IIM – Interuniversity Institute of Myology
University of Padua – cirMYO Interdepartmental Research Center of Myology
University of Chieti – BAMS Department of Basic and Applied Biomedical Sciences

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Abstracts

Lectures

April 26, 2009

Rehabilitation strategies from infrequent paraplegic syndromes to common aging-related muscle weaknesses

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Human muscle in aging share with long-standing denervated muscle similar impairments of the excitation contraction (EC) apparatuses. On the other hand, long-term denervated human muscles recover by FES even when at beginning they hardly respond to electrical stimulation with twitch contractions. We demonstrated that mid-term lower motor neuron denervated muscles present less damaged EC-Coupling apparatus, and respond with tetanic contraction to direct electrical stimulation, while long-standing lower motor neuron denervated muscles need very long and intense impulses to perform twitch contractions, but even in the latter case months of repeated daily training by twitch contraction restored tetanic contractility and improved synthesis and ultrastructural organization of myofibrils and Ca2+ handling membranes [1-3]. Experiments in a rat model of permanent denervation show by Electron Microscpy that the EC-C mechanisms are present and, in part, functional, supporting the hypothesis that, even in absence of ES-induced external-work (that is, visible contraction), ES-induced cyclical Calcium concentration change may drive recovery of the long-term denervated muscles. Our aim is now to translate these results in elderly to shorten recovery time after cast immobilization and other muscle weakness.

[1] Kern H, Carraro U, Adami N, Biral D, Hofer C, Stefan Loefler S, Vogelauer M, Mayr W, Rupp R, Zampieri S. One Year of Home-based Functional Electrical Stimulation (FES) in Complete Lower Motor Neuron Paraplegia: Recovery of Tetanic Contractility Drives the Structural Improvements of Denervated Muscle. Nerurological Research 2009, in press.

[2] Boncompagni S, Kern H, Rossini K, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibres in absence of innervation in humans. Proc Nat Acad Sci USA 2007; 104: 19339-44.

[3] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord 2008; 46(4): 293-304.

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April 27, 2009

Muscle design strategies

Clara Franzini-Armstrong

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The lecture will cover all aspect of muscle structure and function: from myofibril to molecules to membranes asking the question of how muscles have adapted to highly variable functional demands through the animal kingdom.

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Sarcomeric myosin genes: evolution and transcriptional control

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The first part of the lecture will focus on the identification of novel ancient myosins in mammalian striated muscle and on evolutionary tinkering leading to functional diversification of myosin genes. The second part deals with the mechanisms that control the coordinated expression of myosin genes.

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Plasticity and adaptive change: lessons from a paradigm shift

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“Plasticity is the capacity of fully differentiated cells to undergo, in response to a stimulus, a long-lasting change in phenotype that modifies their properties or responsiveness to the same or other stimuli.” [1]. I proposed this definition of plasticity in an attempt to discourage its use for almost everything, including regulation, modulation, and simple cause-and-effect. “Plasticity” it seems, has become the new fashion word of physiology. The scenario was very different fifty years ago, when the notion of a terminally differentiated cell undergoing further change was by no means part of the general thinking. Even when Buller and his colleagues demonstrated the effects of cross-reinnervating fast and slow muscle, the changes were thought of as a quantitative response to some unidentified “trophic” factor in the motor

nerves. The notion that muscle genes could actually be re-expressed was sufficiently new to be strongly resisted in many quarters. Various arguments were put forward to explain the observations in terms of the existing paradigm. But paradigms shift; attention has now moved from the phenomenon to the mechanism, with interest focused on unravelling the intracellular signalling pathways that underlie the re-expression of muscle genes. All the same, it is worth revisiting some of the old counter-arguments, for they have a bearing on the experiments that we do today.

[1] Salmons S: What do we mean by plasticity? Physiology News 2002; No. 47: 7.

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Muscle transcriptional pathways with electrical stimulation

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A large number of transcriptional pathways have been implicated in the response of muscle to an altered activity pattern. This is not surprising in the sense that muscle plasticity involves changes in gene transcription and translation for multiple sub-cellular systems. The transformation of muscle form a fast fatiguable type to a slower more fatigue-resistant type is a paradigm in which the time course and sensitivity to stimulus can be investigated in a systematic manner: by means of an implantable stimulating device the activity dose can be closely controlled. We are searching for thresholds of activity that cause step changes in transcription. The existence of such thresholds would correspond with the observation that muscle fibres fall into a number of classes rather than showing a continuous range of phenotype in vertebrate muscles. We have measured by Q-RTPCR the transcript levels in rat muscle after periods of muscle stimulation having designed QPCR primer pairs to monitor the expression of key transcripts involved in the adaptive response. These include primers for the myosin heavy chains 1, 2A and 2B, IGF-1, MGF, FOXO1A, myostatin, PPARdelta and PGC1alpha. Our first conclusion is that transcript levels for transcription factor change more consistently after 3 hours of changed activity than transcript levels for contractile proteins. After one week of stimulation however, the changes in transcript levels for contractile proteins are also changed consistently. We will investigate the time course of these responses within the first week to identify the earliest time point at which a new stable transcriptional state is achieved and at which it would be appropriate to perform a comparative study of the graded effect on transcription of graded activity patterns.

Malignant Hyperthermia (MH) and Exertional/environmental Heat Stroke (EHS) in mice lacking Calsequestrin-1

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Malignant hyperthermia (MH) and exertional/environmental heat stroke (EHS) in humans present as similar life threatening crises triggered by volatile anesthetics and strenuous exercise and/or high temperature, respectively. Many families (70-80%) diagnosed with MH susceptibility (MHS), and a few with EHS, are linked to mutations in the ryanodine receptor type-1 gene (RYR1), the Ca2+ release channel of the sarcoplasmic reticulum (SR) of skeletal muscle and key protein in excitation-contraction (EC) coupling. However, mutations in the RYR1 gene are not found in all MH families, suggesting that alternative genes remain to be identified. In our laboratory we have recently characterized a novel knockout model lacking skeletal muscle calsequestrin (CASQ1), a SR Ca2+-binding protein that modulates RYR1 function, and investigated whether these mice present a MH/EHS-like phenotype. Ablation of CASQ1 results in remodelling of the EC coupling apparatus and functional changes, which in male mice causes a striking increase in the rate of spontaneous mortality and susceptibility to trigger MH-like lethal episodes in response to halothane- and heat-stress. The demonstration that ablation of CASQ1 result in MH- and EHS-like lethal episodes validates CASQ1 as a viable candidate gene for linkage analysis in MH and EHS families where mutations in RYR1 are excluded.

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Histological characteristics of cultured human muscle tissue: Light- and electron microscopy

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Morphological characteristics of muscle tissue were analyzed by light – and electron microscopy, when cultured as primary muscle tissue culture. Primary muscle tissue cultures were established from diagnostic muscle biopsies. The studies were performed on samples, where the routine histology did

not discover pathological alterations. After one month culture period, the histological investigations were carried out on paraffin -, frozen sections, and electron microscopy.The cultured muscle tissues formed spheroid-like structures. The samples demonstrated myofiber destruction, which was more pronounced in the centre of the spheroids. The degenerating fibers showed ragged-red like alterations, when stained by Gomori-trichrom staining. Cell proliferation was found in the outer layers of the spheroids. The proliferating cells were able to grow and differentiate into myofibers, which could be demonstrated by immunological markers. The new myofibers formed ring like structures around the original, degenerating fibers. The ultrastructural investigation demonstrated damage of the sarcolemma, disarrangement of the myofibrillar structure, alterations of the mitochondria, as well as dividing cells and growing new myofibers around the original fibers. Conclusion: The small, 1-2 mm sized muscle tissue pieces form spheroids in vitro, and survive. The physically damaged and denervated muscle fibers degenerate in this system, which degeneration is accompanied by regenerative changes. The pathomechanism of the morphological changes is further discussed. These experiments demonstrate that it is possible to generate morphological changes in cultured muscle spheroids that are commonly found in diagnostic biopsies, and could help us to understand the pathomechanism of muscle disorders.

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MYH16: the masticatory myosin unveiled

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In human genome, 16 distinct genes code for class II myosin heavy chain subunit (Berg et al . Molecular Biology of the Cell Vol. 12, 780–794, April 2001). Among such genes, MYH16 has been identified as the gene coding for the masticatory or M MyHC (Desjasrdins et al Mol. Biol. Evol. 19(4):375–393. 2002) and subsequently found transcribed but not translated in human masseter and temporalis muscles (Stedman et al Nature 428, 415-418, 2004). In several mammalian species, and among them in carnivores, the gene is transcribed and translated in jaw closer muscles where myosin containing M-MyHC represents the most abundant isoform (Qin et al. J Mol Evol 55, 544-552, 2002). Since only sparse information was available on the contractile properties of muscle fibers expressing M-MyHC (M fibers), we decided to characterized M fibers isolated from the jaw closer muscles (temporalis and masseter) of two species of domestic carnivores, the cat and the dog, in comparison with fibers expressing slow or fast (2A, 2X and 2B) isoforms in trunk and limb skeletal muscles. In each fiber, during maximally calcium-activated contractions at 12°C, we determined isometric specific tension (Po), unloaded shortening velocity (Vo) with the slack test protocol and the rate constant of tension redevelopment (kTR) after a fast shortening-relengthening cycle. At the end of the mechanical experiment, we identified MyHC isoform composition of each fiber with gel electrophoresis. Electrophoretic migration rate of M-MyHC was similar in both species. We found that in both species the kinetic parameters Vo and kTR of M fibers were similar to those of 2A fibers, whereas Po values were significantly greater than in any other fiber types. The similarity between 2A and M fibers and the greater tension development of M fibers were confirmed also in mechanical experiments performed at 24°C. Myosin concentration was determined in single fibers and found not different in M fibers compared to slow and fast fibers, suggesting that the higher tension developed by M fibers does not find an explanation in a greater number of force generators. The specific mechanical characteristics of M fibers was then attributed to a diversity in cross bridge kinetics. Initial study on actin-myosin interaction kinetics showed that the rate of ADP release which is a determinant of maximum shortening velocity (Vo), is a biphasic process with a fast phase comparable to that of fast myosin and a slow phase comparable to that of slow and cardiac myosin. This suggests that ADP release might be strain dependent and this could in turn explain the high tension development and the intermediate kinetics parameters observed in single fibers.

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Some features of the adaptation of man to hypoxia: from the integrative to the molecular level

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Over the last century, studies on humans exposed to hypobaric hypoxia have been centered mainly on the adjustments necessary to assure homeostasis of O2 delivery, both at rest and at high levels of energy turnover. As a result, a large body of information was collected on blood, respiratory gas exchange, cardiovascular parameters such as heart rate and cardiac output and, more recently, on muscle efficiency and maximum power. The available data mainly refer to lowlanders (including second generation altitude populations) undergoing acute (from seconds to hours), subacute (up to a few days), subchronic (up to several weeks) and chronic (over years) low PO2 exposure, as well as to natives, mainly Himalayan and Andeans, born and living permanently at altitude or commuting to higher and/or lower elevations. Such data appear to be often affected by a large variability that is not justified by the characteristics of the research protocols and "state of the art" measuring procedures. The appearance of a new player, the multi-gene transcription protein Hypoxia Inducible Factor (HIF-1), i.e.

the master regulator of cell hypoxic signaling and of hundreds of other genes whose products play a large number of metabolic and transport functions, opens a new scenario for an updated interpretation of earlier results that have been often overlooked. Among the latter, the large scatter of the percentage loss of maximum aerobic power as a function of altitude, the increase in metabolic efficiency of locomotion in chronic hypoxia, the origin and significance of the so-called "lactate paradox" and the functional significance of the muscle mitochondrial mass reduction in both altitude natives and acclimatized lowlanders. This has been done based on work on cell hypoxic signaling by Semenza's group and on metabolic players recently identified by muscle proteome analysis.

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Electrophysiological evaluation of denervated skeletal muscle fibres

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Skeletal muscle inactivity as a consequence of muscle unloading, ageing or denervation, determines a progressive muscle atrophy characterized by reduced i) fibre diameter, ii) T-tubule surface area, iii) plasma-membrane stiffness, iv) expression of protein involved in the excitation-contraction coupling process, v) Ca2+ endingly and vi) loss of the sarcomeric proteins. In isolated single fibres the atrophic state related to above points i-v can be evaluated by using microelectrodes and recording ionic current in current- and voltage-clamp condition. In current-clamp condition it is possible to evaluate a) the resting membrane potential index of increased plasma-membrane leak current, b) the excitability of the fibre as evaluated by the voltage threshold of the action potential (AP) and c) the conduction velocity of the AP. In voltage-clamp condition it is possible to evaluate 1) the passive properties of the sarcolemma as the membrane resistance, the overall surface area of the fibre as well as that of the T-tubule, 2) the active voltage-dependent responsiveness of the fibre as the time course and voltage dependence of intramembrane charge movement, and of Na+ and L-type Ca2+ channel, 3) the reciprocal control between the orthograde action from L-type Ca2+ channel to RyR and the retrograde action from RyR to L-type Ca2+ channel by blocking the ryanodine receptor with ruthenium red, ryanodine or heptanol, 4) the stiffness and mechano sensitivity of the plasma membrane can be evaluated by comparing the data related to points a-c and 1-3 in muscle stretched with muscle at resting length. The observed effects on normal and denervated fibres could be useful to evaluated the atrophic state of the fibre, and for therapies that use the functional electrical stimulation and passive movement of the limbs to repair the damaged denervated human muscle.

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The long-lasting denervated muscle: What we know from experimental animal research?

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Human muscles that have undergone atrophy as a result of persistent lower motor neuron lesion may experience considerable restitution of excitability, force and mass from home-based therapeutical electrical stimulation (TES), though initially they are unable to perform external work [1-3]. For ethical reasons we studied time course of post-denervation effects on the Excitation-Contraction Coupling (ECC), using a rat model as a substitute for human tissue [Squecco R, Carraro U, Kern H, Pond A, Adami N, Biral D, Vindigni V, Boncompagni S, Protasi F, Pietrangelo T, Bosco G, Fanò G, Marini M, Abruzzo PM, Germinario E, Danieli-Betto D, Francini F, Zampieri S. Despite lost contractility, a sub-population of rat muscle fibers maintains an assessable excitation-contraction coupling mechanism after long-standing denervation. J Neuropath Exp Neurol 2009, submitted.]. In this model, it is possible to demonstrate that in long term denervated muscle: i) muscle fibers having "voltage dependent Ca2+ channel activity" are twice those able to contract, ii) isolated muscle, unable to twitch by electrical stimulation, presents slow caffeine contracture, iii) the ECC mechanisms are still present and, in part, functional; and iv) ECC related gene expression is up-regulated in long-term denervated muscles. Importantly, we demonstrate that at any time-point there are muscle fibers that are more resistant than others to denervation atrophy and disorganization of the ECC apparatuses. In summary, our experimental evidence supports the hypothesis that, even in absence of electrical stimulation-induced external work, FES may induce internal calcium concentration alterations in muscle fibers that drive the early recovery of the long-term denervated human muscle.

[1] Carraro U. Modulation of trophism and fiber type expression of denervated muscle by different patterns of electrical stimulation. Basic Appl Myol 2002; 12: 263-272.

[2] Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibres in absence of innervation in humans. Proc Natl Acad Sci. USA 2007;104:19339-19344.

[3] Kern H, Carraro U, Adami N, Biral D, Hofer C, Loefler S, Vogelauer M, Mayr W, Rupp R, Zampieri S. One year of home-based Functional Electrical Stimulation (FES) in complete lower motor neuron paraplegia: Recovery of tetanic contractility drives the structural improvements of denervated muscle. Nerurological Research 2009, in press.

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