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MUSCLE CONTRACTILITY AND PROTEIN TURNOVER

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Report on the Arizona Muscle Symposium

"Muscle Contractility and Protein Turnover"

Goal: To provide a diversified program offering various lectures concerning muscle research with particular emphasis on contraction and turnover of muscle proteins.

Attendance: Researchers from within and outside the University of Arizona attended the Symposium. A total of approximately 125 persons attended the meeting at various times throughout the two days it was held. Besides the speakers from other Universities, there were 39 scientists from elsewhere including 5 from foreign countries. Despite the beautiful weather Saturday afternoon total attendance at the poster sessions was quite good (40-45 people).

Summary of Presentations (see attached program for titles of the talks):

A. Skeletal Muscle Session

Dr. Susan Lowey: Recent studies have considered the nature of the bending in the rod portion of the myosin molecule obtained from calf aorta. Two components of the rod have been identified which have sedimentation constants of 6S and 10S. Under low salt conditions, myosin rod polymers are converted to 10S monomers while higher salt treatment yields the 6S form. It is believed that the 6S monomer is an unbent form of the 10S monomer; the unbending producing changes in hydrodynamic properties of the rod. Other data suggest that the nonphosphorylated myosin generates a nonfunctional 10S whereas phosphorylation makes it functional. Phosphorylation alone, however, is not sufficient to allow switching from 10S to 6S.

Dr. Douglas Stuart: An important consideration in the response of muscle to an external signal for contraction is the orderly recruitment of motor units. Studies on this problem, using biting action as a model, show that orderly recruitment follows the same pattern whether fast biting (twitch) or a slow development is used. Finger movement also shows orderly recruitment of motor units. In muscles with multifunctional roles, reversal of the recruitment order can be detected. Investigation of force development reveals several important observations: a) as force increases so does the firing rate, b) the starting rate is similar at all forces and c) force output of the motor unit is the most important factor in determining threshold. The final important question posed was "what mechanism controls order of recruitment"?

Dr. Jack Wilmore: An important consideration in weight training and strength development is whether the muscle undergoes hypertrophy (increased cell size) or hyperplasia (increased cell or fiber number). Compensatory hypertrophy has been reported in plantaris and soleus muscles of rats following sectioning of the gastrocnemius muscle. With high resistance exercise (but not low resistance) the number of fibers increases in trained limbs of cats. However, in exercised rats muscle mass increases without a change in fiber number.

Dr. X. Joseph Musacchia: The atrophic response of leg muscles of rats or astronauts during spaceflight can be mimicked under gravity conditions by use of a hypokinesia model with rats. During hypokinesia the soleus shows marked atrophy with less dramatic loss of muscle mass in the gastrocnemius and plantaris. The extensor digitorum longus muscles are virtually unaffected. All the muscles grow normally in recovery. These losses of muscle mass are accompanied by increased urinary excretion of urea, ammonia and 3-methylhistidine. The soleus muscle showed loss of total protein and RNA content. It is also apparent that the atrophied muscles have increased fatiguability. These muscles also show evidence of loss of certain enzyme activities (e.g. citrate synthase and cytochrome C) and have an increased number of glucocorticoid receptors/amount of cytosol protein.

Dr. Darrel Goll: The calcium-activated proteinase (CAF) in muscle is located on the Z disk. Hence, it seems to be important in disassembly of the contractile process rather than in the degradation of myofibrillar proteins. Several forms of CAF have been identified; a proteinase activated by high amounts of calcium and two which are activated by low concentrations (one of these is formed by autolysis of the high activation enzyme). An inhibitor has been found in muscle which inactivates CAF and which is also bound to the Z disk. One molecule of inhibitor can block 10 proteinase molecules and calcium is required for binding the inhibitor to the enzyme. It is unclear, however, what factor(s) prevents the inhibitor from blocking CAF at all times under physiological conditions.

Dr. Marc Tischler: Changes in proteolysis correlate with the cell reduction-oxidation state in rat diaphragm and atrium. Protein degradation was measured in the presence of cycloheximide as the linear release of tyrosine into the medium. Intracellular ratios of lactate/pyruvate, total NADH/NAD, and malate/pyruvate were used as indicators of the muscle reduction-oxidation state. Incubation of diaphragms with leucine (0.5-2.0 mM) or its transamination product, sodium α-ketoisocaproate (0.5 mM), resulted in a lower rate of proteolysis and a higher ratio of lactate/pyruvate and NADH/NAD. Unlike leucine, neither isoleucine nor valine alone produced any change in these parameters. Incubation of diaphragms with glucose (20 mM) or atria with sodium lactate (2 mM) produced a diminution of tyrosine release from the muscles and a rise in the ratio of total NADH/NAD. Similarly, in incubated diaphragms of fasted rats, the anabolic effects of insulin, epinephrine and isoproterenol on protein degradation were associated with a higher malate/pyruvate ratio. In catabolic states, such as fasting, cortisol treatment of fasted, adrenalectomized rats or traumatization, enhanced muscle proteolysis was observed. Fresh-frozen diaphragms from these rats had both lower lactate/pyruvate and malate/pyruvate ratios than did muscles from control animals. These data show that diminution of proteolysis in diaphragm is accompanied by an increase of the NAD(P)H/NAD(P) ratios. In contrast to these findings, chymostatin and leupeptin, which inhibit directly muscle proteinases, caused a decrease of the lactate/pyruvate and malate/pyruvate ratios. These results suggest that protein degradation in diaphragm and atrium is linked to the cellular redox state, and that the redox state may mediate the effects of hormones on proteolysis.

B. Cardiac Muscle Session

Dr. Eugene Morkin: An important consideration is the relationship between cardiac performance and protein synthesis. Generally increased work leads to increased protein synthesis and hence mass. There is some belief that norepinephrine released from cardiac sympathetic nerves may be a trigger for cardiac hypertrophy. Along with the alteration of protein synthesis, investigators must consider whether changes in certain isoprotein concentrations might provide some insight into adaptations of myocardial function. For instance, pressure overload is associated with a shift in the lactate dehydrogenase from isozyme H_4 to H_2M_2 resulting in the ability of heart to produce more lactate. Alteration of thyroid hormone levels shifts the proportion of the myosin isozymes. Generally type V_1 increases and V_3 decreases with more thyroid hormone. Recently, it has been found that a high fructose diet induces similar changes in myosin isozymes as does thyroid hormone.

Dr. Radovan Zak: Mammalian ventricle contains two classes of myosin heavy chains: HCα and HCβ. The relative amounts of these heavy chains changes during normal development and in response to altered hormonal and functional states of the heart. One of the factors which clearly influences the expression of these myosin HC's in the heart is thyroid hormone. The rapid increase in the ventricular content of HCα that occurs after birth is closely correlated with the surge in the circulating levels of thyroid hormone at this time. During later development in rabbits, the expression of the HCα gene is independent of normal serum hormone levels, however raising hormone levels 3-fold again results in the accumulation of the HC in the ventricles. As a first step in evaluating the control of myosin HC synthesis, the relative abundance of the mRNAs for HCα and HCβ has been compared with the rates of synthesis of the HC's. Hearts were studied during normal development and during the induction of cardiac enlargement by administration of thyroid hormone to young rabbits. The relative amount of each HC was determined from densitometric scans of Coomassie blue stained gels of myosin separated by electrophoresis in the native state. Total myosin HC was determind by RIA using a monoclonal antibody. The relative amounts of the mRNA for each HC was determined by S1 nuclease protection experiments using two cDNA clones corresponding to HCα and HCβ. DNA sequence analysis showed a 10 percent divergence between these clones in a 350 nucleotide sequence corresponding to a portion of the S-2 fragment of myosin HC. The synthesis rate for each HC were determined from incorporation of ^{3}H-leucine during continuous administration of tracer into the rabbits. The data obtained show a close relationship between the relative amounts of the mRNAs for these HCs and their relative synthesis rates. This strongly suggests that pretranslational events played the major role in regulating the expression of myosin HC in the heart.

Dr. R. John Solaro: One way hearts appear to adjust their contractile dynamics to meet different hemodynamic loads is through alterations in the relations among free sarcoplasmic Ca^{2+}, myofibrillar bound Ca^{2+} and the activity of the myofilaments. In the short term, minutes to hours, the myofilaments are covalently altered by protein phosphorylation which occurs as a result of varying levels of adrenergic stimulation of the cells. In the

long term, the myofilaments appear to be altered by a remodeling process that not only increases the number of filaments per cross-sectional area of the cell, but also the distribution of isoforms of myosin and possibly other myofibrillar proteins. Interestingly short term alterations such as the adrenergic stimulation during exercise result in transiently altered contraction dynamics (increase in peak force and rate of rise and fall of tension) which are the same as those which are sustained following a long term stress such as chronic exercise and altered thyroid status. The heart thus appears to solve the problem of a volume overload by two different routes that lead to the same sort of contractile dynamics. In these studies of short term regulation of myofilament activity, the investigation concerned the determination of which myofibrillar sites are phosphorylated in hearts freeze-clamped in various inotropic states. This work has been done in parallel with detailed studies on the characterization of the relations between the titration of various Ca binding sites on the myofibrils with myofilament activity and how phosphorylation of myosin and troponin affects these relations. During long term stresses on the heart such as chronic exercise and altered thyroid status, we have investigated the nature and the time course of the remodeling process especially with regard to redistribution of the isoforms of myosin and how this affects myofilament activity.

Dr. William Jacobus: An interesting observation in heart muscle is that contraction ceases even when ATP levels are relatively high, as long as the concentration of creatine phosphate is low. Hence, the formation and utilization of creatine phosphate must play an important role in determining whether there is sufficient energy in the muscle for contraction. Calculation of the flux rates for movement of energy-related compounds between mitochondria and the myofibrils shows creatine phosphate flux is only 3-fold greater than ATP flux whereas creatine flux is 900-fold greater than that for ADP. Therefore ADP must be a poor signal to the mitochondria for low energy levels. On the other hand, high concentrations of creatine can act as a shuttle signal. Studies have shown that the mitochondrial creatine phosphokinase is the dominant regulator of ATP production and is coupled to adenine nucleotide transport. Therefore experiments were designed to test whether creatine, creatine phosphate and/or inorganic phosphate controlled this enzyme. By keeping one factor constant and varying the other two, data can be obtained for plotting two varying concentrations against respiration rate in mitochondria on a 3-dimensional graph. These results showed that with phosphate constant, a decrease of creatine phosphate and an increase of creatine under high work conditions was associated with a marked increase in respiration. Therefore ATP production is controlled by the creatine phosphokinase reaction which in turn is regulated by creatine and creatine phosphate.

Dr. Howard White: Conclusions
1. Bovine ventricle-S1 actomyosin binds 40-fold better than does skeletal muscle-S1 actomyosin.
2. Dissociation of ADP is slow enough to be significant during contraction.
3. The association constants of ADP for bovine ventricle and skeletal muscle are similar in the myofibrils and the actomyosin-S1.

4. Binding of ATP brings about fluorescent enhancement as a result of tryptophan in the molecule. Fluorescent enhancement is similar in myofibrils and subfragment-1 and the rate of this enhancement is ADP dependent. High calcium concentration results in a fast decay of the fluorescent enhancement due to more rapid ATP hydrolysis. Low calcium concentration results in a slow decay.

C. Smooth Muscle Session

Dr. James Stull: Calmodulin plays a vital role in permitting calcium to promote myosin phosphorylation. Phosphorylation of the light chain leads to increased isometric tension and then increased velocity of shortening followed by a slow decline with maintenance of tension. Use of anticalmodulin drugs (e.g. fluphenazine) is thought to inhibit myosin light chain kinase (MLCK) and thus leads to a decreased rate of light chain phosphorylation and contraction. Isoproterenol also inhibits phosphorylation and tension development. This reflects a role for β-adrenergic stimulation in relaxation of smooth muscle. This stimulation can be inhibited by inhibition of Ca^{2+}-calmodulin and by inactivating MLCK by phosphorylation. Several mechanisms have been proposed for β-stimulation through increased calcium efflux or decreased calcium influx. Some of the problems to be studied include: a) Concentration of calmodulin is much higher than its K_d for MLCK. b) The MLCK-calcium-calmodulin complex inhibits phosphorylation, not dephosphorylation. How can smooth muscle relax? c) What is the significance of alteration of calmodulin activation properties? d) β-Adrenergic-mediated relaxation does not require MLCK-P dephosphorylation.

Dr. Andrew Somlyo: In relaxed smooth muscle, myosin exists in a filamentous form. The myosin lattice is surrounded by dense bodies which are sites of attachment for the actin filaments which are 100 Å. As a result of cell injury there is an increase of these filaments which is probably due to pressure induced hypertrophy. The regulation of intracellular calcium is another important consideration. Sarcoplasmic reticulum is the most likely storage site for calcium. Recycling of calcium from intracellular sites can promote contraction in the absence of extracellular calcium. Differences in calcium concentrations under relaxed and maximum contraction conditions suggests that buffering is needed.

Dr. David Kreulen: The study of membrane permeability to calcium is important. In smooth muscle a small change in voltage can lead to a much greater change in tension. By grading the depolarization of the membrane you can get proportional changes in voltage and tension. It has been proposed that calcium channels in the membrane are voltage dependent and receptor-operated.

Dr. David Warshaw: The contractility of skeletal and smooth muscle was compared. The velocity of shortening of smooth muscle is less than for skeletal. Although force development is equal for these muscles, smooth muscle has less myosin. The kinetic sequence for both muscles is the same but is 1500 msec for smooth and only 35 msec for skeletal. It was proposed that the length change of smooth muscle is distributed throughout the cell. With a

stimulus, there may be an increased attachment of actin, thereby explaining why less myosin is necessary in smooth muscle. The number of actin-myosin attachments seems to correlate with force development.

Dr. Robert Gore: Diameters of fiber preparations are dependent on temperature and stress (pressure). The observed changes in diameter provide a measure of the viability of the preparation. To calculate tension in the preparation, pressure is altered with an increased potassium and epinephrine concentration. Results indicate that all smooth muscle has a similar length tension curve. As the diameter decreases, the effective mass for tension development also decreases.