TU-PM-SymI-1 REGULATION OF SMOOTH MUSCLE MYOSIN MOVEMENT BY PHOSPHORYLATION.
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This symposium will deal with several aspects of smooth muscle contractility ranging from biochemical characterization of the myosin molecule to studies of intact fiber strips. Smooth muscle contraction is initiated by a rise in sarcoplasmic calcium which results in a transient phosphorylation of the 20 kDa light chain of myosin. This results in a rapid cycling of myosin crossbridges and tension production followed by a period of tension maintainance by slowly cycling, sometimes dephosphorylated myosin crossbridges. This state has been termed "latch" by Murphy and colleagues. It is a state where force can be maintained by slowly cycling crossbridges. The latch state can be maintained even under conditions in which most of the myosin molecules have been dephosphorylated indicating that there is not a simple relationship between myosin phosphorylation and tension production and suggesting that other proteins may be involved in the regulation of smooth muscle contraction.

In my presentation I will discuss the kinetics of the interaction of smooth muscle myosin with actin and also the regulation of the movement of myosin over actin using an in vitro motility assay which appears to simulate the unloaded shortening velocity of a fiber strip. Using this system we can compare biochemical measurements such as MgATPase activity with the rate at which myosin filaments can slide past actin filaments and study the effects of phosphorylation at various residues on the myosin light chain by myosin light chain kinase and protein kinase C. I will also discuss the in vitro effects of caldesmon, an actin binding protein, on smooth muscle regulation.

TU-PM-SymI-2 ENZYMATIC PROPERTIES AND THE CONFORMATION OF SMOOTH MUSCLE MYOSIN. by David J. Hartshorne, Muscle Biology Group, University of Arizona, Tucson, Arizona 85721.

Phosphorylation of serine 19 on the 20,000-dalton myosin light chains increases actin-activated ATPase and is assumed to reflect an increased rate of cross-bridge cycling. Smooth muscle myosin, unlike myosins from striated muscle, can exist in two conformations, a folded 10S state and an extended 6S state. Under suitable ionic conditions phosphorylation of myosin will induce the 10S to 6S transition. The 10S and 6S myosins have distinct enzymatic properties and this is a key point in the hypothesis that the conformation of myosin dictates enzymatic properties and the role of phosphorylation is to modify conformation. It is proposed (Ikebe, M., Hinkins, S., and Hartshorne, D.J. [1983] Biochemistry 22, 4580-4587) that the critical conformational changes occur as part of the 10S-6S transition. The obvious question is which areas of the molecule are altered during the 10S-6S transition and are important in determining enzymatic activity? Using limited proteolysis as a conformational probe several changes were detected during the 10S-6S transition. Two were of particular interest and were localized at the S1-S2 (head-neck) junction, Site B, and at, or close to, the putative actin-binding site, Site A. Site B is thought to be important in determining ATPase activity and a working hypothesis is that phosphorylation alters the conformation at Site B and converts the "inactive" constrained myosin heads to a more flexible "active" state. Several approaches are being investigated to measure the mobility of the S1 units under various conditions. Site A is located at the junction of the central and the C-terminal S1 domains and whether changes at this site can alter actin-affinity is of considerable interest. Supported by NIH grants HL23615 and HL20984.

TU-PM-SymI-3 Ca^{2+}, CROSSBRIDGE PHOSPHORYLATION, AND REGULATION OF THE LATCH STATE. R.A.Murphy, C-M.Hai C.M.Rembold. Dept. of Physiol., Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

We have hypothesized that Ca^{2+}-stimulated crossbridge phosphorylation is both necessary and sufficient to regulate contraction in smooth muscle. However, it is necessary to invoke a type of crossbridge interaction not present in striated muscle to explain the special properties of smooth muscle. These include (1) somewhat low efficiency but high economy of force maintenance compared with striated muscle, and (2) regulation of average crossbridge cycling rates as estimated by shortening velocity at zero load (V_o). Thus we propose a "latchbridge" defined as an attached, force-generating crossbridge which is (1) non-cycling, (2) has a slow detachment rate, and (3) arises by dephosphorylation of an attached (cycling) crossbridge (Hai and Murphy, Am. J. Physiol. 254 (Cell Physiol. 23): Jan., 1988, in press). Two tests of the model's prediction provide further support for the hypothesis. First, experimental measurements show that (1) agonist-induced steady-state changes in myoplasmic [Ca^{2+}] (estimated by aequorin) are correlated with changes in crossbridge phosphorylation, and that (2) there are invariant relationships between phosphorylation and steady-state stress (an estimate of attached crossbridges) and with V_o (an estimate of average crossbridge cycling rates). Second, theoretical studies showed that the addition of a latchbridge to a two-state(free and attached) crossbridge model (which predicted the unique force-velocity relationship characteristic of striated muscle; A.F. Huxley, Prog. Biophys. Mol. Biol. 7: 255-318, 1957) quantitatively predicts (1) the experimentally determined, phosphorylation-dependent family of force-velocity curves describing smooth muscle, and (2) the observed direct dependence of V_o on phosphorylation. Supported by PHS 5 PO1 HL19242 and 5 T32 HL07355, the Lucille P. Markey Charitable Trust and the American Heart Association, Virginia Affiliate.

TU-PM-SymI-4 CROSS-BRIDGE CYCLING IN SMOOTH MUSCLE FOLLOWING LASER FLASH PHOTOLYSIS OF CAGED SUBSTRATES. A.V. Somlyo, Y.E. Goldman, T. Fujimori, M. Bond, *D.R. Trentham and A.P. Somlyo, University of Pennsylvania School of Medicine, Philadelphia, PA and *MRC London, U.K.

The kinetics of the cross-bridge cycle in smooth muscle have been explored by releasing nucleotides through photolysis of "caged" precursors in guinea pig portal vein permeabilized by freeze glycerination. These results and work in progress on the effects of $[Ca^{2+}]$ and of the phosphatase inhibitor, okadaic acid (1), will be reviewed.

Liberation of 3 to 50μM ATP from caged ATP revealed cooperative attachment of unphosphorylated cross-bridges in the absence of Ca^{2+}. In the presence of Ca^{2+}, liberation of 0.5-1mM ATP caused force to develop at $0.4s^{-1}$, a rate similar to that in intact, electrically stimulated muscle. Force development was an order of magnitude faster in muscles that were thiophosphorylated with $ATP\gamma S$ prior to the photochemical liberation of ATP, indicating that physiological force development is rate limited by light chain phosphorylation rather than by intrinsic cross-bridge properties. The rate of force development following the release of ATP in muscles with thiophosphorylated light chains was not significantly affected by the presence or absence of 30μM Ca^{2+}. In conclusion, laser flash photolysis, in addition to its value for kinetic studies, revealed a new property, cooperativity, in vertebrate smooth muscle. We suggest that cooperativity between phosphorylated and non-phosphorylated cross-bridges may be responsible for high tension states attained at low levels of myosin light chain phosphorylation.

(1) Takai, A., C. Bialojan, M. Troschka and J.C. Ruegg. FEBS LETT. 217:81, 1987.

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