst., 20 Staniford St., Boston, MA 02114

Caldesmon (CaD) is a major actin associated protein found in smooth muscle and many non-muscle cells, which also binds to calmodulin in the presence of Ca^{2+} and to tropomyosin. Recent evidence has shown that CaD interacts directly with smooth muscle myosin (Hemric & Chalovich, *J.B.C.* 263, 1878, 1988; Ikebe & Reardon, *J.B.C.* 263, 3055, 1988). In an attempt to locate the myosin-binding domain on CaD we have treated CaD with a number of proteolytic enzymes, and subjected these digestion mixtures to a myosin-Sepharose affinity column. The fractions retained to the column were eluted with high salt buffer, separated on $NaDodSO_4$ gel, and electrophoretically transferred onto an Immobilon (PVDF) membrane. The protein bands were then cut out and subjected to N-terminal sequence analysis. We found that chymotrypsin, papain and CNBr cleavages all resulted in myosin-binding fragments that began with residues in the peptide segment between Asp-2 and Gln-28, suggesting that the major myosin-binding domain is located in the N-terminal portion of the CaD molecule. (Supported by grants from NIH, AHA and MDA)

M-Pos497

MEASUREMENT OF INTRACELLULAR pH FROM STRIPS OF SMOOTH MUSCLE.

C. D. Foster, T. W. Honeyman & C. R. Scheid.
Dept. of Physiology, Univ. of Mass., Medical Centre, Worcester, MA.

To develop a technique for simultaneous measurements of tension and intracellular ion levels in the same population of coupled smooth muscle cells, we utilized strips (1 x 12mm) of toad gastric muscle loaded with the pH sensitive dye BCECF (14μM). Strips were held at a fixed length within a 3ml cuvette and fluorescence emission was monitored at 530nm. The spectrofluorimeter was operated in the ratio mode and the excitation wavelength alternated between 450 and 500nm. Tissues were maintained by perifusing with bicarbonate containing buffers. The introduction of ammonium chloride (40mM) induced a rapid alkalinization (pH 7.3). Washout of ammonia caused rapid acidification (pH 6.9). Restoration of pH was Na^+ dependent and sensitive to amiloride analogues, features characteristic of Na^+/H^+ exchange. This technique allows for "*on-line*" measurements of cell pH from an intact tissue segment under physiological conditions and should prove useful for assessing the involvement of various transport systems (Na^+/H^+ vs HCO_3^-/Cl^-) in cell pH regulation and for assessing changes in other cytosolic ions. Supported by HL 41188.

M-Pos498

POSSIBLE INVOLVEMENT OF ACTOMYOSIN ADP COMPLEX IN REGULATION OF Ca^{2+} SENSITIVITY IN α-TOXIN PERMEABILIZED SMOOTH MUSCLE.

Junji Nishimura and Cornelis van Breemen, Department of Pharmacology, University of Miami, School of Medicine, Miami, Florida 33101

The effects of substrate condition and ADPβS on the pCa^{2+}-tension relationships were investigated, using α-toxin permeabilized rabbit mesenteric artery at 37°C. The Ca^{2+} sensitivity was greatly affected by the substrate condition and increasing the ratio of ATP/CP induced a leftward shift of the pCa^{2+}-tension curve. Addition of 100 μM ADPβS had a similar effect. When the ATP/CP ratio was high, the 0.1 μM Ca^{2+} solution relaxed the tissue precontracted by 10 μM Ca^{2+} solution more slowly showing hysteresis. One mM vanadate, which is reported to relax muscle by forming actomyosin (AM)-ADP-vanadate complex, completely inhibited both contractions induced by 0.18 μM Ca^{2+} solution containing 2 mM MgADP and by 0.3 μM Ca^{2+} solution containing 0.3 μM phorbol 12,13 dibutyrate. These results indicated that the population of AM-ADP complex in the crossbridge had increased due to the accumulation of ADP inside the tissue or activation of PKC and that the inhibition of ADP release from AM-ADP complex may be playing a key role in increasing Ca^{2+} sensitivity of myofilaments.

M-Pos499

NON-HOMOGENEOUS Mn^{2+} QUENCH OF FURA-2 SIGNALS IN CULTURED ARTERIAL MYOCYTES VISUALIZED WITH DIGITAL IMAGING. W.F. Goldman and M.P. Blaustein, Physiology Dept., Univ. of Maryland Med. School, Baltimore, MD 21201

Mn^{2+} has been used to quench extracellular fura-2 signals, although it is known to enter cells through a variety of pathways. We employed digital imaging to study the effects of Mn^{2+} on intracellular fura-2 signals in cultured A_7r_5 cells. The dye was excited at 360 and 380 nm, and the apparent intracellular free Ca^{2+}, $[Ca^{2+}]_{App}$, was determined using ratio methods. Exposure to 2 mM Mn^{2+} induced an initial rise in $[Ca^{2+}]_{App}$ in unstimulated cells that lasted about 20-30 sec. The dye signal then declined in the cell periphery and nucleus, whereas it was more stable in the organelle-rich perinuclear region. Quench of the fura-2 signal was more rapid when cells were depolarized with K-rich media containing Mn^{2+} and Ca^{2+}. However, $[Ca^{2+}]_{App}$ then rose slowly and remained elevated after normal $[K^+]_o$ was restored. These data indicate that Mn^{2+} enters the cells and has complex effects on the fura-2 signals and $[Ca^{2+}]_{App}$. Thus, considerable caution must be used in interpreting fura-2 data when Mn^{2+} is used to quench extracellular dye.

M-Pos500

SPATIAL AND TEMPORAL RESOLUTION OF THE RISING PHASE OF Ca^{2+} TRANSIENTS IN CULTURED ARTERIAL MYOCYTES. W.F. Goldman and M.P. Blaustein, Physiology Dept. Univ. of Maryland Med. School, Baltimore, MD 21201

Ca^{2+} transients (1-2 μM) evoked by serotonin (5-HT) in cultured A_7r_5 cells were studied using fura-2 and digital imaging microscopy. Sequences of video frames of fura-2 fluorescence excited at 360 and 380 nm were acquired and written to a magnetic disc at video frame rates. The apparent intracellular free Ca^{2+}, $[Ca^{2+}]_{App}$, was calculated for individual frames using the ratio method. This enabled us to determine the distribution of $[Ca^{2+}]_{App}$ with a temporal resolution of 33 msec. The onset of the Ca^{2+} transients were manifested first in the periphery as small regions of elevated $[Ca^{2+}]_{App}$ at edges of the cells. This occurred within 2 frames (67 msec) after 5-HT was ejected from a microtube applicator onto the cell surface. The area of elevated $[Ca^{2+}]_{App}$ subsequently expanded until about 600 msec, when peak $[Ca^{2+}]_{App}$ levels were present in virtually all of the non-nuclear regions of the cells. The nucleus was the last area to be affected. Peak $[Ca^{2+}]_{App}$ in the nucleus was only 50-60% of that observed in other regions. This suggests that there is a barrier to diffusion of Ca^{2+} into the nucleus.

M-Pos501

DISTRIBUTION OF CA^{2+}-HANDLING SITES IN SINGLE SMOOTH MUSCLE CELLS.

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The juxtaposition of proteins and organelles responsible for Ca^{2+}-handling is an important determinant of the distribution and time course of changes in cytosolic $[Ca^{2+}]$ which regulate contraction in smooth muscle. Therefore, indirect immunofluorescent staining techniques were used on isolated toad stomach cells to determine the distribution of a plasmalemmal Ca^{2+}-ATPase, the high capacity Ca^{2+}-binding protein calsequestrin (CSQ) and an ER Ca^{2+}-ATPase. 2-D images of immunofluorescence patterns were acquired using confocal microscopy. 3-D images, constructed using computer graphics, were studied to determine the orientation of fluorescent structures inside the cell. Cells labeled with either an antibody against canine cardiac CSQ, one against rabbit cardiac ER Ca^{2+}-ATPase, or the membrane dye $DiOC_6(3)$ showed flourescent strands, aligned parallel to the cell's long axis, mostly near the cell's surface. This labeling pattern suggests that Ca^{2+} storage sites in these smooth muscle cells are organized near the cell's surface.

M-Pos502

MOVEMENT OF CALCIUM IN SMOOTH MUSCLE CELLS: A MODEL BASED ON CURRENT INFORMATION

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A number of processes have been proposed as putative regulators of Ca^{2+} in smooth muscle. While the biological properties of these processes have been studied separately and some functional information can be inferred from such studies it is still not well known how the various parts of the Ca-regulatory system are integrated into an overall homeostatic mechanism. To examine this question and to study the temporal and spatial variations in Ca^{2+} that may occur in smooth muscle cells during activation, we developed a computer model of the cell that included several of the major processes thought to be involved. The cell was assumed to be radially symmetric and the equations for Ca^{2+} diffusion within the cell and through the extracellular space and plasma membrane were solved with an explicit-finite-difference formula. The model included a time dependent change in membrane permeability that governed Ca^{2+} influx and terms describing the plasma membrane Ca-pump and Ca^{2+} release and re-uptake by the sarcoplasmic reticulum. Where possible these processes were modelled with parameters available from the literature. Simulations showed that the plasma membrane Ca-pump could be responsible for the calcium decline following stimulation seen in experiments with Fura-2. On the other hand, the pump appeared to have little influence on the initial rate of rise of intracellular Ca^{2+} immediately after activation. The model also showed that a lower rate of intracellular diffusion could give rise to spatial inhomogeneities of Ca^{2+} in cells especially if the diffusion space near the plasma membrane was limited. Supported by NIH AR39678 and HL14523.

M-Pos503

MECHANISM OF GTPγS-DEPENDENT REGULATION OF SMOOTH MUSCLE CONTRACTION.

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It has recently been proposed that G proteins regulate smooth muscle contractile elements. In saponin skinned tracheal smooth muscle GTPγS enhanced the force response to submaximal Ca^{2+}. The increased tension was associated with a proportional increase in myosin light chain phosphorylation. Two-dimensional phosphopeptide mapping showed phosphorylation primarily at the serine site phosphorylated by myosin light chain kinase (MLCK) in the presence and absence of GTPγS. GTPγS had no effect on the Ca^{2+} sensitivity or total activity of purified tracheal MLCK. GTPγS enhanced the contractile amplitude induced by Ca^{2+}/calmodulin-independent MLCK in the absence of Ca^{2+}, but did not affect the contractile response to okadaic acid, a protein phosphatase inhibitor. These results indicate that GTPγS may inhibit myosin light chain phosphatase activity, increase light chain phosphorylation and thereby enhance smooth muscle contraction. Supported in part by HL23990 and HL32607.

M-Pos504

PHOSPHORYLATION OF SMOOTH MUSCLE MYOSIN LIGHT CHAIN KINASE. M.G. Tansey, K.E. Kamm and J.T. Stull, Dept. of Physiol., UT Southwestern Med. Ctr., Dallas, TX

Purified myosin light chain kinase (MLCK) is phosphorylated by different protein kinases. Phosphorylation at site A, identified by phosphopeptide mapping, leads to an increase in the K_D for calmodulin (K_{CM}). Carbachol and KCl increased force and the extent of phosphorylation at site A by 5 min to 0.71 and 0.8 mol ^{32}P/mol peptide, respectively. In contrast, site A was not phosphorylated in relaxed or PDBu-treated muscle where force was low. Site A phosphorylation correlated to an increase in K_{CM} as estimated by the ratio of MLCK activities in tissue homogenates measured at 1 and 100 μM free Ca^{2+} and 0.4 μM CaM (Miller *et al.*, *Mol. Pharmacol.*, 24:235, 1985). Relaxed (0.64 ± 0.04) and PDBu-stimulated muscle (0.57 ± 0.04) had similar activity ratios. The activity ratio of MLCK was decreased in muscles stimulated with carbachol (0.44 ± 0.02) or KCl (0.33 ± 0.04). Thus, MLCK is phosphorylated in site A with a resultant increase in K_{CM} in contracting tracheal smooth muscle. This phosphorylation would be expected to desensitize MLCK to activation by calcium.

M-Pos506

INWARD CURRENT IN FRESHLY DISPERSED RAT MYOMETRIAL CELLS. M. Yoshino, S.Y. Wang, and C. Y. Kao. Department of Pharmacology, SUNY Downstate Med Ctr Brooklyn, NY 11203.

Whole-cell recordings with Cs^+-filled patch-clamp electrodes (with 1 mM EGTA) show both I_{Na} and I_{Ca} in fresh myocytes from 17-21 day pregnant rat uterus. From HP of -60 mV, I_{Ca} starts at -30 mV, is maximal at 10 mV, and reverses at 80 mV. At 22°C, it peaks at 10 ms, and inactivates with τ of 40 ms. $[Ca^{2+}]_o$ influences its magnitude and reversal potential. Nisoldipine (2 μM) and Co^{2+} (5 mM) block it, and BAY-K 8644 (2 μM) increases it. Half-inactivation is at -34 mV; half-activation at -7 mV. These curves overlap from -30 to 10 mV, showing the presence of a window-current. Inactivation is also markedly affected by $[Ca^{2+}]_i$.

From HP of -80 mV, I_{Na} starts at -30 mV, is maximal at 0 mV, and reverses at 40 mV. It peaks at 3 ms and inactivates with τ of 0.8 ms. It is absent at HP of -30 mV and is blocked by TTX (1 μM). The ratio I_{Ca}/I_{Na} is 2.6 in preterm, and 0.8 in term uterus. So, Na^+ and Ca^{2+} both contribute to the inward current in uterine myocytes, and I_{Na} plays an increasing role in the genesis of fast-rising spikes as parturition approaches. (Supported by NIH grant HD00378).

M-Pos505

TROPOMYOSIN MODULATES THE KINETICS OF THE CALDESMON-INDUCED INHIBITION OF THE GIZZARD ACTO-HMM ATPase. **Kurumi Y. Horiuchi, Mathew Samuel, and Samuel Chacko, Department of Pathobiology, University of Pennsylvania, Philadelphia, PA 19104.**

The inhibition of smooth muscle acto-heavy meromyosin (acto-HMM) ATPase by caldesmon occurs while there is an increased binding of the HMM to the actin, presumably mediated through an interaction between the S-2 region of the HMM and the caldesmon which is bound to actin. This non-productive binding of HMM to actin can be eliminated by utilizing a 38-kDa chymotryptic fragment of caldesmon (38-kDa) that binds to actin and inhibits the actomyosin ATPase. Using the 38-kDa, experiments were carried out to understand the mechanism for the inhibition of gizzard acto-HMM ATPase by caldesmon. The binding of HMM to actin was decreased by 38-kDa both in the presence or in the absence of tropomyosin. The 38-kDa diminished K_{ATPase} (50%) in the absence of tropomyosin, but it had no effect on V_{max}. In the presence of tropomyosin, the 38-kDa caused a 2-fold decrease in the V_{max} with a slight decrease in K_{ATPase}. These data suggest that the tropomyosin plays an important role in the caldesmon-induced inhibition of acto-HMM ATPase. (Supported by HL 22264 & DK 39740)

M-Pos507

SEDIMENTATION, FILAMENT ASSEMBLY, AND ACTIN-ACTIVATED ATP HYDROLYSIS BY SMOOTH MUSCLE MYOSIN. **Mathew Samuel, Prokash K. Chowrashi, Frank A. Pepe, and Samuel Chacko, Departments of Pathobiology and Anatomy, University of Pennsylvania, Philadelphia, PA. 19104.**

Effects of sedimentability, turbidity and filament assembly of myosin on the actin-activated ATP hydrolysis were examined using myosin isolated from the muscular coat of the pig urinary bladder. Raising the free Mg^{2+} increases the turbidity and sedimentability of both phosphorylated (P) and unphosphorylated (U) myosins, and it causes a slight increase in the actin-activated ATPase activity of the U myosin; however, the activity of the P myosin is markedly increased (4 to 10-fold). As seen in electron microscopy, the P myosin assembles into bipolar filaments with tapered ends under the conditions of the ATPase assay while the U myosin forms unorganized sedimentable aggregates of myosin molecules. These data suggest that although the U myosin and the P myosin have the same level of sedimentability and turbidity, the filament assembly present only with the P myosin can be associated with the maximal actin-activation of the Mg-ATPase. Supported by NIH grants DK 39740 & HL 22264 to S.C. and MDA grant to F.A.P.

M-Pos508

THE ROLE OF MYOSIN PHOSPHORYLATION AND FILAMENT FORMATION IN THE REGULATION OF SMOOTH MUSCLE MYOSIN

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The ability of smooth muscle myosin to adopt several monomeric forms (6S and 10S) in addition to filaments has complicated the study of its regulation by phosphorylation. To overcome this difficulty we have cross-linked unphosphorylated turkey gizzard smooth muscle myosin filaments with EDC which results in filaments that are stable in the presence of MgATP and do not depolymerize at high ionic strength. The K^+EDTA and Ca^{++}ATPase activities do not differ from control uncross-linked myosin. The actin activated MgATPase activity of cross-linked phosphorylated myosin is also the same as that of uncross-linked phosphorylated myosin, but that of unphosphorylated cross-linked myosin is activated 2.6 times higher than that of its control. The phosphorylated cross-linked myosin but not the unphosphorylated cross-linked myosin moves actin filaments in the sliding actin filament assay though the nature of movement suggests that there are some rigor-like heads that are created during the cross-linking. In contrast, we find that some preparations of uncross-linked unphosphorylated smooth muscle myosin translocate a fraction of the actin filaments in a manner that is qualitatively and quantitatively different from that of phosphorylated myosin. In addition we find that smooth muscle myosin monomers can also support movement of the actin filaments. The possible implications of these results will be discussed.

M-Pos509

QUANTITATION OF MYOSIN 20 kD LIGHT CHAIN PHOSPHORYLATION USING COLLOIDAL GOLD STAINED WESTERN BLOTS.

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We examined several methods for protein detection following two-dimensional gel electrophoresis to quantitate myosin light chain phosphorylation in small (10 μg wet weight) smooth muscle tissue samples. These measurements require a linear response in the range of 1 to 100 ng LC_{20}. Colloidal gold staining of nitrocellulose blots was a significant improvement over silver staining of gels or radioimmunoblotting with LC_{20} specific antisera. This was due to a fortuitous high affinity of colloidal gold for myosin light chains relative to most other proteins and a linear response over nearly 3 orders of magnitude. Phosphorylation values measured by this method closely match those obtained from scanning Coomassie Blue R250 stained gels at 100-fold higher loadings. This technique will facilitate the measurement of phosphorylation in small tissue samples, isolated cells or in cultured cells. Supported by USPHS grant 5-P01-HL19242.

M-Pos510

POSSIBLE CAUSE OF DISCREPANCIES IN POTENCIES OF POTASSIUM CHANNEL ACTIVATORS IN VASCULAR SMOOTH MUSCLE: ROLE OF CONTRACTING AGENT ON RELAXATION.

Diane Henry Normandin and John R McCullough (intro. by Stephen F Flaim), Department of Pharmacology, Squibb Institute for Medical Research, Princeton, NJ 08543-4000.

The sensitivity of relaxation responses for the potassium channel activators cromakalim and pinacidil were equivalent in aortic rings contracted by equieffective concentrations of phenylephrine or methoxamine, although higher concentrations of methoxamine were needed to cause contractions. The IC50 values for cromakalim were 0.06 μM and 0.06 μM versus phenylephrine (0.01 μM) and methoxamine (0.3 μM), respectively; for pinacidil 0.19 μM and 0.07 μM, respectively. At higher concentrations of phenylephrine and methoxamine, the concentration relaxation response curves to cromakalim and pinacidil were shifted to the right. The maximum relaxation to cromakalim, but not pinacidil was attenuated. In the portal vein the IC50 values obtained using longitudinal strips were approximately 4-fold more potent than the values obtained using intact veins. These IC50 values were equivalent to those seen using submaximal concentrations of phenylephrine or methoxamine. These results suggest that similar potassium channels are present and functional throughout the vasculature. In addition, the ability of pinacidil but not cromakalim, to completely relax maximally contracted tissues suggests an additional mechanism of action for pinacidil which may be indirectly dependent upon the mechanism of calcium movement into the cell and/or the release of intracellular calcium.

M-Pos511

RELAXATION OF AIRWAY SMOOTH MUSCLE (ASM): ISOTONIC.

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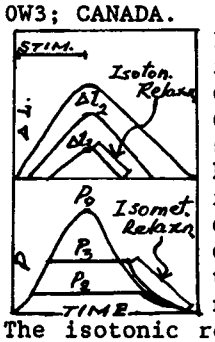

If the major function of ASM is to regulate airway conductance, then length change must be studied in studying relaxation in vitro. Force-velocity data are shown in the figure. After stimulus cessation, isotonic relaxation occurs, followed by isometric, the latter representing resolution of wall stiffness. The isotonic relaxation records were curve fitted and relaxation rate constants obtained. These were, however, load dependent; therefore the constants were plotted against load and a load-independent value of the constant obtained. This will enable comparison of isotonic relaxation in antigen sensitized and control ASM. Using abrupt load clamping at the peak of a lightly loaded isotonic contraction, a relaxation constant could be obtained that was not only load-independent but also independent of initial contractile element length. The contribution to early relaxation from the compressed "internal resistor" was also evaluated and found to be significant. (Supported by the Manitoba Lung Association.)

M-Pos512

SMOOTH MUSCLE ENERGETICS: COMPATIBLE WITH "LATCH"? Richard J. Paul, Colin Gibbs and Igor Wendt. Depts. of Physiology and Biophysics, University of Cincinnati, Col. of Medicine, 45267-0567 USA and Monash University, Clayton, 3168, Australia.

Hai and Murphy's (AJP 254:C99, 1988) model ascribes the high economy of smooth muscle to a slow crossbridge detachment if dephosphorylated when attached to actin ("latch state"), and its lower efficiency to a high rate of myosin phosphorylation/dephosphorylation (MLC p/dp) ATPase relative to crossbridge ATPase, which is yet controversial (Ann. Revs. Physiol. 51:331, 1989). Using SCoP (Nat. Biomed. Simulation Res., Duke U.) we tested: 1) is a high rate of MLC p/dp ATPase necessary? 2) Is latch required, particularly to fit declining MLC-P_i with increasing stress? 3) Is this model compatible with the observed linearity between steady state J_{ATP} and stress? Our answers in all cases are negative. We tested whether MLC p/dp ATPase is the major energy cost using measurements of heat production (Q) in living and "skinned" guinea pig taenia coli. Comparison of Q in KCl contractures to those in skinned fibers elicited by Ca^{2+} or ATPγS indicates that crossbridge ATPase is the major cost in intact smooth muscle. Moreover when stress in skinned fibers was varied by controlled exposure to ATPγS, velocity was a function of stress (number of γS-bridges), which is not consistent with the latch model. A high attachment:detachment rate ratio for smooth muscle actin-myosin interaction may explain these observations. Supported by NIH HL 23240, HL22619 & Fogarty Senior Int. Fellow. (RJP).

M-Pos513

EFFECTS OF KINASE INHIBITORS H8 AND ML9 ON MYOSIN PHOSPHORYLATION (MLC-P_i), ISOMETRIC FORCE (F_o), AND SHORTENING VELOCITY (V_{us}) IN SKINNED GUINEA PIG TAENIA COLI (TC). J.D. Strauss and R.J. Paul. Dept. of Physiology and Biophysics, College of Medicine, University of Cincinnati, OH 45267-0576.

An isoquinoline derivative (H8) and a diazepine-derived compound (ML9) are inhibitors reported to be more specific for myosin light chain kinase (MLCK) than other protein kinases (Hidaka et al., Biochem. 23:5036, 1984 and Saito et al., J. Biol. Chem. 262:7796, 1987). Both reduced F_o and V_{us}, however, the dose-response curves were markedly dissimilar. H8 (μM) significantly inhibited V_{us}, however, F_o was greater than 50% of control even at [mM H8]. These effects were reversible. ML9, in contrast, had similar effects on V_{us}, but F_o was also reduced in a parallel manner. H8 inhibited thiophosphorylated, Ca^{2+}-insensitive fibers, indicating a mechanism that may not involve MLCK. No changes in MLC-P_i were measured, further suggesting that H8 is ineffective against MLCK in these fibers. The inhibition by H8 was dependent on [ATP] suggesting that H8 acts directly on the actomyosin ATPase. Consistent with its reported mechanism of action, the effects of ML9 were also ATP dependent. However, ML9 was not effective in thiophosphorylated fibers and reduced MLC-P_i. In skinned TC, H8 may be an effective probe of the actin-myosin interaction whereas ML9 is likely to act through MLCK. Supported by NIH HL22619, TG HL07571, and the Albert Ryan Foundation (JDS).

M-Pos514

G PROTEINS MAY REGULATE THE [Ca^{2+}]-SENSITIVITY OF MYOSIN PHOSPHORYLATION IN INTACT SWINE CAROTID ARTERY. Christopher M. Rembold. Dept. of Int. Med., Univ. of VA, Charlottesville, VA 22908 USA

Agonist stimulation of vascular smooth muscle, when compared to high KCl depolarization, is associated with an increase in [Ca^{2+}]-sensitivity of phosphorylation (MP). I evaluated myoplasmic [Ca^{2+}] with aequorin and MP with 2D IEF/SDS electrophoresis in swine carotid media stimulated by NaF, a nonspecific activator of G proteins. The relative [Ca^{2+}]-sensitivity of MP depended on the type of stimulus (ranked high to low sensitivity): histamine = mixture of histamine and NaF > NaF alone = mixture of NaF and KCl = mixture of histamine and KCl > KCl alone. The addition of either histamine or NaF (an activator of G proteins) to KCl depolarized tissues produced similar increases in the [Ca^{2+}]-sensitivity of MP, suggesting that NaF-dependent activation of G proteins can mimic contractile agonist induced increases in the [Ca^{2+}]-sensitivity of MP.

M-Pos515

HETEROGENEOUS DISTRIBUTION OF CALDESMON AMONG THIN FILAMENTS IN SMOOTH MUSCLE Joe R. Haeberle and David R. Hathaway, Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405

Recent studies by Lehman et al. (J.Mus.Res.Cell Motil., 10:101-112, 1989) suggest that two populations of actin filaments are present in chicken gizzard smooth muscle: one with and one without caldesmon. We have measured the relative amounts of actin and caldesmon present in whole muscle extracts of vascular, uterine, and intestinal smooth muscles and in native thin filaments which have been purified from vascular smooth muscle by immunoprecipitation with anti-caldesmon antibodies. For whole muscle extracts, proteins were extracted by boiling in SDS and actin was quantified by densitometric scanning of Coomassie blue-stained gels. Caldesmon was measured by quantitative immunostaining of nitrocellulose blots. Native thin filaments were purified by low salt extraction of glycerinated vascular smooth muscle in the presence of MgATP and Triton X-100. The molar ratios of caldesmon:actin (Mr actin = 43,000, caldesmon = 87,000) for the whole tissue homogenates were: 1:82 - bovine aorta, 1:85 - porcine carotid artery, 1:41 - rat uterus, and 1:17 - rabbit ileum; the molar ratio for the carotid artery native thin filaments was 1:15. This suggests that there may be a significant population of caldesmon-free filaments in vascular smooth muscles which could be as high as 80% of the total actin filament population. In contrast, a much larger portion of the thin filaments in the rabbit ileum must contain caldesmon. These findings suggest that caldesmon may play a more prominent role in the regulation of uterus and ileum smooth muscles than tonic vascular smooth muscles like the carotid artery and aorta.