Second Meeting of the Interuniversity Institute of Myology

Casciana Terme - Pisa, October 21-23, 2005
Local Organizers: Prof. P. Bruni and Prof. G. Cecchi
Università degli Studi di Firenze

Friday October 21, 2005

15.00 *Welcome Address*, G. Fanò, P. Bruni and G. Cecchi

REGULATORY MECHANISMS OF MYOGENESYS 1
Chair: G. Fanò and A. Musarò

15.15 MURINE MACROPHAGE CONDITIONED MEDIUM (mMCM) EMPROVES IN VIVO MUSCLE REGENERATION AFTER MASSIVE TISSUE ABLATION
L. Martelli, A. Malerba, M. Frigo, I. Scambi, D. Segat, L. Boldrin, P. De Coppi, R.G. Bellomo, L. Vitiello and M.D. Baroni

15.30 THE PROMYOGENIC EFFECT OF TUMOR NECROSIS FACTOR ALPHA IS MEDIATED BY THE CYTOKINE-INDUCED ACTIVATION OF SPHINGOSINE KINASE
C. Donati, P. Nincheri, F. Cencetti, E. Meacci and P. Bruni

15.45 S100B-DEPENDENTINHIBITION OF MYOBLAST DIFFERENTIATION: MOLECULAR MECHANISM
F. Riuzzi, G. Sorci and R. Donato

16.00 RAGE MODULATES MYOBLAST PROLIFERATION, APOPTOSIS, ADHESIVENESS, MIGRATION AND INVASIVENESS
F. Riuzzi, G. Sorci and R. Donato

16.15 TIME-COURSE PROFILE OF MUSCLE ACTIN ISOFORMS EXPRESSION IN DIFFERENTIATING HUMAN SATELLITE CELLS ISOLATED FROM DONORS OF DIFFERENT AGE
H. Lancioni, L. Lucentini, A. Palomba, S. Fulle, M.R. Micheli and F. Panara

16.30 MHCs GENE CLUSTER ORGANISATION AND THEIR mRNA EXPRESSION IN DOG SKELETAL MUSCLES
L. Maccatrozzo, M. Patruno, F. Caliaro, L. Toniolo, C. Reggiani and F. Mascarello

16.45 LEVELS OF mRNA GROWTH FACTORS IN FOETAL AND NEWBORN PIGLETS
M. Patruno, F. Caliaro, L. Maccatrozzo, L. Toniolo, C. Reggiani and F. Mascarello

17.00 *Coffee Break*

17.30 MHC ISOFORM COMPOSITION IN TRUNK, LIME AND SPECIALISED CANINE MUSCLES
L. Toniolo, L. Maccatrozzo, M. Patruno, F. Mascarello and C. Reggiani

17.45 NF-KB KO MICE ANALYSIS
R. Adami, F. Mourkioti, V. Parente, R. Bottinelli, M. Pasparakis and N. Rosenthal

18.00 mMCM ENHANCED THE PROLIFERATION OF A SUB-POPULATION OF MUSCLE-DERIVED CELLS OBTAINED BY SERIAL PLATINGS
A. Malerba, L. Martelli, M. Frigo, D. Segat, L. Boldrin, P. De Coppi, R.G. Bellomo, L. Vitiello and M.D. Baroni

18.15 MYOTUBES AND FIBERS CULTURES ON TRANSISTOR MICROCHIPS: INNOVATIVE WETWARE DEVICES FOR SINGLE CELL AND NON-INVASIVE STEMULATION, REGISTRATION AND TRANSFECTION
M. Quarta, M. Canato, S. Vassanelli and C. Reggiani

18.30 MORPHOLOGICAL CHANGES OF THE EXCITATION-CONTRACTION COUPLING APPARATUS IN AGEING HUMAN SKELETAL MUSCLE: A POSSIBLE ROLE IN THE DECLINE OF MUSCLE PERFORMANCE
S. Boncompagni, L. d'Amelio, S.Fulle, G.Fanò and F. Protasi

18.45 PLD ACTIVITY REGULATION AT THE EARLY STAGE OF MYOGENESIS
F. Naro, S. Mebarek, H. Komali, A.F. Prigent and G. Némoz

19.00 CULTURE OF SATELLITE CELLS IN AN ELECTRICALLY-STEVfULATED 3D COLLAGEN SCAFFOLD FOR THE CREATION OF ENGINEERED SKELETAL-MUSCLE GRAFTS
E. Serena, P. Campagnolo, L. Boldrin, E. Cimetta, P. G. Gamba, L. Vitiello, P. De Coppi and N. Elvassore

19.15 CASPASES REGULATE SKELETAL MUSCLE REGENERATION
V. Moresi, A. Pristerà, S. Adamo, M. Molinaro and D. Coletti

20.30 *Cena*

21.30 Consiglio Direttivo

Saturday October 22, 2005

REGULATORY MECHANISMS OF MYOGENESYS 2
Chair: G. Fano and A. Musarò

9.00 *Presentazione della Dr.ssa F. Rovetta Vincitrice del Premio "Giovane Ricercatore" dell'IIM*

EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF) ON *IN-VITRO* DIFFERENTIATION OF MUSCOLAR CELLS
F. Rovetta, C. Morabito, N. Steimberg, J. Boniotti, G. Fanò, M. A. Mariggiò and G. Mazzoleni

9.30 MYOBLAST DIFFERENTIATION: RELEVANCE OF CYTOSKELETAL REMODELLING AND SACs ACTIVATION. I - MORPHOFUNCTIONAL REMARKS
C. Sassoli, D. Nosi, E. Meacci, P. Bruni, S. Zecchi-Orlandini And L. Formigli

9.45 MYOBLAST DIFFERENTIATION: RELEVANCE OF CYTOSKELETAL REMODELLING AND SACs ACTIVATION. II - ELECTROPHYSIOLOGICAL RESULTS
R. Squecco, C. Sassoli, S. Zecchi, L. Formigli and F. Francini

STRUCTURAL AND FUNCTIONAL MUSCLE MOLECULES
Chair: C. Reggiani and F. Trimarchi

10.00 REDOX STATUS AND CA^{2+}-TRANSPORT SYSTEM IN SKELETAL MUSCLE OF MLC/mIGF-1 TRANSGENIC MOUSE: A PRELEMINARY REPORT
S. Beccafico, G. Fanò, G. Dobrowolny, A. Musarò and S. Belia

10.15 SEARCHING FOR NEW PROTEINS PREFERENTIALLY EXPRESSED IN SKELETAL MUSCLE
J. Vujovic, A. Santuccione and V. Sorrentino

10.30 ORGANIZATION OF SARCOPLASMIC RETICULUM PROTEINS IN DIFFERENTIATING MYOTUBES
V. Cusimano, E. Giacomello and V. Sorrentino

10.45 HIF-1 ACTIVITY IN RAT FAST AND SLOW SKELETAL MUSCLE EXPOSED TO SYSTEMIC HYPOXIA
N. Cacciani, A. Matsakas, M. Murgia, S. Schiaffino and C. Reggiani

11.00 *Coffee Break*

11.30 ROLE OF CAMKII IN REGULATION OF GLYCOGEN METABOLISM IN MAMMALIAN FAST TWITCH SKELETAL MUSCLE
E. Damiani, R. Sacchetto and E. Bovo

11.45 REDOX REGULATION OF BETA-ACTIN
T. Fiaschi, G. Cozzi, G. Raugei, G. Ramponi and P. Chiarugi

12.00 SPHINGOLIPID SIGNALING IN DENERVATED SKELETAL MUSCLE
M. Zanin, E. Germinano, D. Biral, RA Sabbadini, R. Betto and D. Danieli

12.15 PROTEOMIC ANALYSIS OF SKELETAL MUSCLES IN *MDX* AND α-SARCOGLYCAN-NULL MUTANT MICE
L. Brocca, O. Pansarasa, A. Bachi, R. Bottinelli and M.A. Pellegrino

12.30 LACK OF SKELETAL CALSEQUESTRIN DETERMINES A CHANGE IN THE ULTRASTRUCTURE OF CALCIUM RELEASE UNITS
C. Paolini, M. Quarta, M. Dainese, C. Reggiani, P.D. Allen and F. Protasi

12.45 ANY ROLE FOR HOMER IN ADAPTATION AND SIGNAL TRANSDUCTION OF SKELETAL MUSCLE?
E. Bortoloso, N. Filati, A. Megighian, E. Tibaldo, D. Sandonà, G. Valle, A. Nori and P. Volpe

13.00 GENE EXPRESSION IN RAT SKELETAL MUSCLES AFTER MEDIUM-AND LONG TERM-DENERVATION
R. Lapalombella, A. Scordari, P.M. Abruzzo, M. Marini and Ugo Carraro

13.30 *Pranzo*

CONTRACTION KINETICS AND REGULATION
Chair: R.. Bottinelli and F. Protasi

15.00 FORCE GENERATION AND THE EFFECT OF INORGANIC PHOSPHATE IN SKINNED FIBRES FROM RABBIT PSOAS
M. Caremani, C. Piperio, V. Lombardi and M. Linari

15.15 TWO INDEPENDENT MECHANICAL EVENTS IN THE INTERACTION CYCLE OF SKELETAL MUSCLE MYOSIN WITH ACTIN
M. Capitanio, M. Canepari, P. Cacciafesta, V. Lombardi, R. Cicchi, M. Maffei, F.S. Pavone and R. Bottinelli

15.30 CROSSBRIDGE FORCE AND EXTENSION IN HYPERTONIC SOLUTION
B. Colombini, M.A. Bagni and G. Cecchi

15.45 TROPONIN COMPOSITION AFFECTS MYOFILAMENT CALCIUM SENSITIVITY BUT NOT CROSS-BRIDGE CYCLE KINETICS
B. Scellini, A. Belus, N. Piroddi, C. Tesi and C. Poggesi

16.00 AFM-IMAGING AND ELASTICITY MEASUREMENTS OF THE SARCOLEMMA IN CULTURED SKELETAL MUSCLE FIBERS
M. Canato, E. D'Efranchi, E. Bonaccurso, M. Tedesco, E. Pavan, R. Raiteri and C. Reggiani

16.15 *Coffee Break*

16.30 MECHANICAL PROPERTIES OF FAST AND SLOW SKELETAL MUSCLE IN MLC/mIgf-l TRANSGENIC MICE: AN *IN VITRO* APPROACH

E. Rizzuto, C. Nicoletti, Z. Del Prete, M. Molinaro and A. Musarò

16.45 PHYSIOLOGICAL CHARACTERIZATION OF 2-WEEK REGENERATED SOLEUS MUSCLE FIBRES
E. Germinario, A. Esposito, M. Zanin, P.T. Palade, R. Betto and D. Danieli

17.00 CROSS-BRIDGE RECRUITMENT BY STRETCH OCCURS IN THE SUBMILLISECOND TIMESCALE
M. Reconditi, E. Brunello, R. Elangovan, T. Narayanan, M. Irving, M. Linari, G. Piazzesi, and V. Lombardi

17.15 ACUTE PASSIVE STRETCHING ALTERS THE MECHANICAL BUT NOT THE ELECTRICAL PROPERTIES OF CALF MUSCLES IN HUMANS
F. Esposito, E. Cè, P. Alfieri, G. Pizzini and A. Veicsteinas

17.30 AUXOLOGY AND MOUNTAIN SPORTS FITNESS PROFILES
F. Coscia, P.V. Gigliotti, A. Cavicchioli, A. Biscarini and G.E. De Medio

18.00 *Open Convention*

20.00 *Cena*

21.30 *Jazz Session*

Sunday October 23, 2005

MUSCLE DISEASES

Chair: *V. Sorrentino and A. Uncini*

9.00 PREVALENCE OF ASYMPTOMATIC HYPERCKEMIA
M.V. De Angelis, A. Di lorio, A. Di Muzio, L. Ferrucci, M. Capasso, G. Abate and A. Uncini

9.15 STRUCTURAL ALTERATIONS OF THE EXTERIOR MEMBRANE SYSTEM IN SKELETAL MUSCLE FIBERS ARE PRESENT IN IDIOPATHIC HYPERCKEMIA (IH)
S. Boncompagni, A. Di Muzio, M. Capasse, M.V. De Angelis, M. Paci, F. Protasi and A. Uncini

9.30 KINETICS OF CONTRACTION AND RELAXATION IN SINGLE MYOFIBRILS FROM FAMILIAL HYPERTROPHIC CARDIOMYOPATHY PATIENTS
A. Belus, N. Piroddi, C. Tesi and C. Poggesi

9.45 CLINICAL AND FUNCTIONAL CHARACTERIZATION OF COMPOUND HETEROZYGOUS CASQ2 MUTATIONS ASSOCIATED WITH CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHICARDIA (CPVT)
A. Nori, M. R. di Barletta, S. Viatcheko-Karpinski, M. Memmi, D. Terentyev, F. Turcato, G. Valle, C. Napolitano, S. Gyorke, P. Volpe and S. G. Priori

10.00 SATELLITE CELLS DELIVERED BY POLYMER: A NEW STRATEGY IN MUSCLE CELL THERAPY
L. Boldrin, A. Malerba, M. Flaibani, E. Cimetta, M. Piccoli, M. Pozzobon, C. Messina, L. Zanesco, P.G. Gamba, L. Vitiello, N. Elvassore and P. De Coppi

10.15 EXTRAPYRAMIDAL SYMPTOMATOLOGY DUE TO CHRONIC EXPOSURE TO MANGANESE: POSSIBLE INVOLVEMENT OF DIRECT MYOPATHY
J. Boniotti, F. Rovella, N. Steimberg, P. Apostoli, M. A. Mariggiò and G. Mazzoleni

10.30 MACROPHAGE-SECRETED FACTORS ENHANCE THE PROLIFERATION RATE OF DUCHENNE MUSCLE DYSTROPHY MYOBLASTS
M. Frigo, A. Malerba, L. Martelli, D. Segai, L. Boldrin, P. De Coppi, R.G. Bellomo, M.D.Baroni and L. Vitiello

10.45 ROLE OF INFLAMMATION IN MUSCLE REGENERATION
L. Pelosi, C. Giacinti, C. Nardis, L. Barberi, G. Dobrowolny, M. Molinario and A. Musarò

11.00 *Coffee Break*

11.15 SATELLITE CELLS DERIVED FROM *VASTUS LATERALIS* MUSCLE OF CFS PATIENTS: FIRST DATA
C. Puglielli, T. Pietrangelo, R. Mancinelli, J. Vecchiet, L. Vecchiet, G. Fano and S. Fulle

11.30 FUNCTIONAL AMELIORATION OF DYSTROPHIC PHENOTYPE IN A DOG MODEL OF DUCHENNE MUSCULAR DISTROPHY THROUGH INTRA-ARTERIAL DELIVERY OF MESOANGIOBLASTS
C. Rinaldi, O. Pansarasa, G. D'Antona, M. Sampaolesi, S. Blot, R. Bottinelli and G. Cossu

11.45 ANTISENSE OLIGONUCLEOTIDES AS A THERAPEUTIC TOOL FOR DYSTROPHIC MUSCLE: IN VIVO EXPEREMENTING IN THE MURINE MDX MODEL
A. Malerba, P.A. Ditadi, N. Bassi, P. Campagnolo, P. Gamba, E. Zaccariotto, M.D. Baroni, C. Reggiani and L. Vitiello

12.00 SYSTEMIC DELIVERY OF AAV VECTOR EXPRESSING ANTISENSE-U1 SNRNA RESTORES DYSTROPHIN EXPRESSION AND FIBRES FUNCTION IN SKELETAL MUSCLES OF *MDX* MICE
O. Pansarasa, G. D'Antona, M. Denti, A. Rosa, F. De Angelis, V. Parente, O. Sthandier, A. Auricchio, Bottinelli R and I. Bozzoni

12.15 THE CONTRIBUTION OF HYPERTROPHIC SKELETAL MUSCLE TO MOTOR NEURON SURVIVAL IN A MOUSE MODEL OF ALS
G. Dobrowolny, C. Giacinti, L. Pelosi, C. Nicoletti, M. Molinaro and. A. Musarò

12.30 GENETIC SCREENING OF THE ENTIRE RYR1 GENE AND EXPRESSION OF MUTATED PROTEINS
L. Galli, D. Rossi, P. De Smedt, A. Orrico, D. Franci, F. Petroli, S. Lorenzini, V. Tegazzin and V. Sorrentino

13.00 *Pranzo*

Abstracts

NF-KB K-O MICE ANALYSIS

Adami R. (1), Mourkioti F. (2), Parente V. (1), Bottinelli R. (1), Pasparakis M. (2) and Rosenthal

(1) Dept. Experimental Medicine, University of Pavia, v. Forlanini 6, 27100, Pavia; (2) EMBL Mouse Biology Unit, v. Ramarini 32, 00016 Monterotondo-Scalo, Roma

The transcription factor Nuclear Factor kappa enhancer Binding protein (NF-kB) regulates the expression of many genes that are involved in innate immune reaction, inflammatory responses and apoptosis.

In order to study a potential role of NF-kB in muscle homeostasis we have exploited a muscle tissue targeted gene deletion of an essential element of its regulatory cascade, the IKK2 (or IKKb) subunit of the IkB Kinase (IKK) complex.

Analysis of gene knock-out (K-O) mice revealed a significant reduction of the IKK2 protein specifically in the skeletal muscles.

Comparison of wild type and K-O mice showed that the K-O mice had an improved muscle force development.

These preliminary findings confirm that the NF-kB regulatory pathway has important functions in muscle homeostasis.

REDOX STATUS AND CA2+-TRANSPORT SYSTEM IN SKELETAL MUSCLE OF MLC/MIGF-1 TRANSGENIC MOUSE: A PRELIMINARY REPORT

Beccafico S. (1), Fanò G. (1), Dobrowolny G. (2), Musarò A. (2), Belia S. (1)

(1) CeSI – Centro Studi Invecchiamento Università "G. D' Annunzio" Chieti –Pescara; (2) Dipartimento di Istologia ed Embriologia Medica Università di Roma "La Sapienza"; (1, 2) Istituto Interuniversitario di Miologia

Insulin-like growth factor 1 (IGF-1), is critical in promoting growth of skeletal muscle. It has been shown that muscle specific IGF-1 (mIGF-1) overexpression, in MLC/mIgf-1 transgenic mouse, reduces age-related skeletal muscle atrophy and improves muscle mass and strength (Musarò et al Nature 1999; 400:581-5). The aim of this work was to verify whether the increased muscle activity induces an increase in Reactive Oxygen Species (ROS) production from mithocondria and this in turn modifies some aspects of muscle functionality, e.g. EC coupling (Fulle et al Free Radical Biology & Medicine 2000; 29:1252-9, Fulle et al Neuromuscular Disorders 2003; 13: 479-84).

We performed binding studies and enzymatic activity measures (in cell-free systems), to determine some aspects of Ca2+-transport (expression and activities of EC-Coupling receptors) and the modifications of the antioxidant scavenger enzyme activities (Glutathione Transferase, Glutathione Reductase, Catalase) in both wild type and MLC/mIgf-1 transgenic mice. The results reported here, concerning the functional activity, show that in the MLC/mIgf-1 muscles: i) the number and/or the activity of DHPR channels increase, ii) the Sarcoplasmic Ca2+ pump and the presence of Ryanodine receptor are not modified. For that concerning the redox status: i) specific activity of Glutathione Transferase is reduced in MLC/mIgf-1 muscle, while the Glutathione Reductase and Catalase do not show significant differences, ii) only a little appearance of lipid peroxidation it is possible to note in the sarcolemmal membranes.

We also investigated the same parameters in undifferentiated satellite cells derived from MLC/mIgf-mice, obtaining results qualitatively comparable to those obtained in the whole muscle.

In conclusion, from these preliminary results, it is possible to hypothesize that muscle hypertrophy does not induce negative modifications related to the oxidative stress.

KINETICS OF CONTRACTION AND RELAXATION IN SINGLE MYOFIBRILS FROM FAMILIAL HYPERTROPHIC CARDIOMYOPATHY PATIENTS

Belus A., Piroddi N., Tesi C., Poggesi C.

Dipartimento di Scienze Fisiologiche, Università di Firenze

Familial Hypertrophic CardioMyopathy (fHCM) is an autosomal–dominant disease of the cardiac sarcomere. It is a single gene disorder for which at least 10 genes encoding contractile proteins have been identified. The mechanical properties of cardiac sarcomeres from fHCM patients have never been directly investigated. We used the single myofibril technique (Tesi et al., Biophys. J., 2002, 83, 2142-2151) to compare the kinetics of contraction and relaxation of myofibrils isolated from ventricular biopsies of 3 fHCM patients carrying mutations in cardiac myosin binding protein C- or myosin heavy chain- genes, 4 patients with left ventricular hypertrophy due to aortic stenosis (AoSt), and 4 patients with no left ventricular hypertrophy (Ctrl). Preparations, mounted in a force recording apparatus (15 °C), were maximally Ca2+-activated (pCa 3.5) and fully relaxed (pCa 8) by rapid (<10 ms) solution switching. Both maximal isometric tension and apparent rate of tension generation were about 30% lower in fHCM as compared to non hypertrophic controls (Ctrl). Similar changes were found in left ventricular hypertrophy by increased work load (AoSt vs. Ctrl). Force relaxation kinetics upon Ca2+ removal were faster in fHCM than in both Ctrl and AoSt myofibrils indicating that the apparent rate with which cross-bridge leave the force generating states is accelerated in fHCM preparations. This suggests an inefficient energy utilization by fHCM sarcomeres and is consistent with the central role of compromised energetics suggested for fHCM. [Supported by Telethon grant GGP02428].

SATELLITE CELLS DELIVERED BY POLYMER: A NEW STRATEGY IN MUSCLE CELL THERAPY

Boldrin L. (1), Malerba A. (2), Flaibani M. (3), Cimetta E. (3), Piccoli M. (1), Pozzobon M. (1), Messina C. (1), Zanesco L. (1), Gamba P.G. (1), Vitiello L. (2), Elvassore N. (3), De Coppi P. (1)

(1) Stem Cell Processing Laboratory, Dept. of Paediatric Oncohematology, (2) Biology, (3) Chemical Engineering, University of Padova, Italy

Myoblast transplantation is a promising therapeutic tool in muscle diseases. However poor results have been obtained with both injection and systemic delivery of the cells in order to regenerate muscle tissue in vivo. In our study we have combined cell biology and polymer chemistry to create in vitro the most appropriate microenvironment for in vivo cell transplantation. Satellite cells were obtained from flexor digitorum brevis of C57BL/6-TgnEGFP transgenic mice and from human muscle biopsies. After muscle enzymatic digestion, single muscle fibers were plated in matrigel-coated dishes and then mouse and human satellite cells were expanded and seeded onto poly-glycolic acid (PLGA) polymers. Immunostaining, flow citometry and RT-PCR analyses testified satellite cells myogenicity and verify also polymer biocompatibility. In parallel, cellularized polymers and free satellite cells were implanted and injected in tibialis anterior muscles respectively of GFP–ve, syngenic mice and of CD1 nude mice. At 1, 2 and 4 weeks the animals have been sacrificed and serial sections of treated muscle have been analyzed with immunofluorescence and western blot analyses. Transplanted satellite cells were able to form fully differentiated muscle fibers and to migrate from the polymer to the damaged situ. Western blot analyses showed higher cell survival of satellite cells delivered by polymer than those injected. We describe for the first time the possibility of delivering satellite cells to the damaged muscle through a polymeric scaffolds. This strategy could be used in the future as therapeutic tool for degenerative muscle diseases.

MORPHOLOGICAL CHANGES OF THE EXCITATION-CONTRACTION COUPLING APPARATUS IN AGEING HUMAN SKELETAL MUSCLE: A POSSIBLE ROLE IN THE DECLINE OF MUSCLE PERFORMANCE

Boncompagni S. (1), D'Amelio L. (2), Fulle S. (1), Fanò G. (1), Protasi F. (1)

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The ability of skeletal muscle to generate force and produce movement declines with age. Recent findings indicates that the excitation-contraction (EC) coupling, the mechanism linking the depolarization of the surface membrane to the release of intracellular Ca2+ from the sarcoplasmic reticulum (SR), may be an important factor contributing to muscle dysfunction: EC un-coupling theory. Using transmission electron microscopy, we have studied the frequency, localization and ultrastructure of calcium release units (CRUs), the structures that mediates EC coupling, in humans muscle biopsies from males and females subject from 28 to 83 years of age. Although we did not find differences in the measure of the witdh TT/SR junctional gap between young and old humans, we observed significant alterations in the arrangement of the transverse T-tubules, in the structure and disposition of CRUs and also a significant decrease in their frequency between controls and aged samples: 24.4/100mm2 (28 and 34 years of age, n = 2) vs. 11.6/100mm2 (71 to 83 years of age, n. = 7). These data indicates the EC coupling apparatus in humans undergoes a partial disarrangement and a spatial re-organization as a function of age. A low number of EC coupling units in the muscle fiber myoplasm could definitely interfere with an efficient delivery of Ca2+ ions to the contractile proteins and may in fact be an important factor to understand the mechanisms that lead to the functional decline of ageing musculature.

EXTRAPYRAMIDAL SYMPTOMATOLOGY DUE TO CHRONIC EXPOSURE TO MANGANESE: POSSIBLE INVOLVEMENT OF DIRECT MYOPATHY

Boniotti J. (1), Rovetta F. (1)*, Steimberg N. (1)*, Apostoli P. (2), Mariggiò M.A. (3)*, Mazzoleni G. (1)*

(1) General Pathology and Immunology Unit and (2) Institute of Occupational Health, School of Medicine - University of Brescia, viale Europa, 11, 25123 Brescia

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**IIM, Interuniversity Institute of Myology*

Manganese (Mn) is both an essential nutrient needed for the activation of critical cellular enzymes and a potent neurotoxicant (chronic exposure). It also shows specific organotrophism in vivo (acute/chronic effects). Typical signs of Mn neurotoxicity include neurobehavioral deficits and extrapyramidal motor system impairments that, associated to an atrophy/gliosis of basal ganglia structures, produce a complex syndrome (manganism). Even if widely investigated, the molecular mechanisms responsible for Mn toxicity remain to be clarified.

In this study we compared the effect of Mn2+ on 3 in vitro models representative of the main target organs for Mn toxicity in vivo. HepG2 and MDCK cell lines were selected for liver and kidney, respectively; glial GL15 and neuronal SH-SY5Y cells were used as model of CNS components. Even if no evidence of Mn toxicity on skeletal muscle has been reported, C2C12 myoblasts were also included. Cell cultures were exposed for different time periods (1 to 7 days) to MnCl2 or Mn(NO3)2 at concentrations ranging from 0,1 μM to 1 mM. Because of the high affinity of Mn to serum proteins, the effective amount of Mn2+ in the culture media used for each treatment was verified by Atomic Absorption Spectrometry (AAS). The effect of Mn on cell viability and proliferation rate was used as an index of its toxicity; the study

was integrated with morphological analyses (optical microscopy).

The results obtained demonstrate that all the first 4 cell types analysed react to Mn in a time- and dose-related manner, strictly mirroring the in vivo response of the tissues they represent. This confirms that these cell lines are suitable models to study the molecular pathways of Mn toxicity. Interestingly, also C2C12 cells showed a toxic response to Mn, preferential targets being differentiated myotubes. Even if still preliminary, these findings might suggest that a Mn-induced direct myopathy could contribute to the pathogenesis of Mn-induced motor system impairments.

ANY ROLE FOR HOMER IN ADAPTATION AND SIGNAL TRANSDUCTION OF SKELETAL MUSCLE?

Bortoloso E., Pilati N., Megighian A. (1), Tibaldo E., Sandonà D., Valle G., Nori A., Volpe P.

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The proteins of the Homer family (Homer 1, 2, and 3), in neurons, regulate multimeric complexes involved in signal transduction. Some Homers have been detected in skeletal muscles; the constitutive expression of mRNAs for Homer 1, 2 and 3 was reported in murine muscles; the complete ORF and full-length cDNA clones of Homer 1a and 1c were obtained from rat muscle. Homer 1 isoforms have been localized by immunofluorescence at the I band. Moreover, Homers were found to bind to a proline-rich motif of IP3R, which is also present in ryanodine receptor (RyR)/Ca2+ release channel, and, on the basis of immune-precipitation and GST pull-down experiments, a direct association of Homer 1c and RyR1 was postulated. Lastly, Homer 1 isoforms have been reported to influence in vitro the gating kinetics of RYR1.

The presence of Homer isoforms, referable to 1a, 1b/c/d, 2b and 3, was investigated in rat skeletal muscles under resting conditions. Transition in Homer isoforms compositon was studied under experimental conditions of short-term and long-term adaptation, e.g., fatigue and regeneration. Homer 1a was constitutively expressed and was transiently up-regulated during regeneration. In C2C12 cell cultures, Homer 1a was also up-regulated during formation of myotubes. No change of Homer 1a was observed in fatigue; Homer 1b/c/d and Homer 2b were positively and linearly related to muscle mass change during regeneration; Homer 3 was not detectable under resting conditions but was transiently expressed during regeneration and differentiation of C2C12 myotubes, although with a temporal pattern distinct from that of Homer 1a. It is concluded that Homer 1a and Homer 3 seem to play a pivotal role in muscle differentiation and regeneration, whereas Homer 1b/c/d and Homer 2b appear to be part of the muscle growth program. Homers may play a role not only in signal transduction of skeletal muscle, in particular regulation of Ca2+ release from sarcoplasmic reticulum, but also in adaptation.

PROTEOMIC ANALYSIS OF SKELETAL MUSCLES IN MDX AND A-SARCOGLYCAN-NULL MUTANT MICE

Brocca L. (1), Pansarasa O. (1), Bachi A. (2), Bottinelli R. (1) Pellegrino M.A. (1)

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Muscular dystrophies (MD) are human and animal diseases well known to determine a variable, but often dramatic deterioration of structure and function of skeletal muscle leading to progressive muscle weakness. However, there is surely a gap between the understanding of the molecular defects of MDs and the manifestations of the disease.

With the attempt to define the cascade of molecular events that lead to skeletal muscle alteration, two muscular dystrophies with known primary biochemical defects, were studied by a proteomic approach. The protein profiles of hindlimb (soleus and gastrocnemius) and respiratory (diaphragm) muscles were investigated in mdx and a-sarcoglycan-null mutant mice by using 1- and 2-dimensional gel electrophoresis (1-DE and 2-DE). Fiber type distribution was assessed on the basis of myosin heavy chain (MHC) isoform composition. By using 2-DE, proteomic maps were generated and more than 500 protein spots on each gel were detected by silver staining. Almost 50 protein spots were excised and characterised by mass spectrometry (MS). The 35% of the identified spots corresponded to oxidative enzymes, the 15% to glycolytic enzymes, the 10% to transport proteins, the 12% to cytoskeletal proteins and the 28% to "other proteins". Differences in protein expression between dystrophic and control muscles were defined.

Histological, immunohistochemestry and immuno-fluorescence analysis were also performed in order to evaluate muscle morphology in terms of degeneration and/or regeneration, atrophy and/or hypertrophy, connective infiltration.

HIF-1 ACTIVITY IN RAT FAST AND SLOW SKELETAL MUSCLE EXPOSED TO SYSTEMIC HYPOXIA

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Incresing evidence suggest that cellular responses to hypoxia are mediated by the hypoxia inducible factor-1 (HIF-1), a transcription factor playing a crucial role in oxygen homeostasis. In this study we aimed to assessed whether HIF-1 activity is the same in slow oxidative muscles, like soleus,

and fast glycolytic muscles as EDL. Rat soleus and EDL were transfected in vivo with a reporter construct carrying luciferase under control of concatamers of the hypoxia responsive element (HRE). Transcriptional activity of the HIF-1 was measured with luciferase assay in homogenates of soleus and EDL of rats breathing spontaneously in normoxia (21% oxygen) or hypoxia (10% oxygen). In normoxic conditions HIF-1 activity in the soleus was nearly double than in the EDL. The difference between soleus and EDL disappeared after 24 hours of denervation suggesting a role of muscle contractile activity in determining the higher HIF-1 activity in soleus. In hypoxic conditions HIF-1 transcriptional activity increased both in EDL and soleus. While in soleus HIF-1 activity remained high, in EDL it returned to the initial values after approximately 24 hours.

AFM-IMAGING AND ELASTICITY MEASUREMENTS OF THE SARCOLEMMA IN CULTURED SKELETAL MUSCLE FIBERS

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Atomic Force Microscopy (AFM) combined with optical microscopy was used to study three-dimensional structure and elastic properties of the sarcolemma of adult, fully differentiated, skeletal muscle fibers.

Single fibers were enzymatically and mechanically dissociated from flexor digitorum brevis (FDB) muscle of adult mice. On the sixth days after dissociation, the upper surface of intact fibers was analysed with AFM and the most prominent features in images were periodic transversal foldings with an intervals that corresponded to the sarcomere length. Further analysis of the topography profile showed that the depth of the foldings decreased with increasing sarcomere length and that the crests of the foldings corresponded to the z-lines. AFM images also showed deep depressions on the sarcolemma likely corresponding to openings of T tubules and caveolae. Two-dimensional elasticity maps were obtained using AFM as an indenter and showed that the crests of the transversal foldings corresponded to higher stiffness regions.

This study provides a complete three-dimensional topography and mechanical characterization of intact, living skeletal muscle fibers and might form the basis for further investigations aimed to compare healthy and dystrophic muscles.

FORCE GENERATION AND THE EFFECT OF INORGANIC PHOSPHATE IN SKINNED FIBRES FROM RABBIT PSOAS

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In the motor protein myosin II the unitary force or length step is associated to the release of inorganic phosphate (Pi) (Hibberd et al. Science 228:1317, 1985). We reinvestigate the effect of Pi on the kinetics and mechanics of the actin-myosin interaction in situ in skinned fibres from rabbit psoas (sarcomere length, 2.4 μm) activated by using a system that allows to rise the fibre temperature from 0-1 to 12°C (T-jump) in less than 5ms, with preservation and control of sarcomere length (Linari et al. J Physiol. 554:335, 2004). In control conditions (low Pi), we found that the rate of isometric force development (kTD) following the T-jump was 24.3 ± 1.5/s (mean ± SEM, 6 fibres) and the rate of force redevelopment following a period of unloaded shortening (kTR) was 24.0 ± 1.8/s. The addition of 10 mM Pi reduces the isometric force (T0) to 0.40 ± 0.01 the control and increases kTD and kTR to 37.6 ± 1.9/s and to 39.4 ± 2.7/s respectively. In a separate series of experiments the effect of Pi on half-sarcomere stiffness (e0) was determined. Addition of 10 mM Pi reduced T0 to 0.51 ± 0.02 (4 fibres) and e0 to 0.65 ± 0.02. After correction for filaments compliance, cross-bridge stiffness results to reduce in proportion to isometric force. These results indicate that i) independent of the nature of the intervention that inhibits force, either temperature or mechanics, the new equilibrium distribution of force generating heads is attained through the same rate limiting step and this step is sensitive to Pi; ii) Pi reduces the isometric force by reduction in the number of force-bearing heads, with no change in force per head.

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CROSSBRIDGE FORCE AND EXTENSION IN HYPERTONIC SOLUTION

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During fast stretching of activated muscle fibres, tension rises steeply to a peak (critical tension, Pc) at which the resistance to elongation greatly diminishes and the tension falls to a much lower level. This indicates a sudden fall of fibre stiffness caused by forced crossbridge detachment. Our previous results (Bagni et al., J Physiol. 565.1: 261-268, 2005) showed that during the tetanus rise Pc was directly proportional to the tension developed, thus providing a valid method to estimate the number of attached crossbridges not influenced by the myofilament series compliance. This technique was extended here to study the mechanism of tetanic tension inhibition in single muscle fibres perfused with hypertonic solution (1.6T obtained by adding NaCl). Fast stretches (9.5-33 sarcomere lengths s-1 and 16-25 nm hs-1

amplitude) were applied to activated muscle fibres from the tibialis anterior muscle of the frog (Rana esculenta) at the tetanus plateau and during the tetanus rise in Ringer and hypertonic solution, at 14°C. Force was measured with a fast transducer and sarcomere length was measured by a striation follower device. The results showed that the ratio of critical tension over isometric tension (Pc/P) increased in hypertonic solution by about 25%. This increase is almost exactly accounted for by the reduction of about 20% of the isometric tetanic tension observed in hypertonic solution. This result indicates that, in agreement with previous results, tension inhibition in hypertonic solution is not caused by a decrease of attached crossbridge number but by a reduction of the individual crossbridge force. In accordance with this idea, the sarcomere elongation needed to cause forced crossbrige detachment (Lc) was greater in hypertonic than in Ringer solution.

AUXOLOGY AND MOUNTAIN SPORTS FITNESS PROFILES

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Environment sports offer the possibility of multidisciplinary approach to all physical activities and thus become a support of training for all other sports activities. They facilitate the development of different physical capacities related to the specific period of growing up. They teach the youth to compare themselves with the environment. The nature that finally dictates the rules and regulations of the games even re-dimensioning those who are stronger.

To help the physiological development in the time being, means to create the capacity that remains unchangeable in the human body for the rest of the life. To put in actice this kind of concept, it is necessary that the instructors, sports doctors teachers and relatives etc know the opportunity that is offered by mountain schools, sailors schools and all the stages in the environment. For this reason, Scienze Motorie e Sportive University of Perugia has also a teaching about Mountains Sports in collaboration with Truppe Alpine Esercito Italiano.

- To teach sports in accord with the auxology means:
- to develop physical and sports exercises that help develop the physical capacity in the moment of physiological development.
- To teach a specific sports technique that is responsible in their own relationship and that of the others, through the rules of sports and that of athletic sports.
- Induce a mentality ready to the welfare through sports and physical exercises in such a way that it can create an adult who will never be an 'ex-athlete'.
- The activities in the environment involve these necessities whereby.
- Climbing, nord skiing, mountaineering skiing, sailors sports, help in the coordination, mobility and reactivate the limbs.
- Hiking, nord skiing/long distance racing and mountain skiing help in the development of the aerobic resistance capacity and above all, in the youths, teach to dose the individual capacities compared to prolonged time of exercises.

The sports in the environment, finally, reduce the risk of cronic fatigue.

We want to emphasize the importance also for the young people of our study about the "Fitness profile and Safety in Mountain". To understand your own physical limit and use a specific training program to better your physical capacities, this will provide a higher level of safety in the mountain.

ORGANIZATION OF SARCOPLASMIC RETICULUM PROTEINS IN DIFFERENTIATING MYOTUBES

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In mature skeletal muscle fibers the sarcoplasmic reticulum (SR) is a highly organized endomembrane system, which with its terminal cisternae and longitudinal tubules surrounds the contractile apparatus. The different domains of the SR are characterized by specific proteins responsible for different functions. In the terminal cisternae are localized, among others, the ryanodine receptors (RyR), triadin and calsequestrin, while the longitudinal tubules of the SR are mainly enriched in SERCA. The small ankyrin isoform ank1.5 and the InsP3R, are also localized in the SR, but the exact site is not well defined. We have analysed the organization of different SR proteins (RyR, triadin, SERCA, ank1.5, InsP3R) and of proteins of the contractile apparatus and cytoskeleton (α-actinin, obscurin, ankyrin B) in primary skeletal muscle cells at different days of differentiation. In double immunohistochemical stainings we compared the reciprocal localization of these proteins in order to obtain a time course of their organization during differentiation. In general, proteins of the contractile apparatus and of the cytoskeleton (α-actinin, obscurin, ankyrin B) appear to be organized earlier than SR proteins. Among SR proteins, those of the longitudinal tubules (ank1.5, SERCA, InsP3R) are organized in a pattern comparable with that of mature muscle earlier than proteins of the terminal cisternae (RyR, triadin).

ROLE OF CAMKII IN REGULATION OF GLYCOGEN METABOLISM IN MAMMALIAN FAST-TWITCH SKELETAL MUSCLE

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In mammalian fast-twitch skeletal muscle, multifunctional CaMKII is distributed within junctional and non-junctional sarcoplasmic reticulum (SR). Junctional SR-bound CaMKII participates in regulation of RyR1/Ca2+-release channel. Viceversa, the functional role of non-junctional SR-bound CaMKII, that does not regulate Ca2+ transport, remains still unclarified.

It is, however, known that: a) skeletal muscle contains a CaM-dependent glycogen synthase (GS) kinase activity; b) purified GS from skeletal muscle is phosphorylated by purified CaMKII; c) glycogen particles containing GS associate to SR, in register with I band; d) increases of [Ca2+]i.activate CaMKII in skeletal muscle; e) in liver, CaMKII was shown to regulate GS activity. Therefore, we hypothesize that CaMKII bound to non-junctional SR participates in regulation of glycogen metabolism, during conditions of raised [Ca2+]i.

We show that GS binds to SR, only if rabbits are treated with b-blocker propranolol. Experiments of glycogenolysis in vitro confirm that glycogen is largely responsible for binding of GS to SR. However, GS is also targeted to SR through binding to glycogen- and PP1c-targeting protein GM. Within SR, GS localizes to the Ca2+-pump membrane of non-junctional SR.

Phosphorylation experiments with [g-32P]ATP show, that both GS and GM are substrates of SR-bound CaMKII. However, no evidence for a structural interaction between the GS/GM complex and CaMKII was obtained.

SR-bound GS is in a high activity state. However, direct phosphorylation of GS by CaMKII do not appreciably reduce GS activity, suggesting that CaMKII might act sinergistically with other endogenous PK. These results support a central role of CaMKII in regulation of glycogen metabolism during muscle contraction.

PREVALENCE OF ASYMPTOMATIC HYPERCKEMIA

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Objective: Asymptomatic hyperCKemia (increased CK without symptoms and signs) is alarming for the affected subjects, a puzzling diagnosis for physicians and an increasing cause of referral to Neuromuscular Centres. Despite the fact that persistently increased serum CK levels are commonly encountered in healthy individuals, prevalence data do not exist. The aims of this study were to determine in a large and representative population: 1) the prevalence of asymptomatic hyperckemia; 2) the influence of age and gender on the CK levels.

Subjects and methods: We analysed the data of 1271 Italian subjects belonging to the Inchianti epidemiological study performed in two Italian towns located in the Chianty countryside: Greve in Chianti (11,709 inhabitants; rural area) and Bagno a Ripoli (village of Antella, 4704 inhabitants; just outside the urban area of Florence). The participants were all European of Caucasian race. The presence of possible causes of increased CK level (drugs, cancer, alcoholism, physical exercise, thyroid disease, cardiac disease, trauma) was determined by a questionnaire. A neurological examination was performed at the inclusion in the Inchianti study.

Results: CK were elevated in 117 subjects (9.2%) with normal neurological examination. Mean CK value was 284+ 174 UI/l (181-1763); 66% of subjects had CK < 259 UI/l and only 10% had CK > 405 UI/l. Fiftyseven subjects (4.48%) had at least one possible cause for hyperCKemia; yet in sixty subjects (4.72%) hyperCKemia was unexplained. The levels of CK were highest among the youngest age groups. No influence of gender on the CK level was found.

Conclusions: The prevalence of asymptomatic hyper-CKemia in the Italian population is 9,2%.

Two thirds of subjects have only very mild hyperckemia (less than one and half the control value).

This kind of subjects currently crowds the Neuromuscular Centres and only few of these may have an asymptomatic muscle disorders.

STRUCTURAL ALTERATIONS OF THE EXTERIOR MEMBRANE SYSTEM IN SKELETAL MUSCLE FIBERS ARE PRESENT IN IDIOPATHIC HYPERCKEMIA (IH)

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Objective: Persistent high levels of serum creatine kinase (CK) are considered a hallmark of neuromuscular diseases. However, chronic elevations of CK (hyperCKemia) may be occasionally encountered in apparently healthy individuals with normal examination, normal EMG and normal or mild, not specific, alterations in the muscle biopsy. This condition is defined idiopathic hyperCKemia (IH). Two recent reports showed an association between IH and muscle Caveolin-3 deficiency. While we believe that caveolin-3 deficiency is probably not a frequent cause of hyperCKemia, it is extremely interesting that a mutation of an exterior-membrane protein has been correlated to IH. For this reason, we have

hypothesized that persistently high level of serum CK could be also caused by still unknown alterations of exterior-membrane components.

Materials and methods: in muscle biopsies of four non related patients with IH and normal caveolin-3 immunohistochemistry (four men, range: 28-64 years) and three healthy controls (three men, range: 22-34) we analyzed, in six muscle fibers for subject, the arrangement of the internal T-tubule network and the frequency of caveolae.

Results: the ultrastructural study showed only in the patients a) unusual longitudinal orientation of the T-tubules network; b) abnormal swelling of the T-tubule profiles; c) unusual vesciculation of T-tubules. The frequency of caveolae was reduced of about 40-50% compared to control samples.

Discussion: this preliminary study suggests the presence of structural alterations of the muscle fibers exterior-membrane system (T-tubules and sarcolemma) and a significant decrease in the overall number of caveolae in the patients with IH. Interestingly a reduced number of caveolae was also found in patients affected by a Caveolin-3 deficiency and in Caveolin-3 knockout mice.

Conclusion: our findings suggest a correlation between IH and structural alterations of the exterior-membrane system.

THE CONTRIBUTION OF HYPERTROPHIC SKELETAL MUSCLE TO MOTOR NEURON SURVIVAL IN A MOUSE MODEL OF ALS

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Amyotrophic Lateral Sclerosis is a neuromuscular disease characterized by degeneration of both upper and lower motor neurons, associated with muscle atrophy, speech deficit and respiratory failure. Mutations in the gene encoding superoxide dismutase 1 (SOD1) has been associated to familial ALS disease. Transgenic mice ubiquitously overexpressing human SOD1 mutants develop the pathological features of ALS disease. However, the etiology of the disease and its cellular origin still remain to be defined. Recent studies suggested a possible role of non neuronal cells in the development of ALS disease; notably, the restriction expression of SOD1 mutant in postnatal motor neurons doesn't induce detectable signs of ASL pathology.

We recently investigated the contribution of hypertrophic skeletal muscle to motor neuron survival in a mouse model of ALS.

We observed that muscle-restricted expression of a localized mIGF-1 isoform maintained muscle integrity and enhanced satellite cell activity, stabilized neuromuscular junctions, reduced inflammation in the spinal cord, and enhanced motor neuronal survival in SOD1G93A mice, delaying the onset and progression of the disease. These studies establish skeletal muscle as a primary target for the dominant action of inherited SOD1 mutation and suggest ALS is emerging as a "multi-systemic" disease in which the alteration in structural, physiological and metabolic parameters in different cell types (muscle, motorneuron, glia) may act synergistically to exacerbate the disease.

THE PROMYOGENIC EFFECT OF TUMOR NECROSIS FACTOR ALPHA IS MEDIATED BY THE CYTOKINE-INDUCED ACTIVATION OF SPHINGOSINE KINASE

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A large body of experimental evidence is in favour of a complex role exerted by tumor necrosis factor alpha (TNF) in skeletal muscle. Indeed the inflammatory cytokine appears able to induce muscle wasting by stimulating protein degradation (1), but acting as an autocrine cue, it can also affect biological events such as myoblast proliferation, migration and differentiation, critical for correct tissue regeneration (2).

In this study we at first examined the effect of TNF on myogenesis by evaluating the expression of the early myogenic marker myogenin in C2C12 cells. In agreement with previous observations we found that low concentrations of the cytokine (up to 0.2 ng/ml) markedly enhanced the myogenin content whereas higher doses significantly reduced it, demonstrating the occurrence of a bimodal regulation of myogenesis by TNF. Given that in other cell systems the cytokine-directed activation of sphingosine kinase (SphK), that leads to sphingosine 1-phosphate (S1P) formation, is required for some of the cytokine effects and recently a pro-differentiating action of S1P in C2C12 cells was demonstrated (3), the possible role of TNF-induced S1P in the biological response to the cytokine was then examined. TNF was found to induce a sustained activation of SphK activity in C2C12 cells at concentration as low as 0.1 ng/ml, in favour of a role for the enzyme as effector of TNF also in myoblasts. Moreover, by employing the SphK inhibitor dimethyl-sphingosine and by overexpressing the wild-type or the dominant negative mutant of SphK1, it was shown that TNF-induced activation of SphK is required for the pro-myogenic action of the cytokine, supporting th notion that S1P generated in response to cytokine challenge accounts for this important physiological action exerted by TNF.

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S100B-DEPENDENT INHIBITION OF MYOBLAST DIFFERENTIATION: MOLECULAR MECHANISM

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Myogenesis is a multistep process in which the precursors of myofibers, the myoblasts, first proliferate and then differentiate into fusion-competent cells that form myotubes. S100B, a member of a multigenic family of Ca2+-modulated proteins of the EF-hand type, is expressed in several cell types some of which release the protein. Thus, S100B is found in serum and extracellular fluids, and its extracellular concentration increases under certain physiological and pathological conditions. S100B inhibits rat L6 myoblast differentiation by binding with high affinity (ca. 40 pM) and reversibly to an unidentified receptor (1). We show here that S100B independently inhibits the promyogenic MKK6-p38 MAPK pathway and stimulates the Ras-MEK-ERK1/2 pathway. S100B-induced inhibition of p38 MAPK causes a defective induction of the transcription factor myogenin and the antiproliferative factor p21WAF1, while S100B-induced activation of ERK1/2 causes stimulation of myoblast proliferation via cyclin D1 induction and Rb phosphorylation and protection against apoptosis via activation of NF-kB transcriptional activity. Thus, S100B might participate in the regulation of myoblast differentiation by stimulating myoblast proliferation, protecting myoblasts against apoptosis and modulating myotube formation. These effects of S100B might be important during embryogenesis, when myoblasts must attain a critical density for fusion into myotubes as well as in skeletal muscle regeneration, when satellite cells should exit the quiescent state and proliferate before fusion with the damaged myofibers. In both cases, the inhibitory effect of S100B on the promyogenic p38 MAPK could contribute to inhibit excessive myotube formation.

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RAGE MODULATES MYOBLAST PROLIFERATION, APOPTOSIS, ADHESIVENESS, MIGRATION AND INVASIVENESS

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We reported that RAGE (receptor for advanced glycation end products), a multiligand receptor of the immunoglobulin superfamily expressed in rat L6 myoblasts, if activated by its ligand amphoterin (HMGB1), stimulates myogenic differentiation via Cdc42-Rac-MKK6-p38 MAPK, and that RAGE expression in rat skeletal muscles is developmentally regulated (1). We show here that inhibition of RAGE function via overexpression of a signaling-deficient RAGE mutant (RAGEDcyto) results in increased myoblast proliferation, migration and invasiveness and decreased apoptosis and adhesiveness, while the opposite occurs in myoblasts overexpressing RAGE, compared with mock-transfected myoblasts. A decreased induction of the proliferation inhibitor, p21WAF1, and expression of adhesion molecules that are important for myogenesis and increased induction of cyclin D1, extent of Rb, ERK1/2 and JNK phosphorylation and matrix metalloproteinase 1 and 2 activity are detected in L6/RAGEDcyto myoblasts, while the opposite occurs in L6/RAGE myoblasts, compared with mock-transfected myoblasts. Neutralization of culture medium amphoterin negates all effects of RAGE activation in myoblasts. Effects of RAGE in myoblasts depend on p38 MAPK activation, because the p38 MAPK inhibitor, SB203580, reduces them in L6/RAGE and L6/mock myoblasts. Thus, the amphoterin/RAGE pair stimulates myoblasts differentiation by a combination of inhibition of proliferation and stimulation of differentiation. Also, based on the present findings, we speculate that deregulation of RAGE expression and/or function might contribute to the increased motility and invasiveness of myoblast neoplastic counterparts.

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ACUTE PASSIVE STRETCHING ALTERS THE MECHANICAL BUT NOT THE ELECTRICAL PROPERTIES OF CALF MUSCLES IN HUMANS

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An acute bout of passive muscle stretching has been shown to diminish maximal force and power output. Two mechanisms have been suggested to explain these findings: a mechanical alterations in the stretched muscle and an impaired neural activation. Thus, the aim of this study was to evaluate the stretching-induced changes in the electrical and mechanical properties of the muscle fibres during maximal electrically elicited contractions of the calf muscles. Twelve subjects (age 22±1 years, mean±s.e.m.; body mass 75±2 kg; stature 179±2 cm) underwent 6 single twitch electrical stimulation at maximal intensity before and after a bout of passive stretching. During contractions, the force, surface EMG and mechanomyogram (MMG) were simultaneously recorded from the medial gastrocnemius muscle. From the analysis of the 3 signals, after stretching it resulted that: i) the force peak, time to peak and the peak rate of force production significantly decreased by 12.6±2.9%, 3.5±1.0% and 13.6±4.9%, respectively; ii) the MMG amplitude (peak-to-peak) also decreased (-3.6±1.3%; $p<0.05$), and iii) no differences were found in EMG parameters. In conclusion, acute passive stretching affected the mechanical but not the electrical properties of the maximally contracting muscles, suggesting an alteration of the musculotendinous stiffness, but not a depression of muscle activation.

REDOX REGULATION OF BETA-ACTIN

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Intracellular reactive oxygen species (ROS), including hydrogen peroxide and superoxide anion, have a key role in regulating intracellular processes such as growth factor signal transduction, gene transcription and cytoskeleton rearrangement. We previously reported that in fibroblasts upon integrin engagement there is a dramatic rise of ROS production and we therefore planned to identify the redox regulated proteins during this process. We used a proteomic approach involving protein labelling with N-(biotinoyl)-N'-(iodoacetyl)ethylenediamine (BIAM), a sulfhydryl-modifying reagent that selectively probes the thiolate form of reactive cysteine residues. We have identified two proteins as targets of integrin-generated ROS: β-actin and Non-Muscle Myosin Heavy Chain (nmMHC). Confocal analysis of actin polimerization shows that the treatment of the cells with several antioxidants, including NADPH-oxidase and 5'-LOX specific inhibitors and general scavengers, completely impedes actin fiber formation during cell adhesion leading to a round cell shape. Furthermore, the inhibition of GSH production by buthionine sulfoximine leads to a dramatic disorganization of actin cytoskeleton. β-actin Cys374 is the target of integrin-generated ROS, since overexpression of a Cys374 to Ala mutant form of β-actin dramatically affects both cell spreading and actin stress fibers organization. Hence, ROS produced by integrins during cell adhesion induces a GSH-mixed disulfide involving cys374 of β-actin, leading to actin stress fibers formation and cell spreading. These studies open a new prospective for the regulation of cytoskeletal proteins during several circumstances, including chemotaxis, myogenesis, neurogenesis and metastasis dissemination.

MACROPHAGE-SECRETED FACTORS ENHANCE THE PROLIFERATION RATE OF DUCHENNE MUSCLE DYSTROPHY MYOBLASTS

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Duchenne Muscle Dystrophy (DMD) is an X-linked genetic disease caused by mutations of the Dystrophin gene. The lack of this cytoskeletal protein produces cycles of muscle degeneration-regeneration, which in turns lead to a progressive depletion of satellite cells. Cell transplantation is considered to be one possible therapeutic approach for the disease. Such approach could be based either on the use of myogenic stem cells or on the re-transplantation (within or not a biocompatible scaffold) of the patient's myoblasts after ex vivo expansion and genetic modification.

We have already shown that a murine macrophage conditioned medium (mMCM) can enhance rat muscle cells proliferation. Here we report that mMCM can also increase the proliferation rate of myoblasts obtained from a DMD patient's biopsy. In particular, the average doubling time dropped from 38.8 hours in untreated cells to 31.3 hours in mMCM-treated cells. In other terms, while in 51 days a control cell generated about 106 myoblasts (20 duplications), under mMCM treatment it produced more than 1010 cells (33 duplications). In both cases, cell cultures showed a good differentiation ability if seeded at high density. DMD cells expanded in the presence of mMCM were also injected in nude mice muscles after a Bupivacaine injury. Their grafting efficiency is presently under evaluation.

In parallel, we are evaluating the effects of a human macrophage conditioned medium (hMCM) on the same experimental model. Preliminary results suggest that the hMCM-induced effects on DMD cell proliferation could be less prominent in comparison to mMCM.

PHYSIOLOGICAL CHARACTERIZATION OF 2-WEEK REGENERATED SOLEUS MUSCLE FIBRES

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The functional properties of 14-day regenerated rat soleus muscle was investigated in chemically skinned fibres. Regeneration was induced by myotoxic injury with bupivacaine. Myofibrillar and sarcoplasmic reticulum properties of single fibres were correlated to the expression of specific protein isoforms.

The maximal specific tension of regenerating fibres was not different from that of controls, despite the smaller CSA (about 45%) of regenerating fibres. The 14-day regenerating soleus fibres expressed type 1 or 1+2A myosin heavy chain isoforms in addition to residual embryonic and/or neonatal isoforms. The regenerating fibres showed a significant right shift of pCa-tension relationships and a higher pCa threshold. SDS-PAGE analysis showed regenerated fibres containing the slow troponin C isoform as well as fibres with both the slow and fast isoform. The presence of the fast isoform was correlated to the higher pCa threshold of fibres.

The caffeine threshold concentration of sarcoplasmic reticulum Ca2+ release was significantly higher in regenerated than in control fibres. Consistent with the lower sensitivity to caffeine of RyR-3, regenerated muscles expressed a significant higher level of this RyR isoform. The amount of Ca2+ released at maximally-activating caffeine concentration

(20 mM) was higher than in controls. Moreover, sarcoplasmic reticulum Ca2+ capacity, measured by a light-scattering method, was higher in regenerated fibres than in controls. In contrast, Western blot analysis in the whole muscle did not show appreciable differences in SERCA isoform expression between regenerated and control muscles, suggesting that the large sarcoplasmic reticulum Ca2+ capacity of regenerating fibres is likely due to a larger volume. Consistently, the rate of Ca2+ uptake, was not different in 14-day regenerating fibres with respect to control. Finally, the regenerated muscle shows a larger amount of the cardiac isoform of DHPR with respect to the control.

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TIME-COURSE PROFILE OF MUSCLE ACTIN ISOFORMS EXPRESSION IN DIFFERENTIATING HUMAN SATELLITE CELLS ISOLATED FROM DONORS OF DIFFERENT AGE

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Myogenesis is a complex and highly coordinated process, mainly sustained by a subpopulation of myogenic cells known as satellite cells (SC). The progressive loss of contractile efficiency and metabolic activity, together with the reduced capability of muscle regeneration that occurs during aging, can be explained by the decrease of the SC number and/or their limited proliferative and differentiative capacity with age. In order to elucidate the molecular mechanisms underlying muscular senescence, we have performed primary cultures of satellite cells from skeletal muscle of different aged humans (a 5-days newborn, a 34-year-old young adult and a 58 and 71-year-old elderly). All these cultures were characterized in the mean lifespan, and in the proliferative and differentiative capability. Myogenicity was assayed using desmin, Pax7, myosin heavy chain, and actin expression analyses. Time-course expression of a-smooth muscle (aSMA) and a-sarcomeric muscle (aSRA) actin isoforms was studied using immunocytochemistry, western blotting and reverse transcriptase polymerase chain reaction. The two actin isoforms were expressed in proliferating and differentiating cells derived from donors of all examined ages. aSMA was present at the myoblast stage and it was down-regulated during differentiation, cell fusion, and myofiber organization, while aSRA was up-regulated in myotubes. No significant differences were observed in newborn, young, and elderly normal human SC cultures. Studies carried out with the myogenic established C2C12 cell line, used as myoblast differentiation model, confirmed the results. Eventhough aSMA is not a tissue-specific protein, our data suggest that this actin isoform could play an important role in the morphological changes that lead to myogenic cell fusion and myofiber organization, so it can be used as a molecular marker of myogenic differentiation.

GENE EXPRESSION IN RAT SKELETAL MUSCLES AFTER MEDIUM- AND LONG TERM-DENERVATION

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Traumatic injuries, neurodegenerative diseases or aging may determine skeletal muscle denervation and induce an atrophy-dependent loss of fibers and a number of morphological, biochemical and physiological modifications. Upregulation of mRNAs encoding for myogenic transcriptional factors and for the protein degradation machinery is well documented after medium-term denervation1.

We decided to evaluate by Real Time RT-PCR a number of mRNAs from rat skeletal muscles after medium- (2-3 months) or long- (9 months) term denervation, in order to extend the observations reported in literature to a longer time interval, and to include a number of previously undescribed mRNAs.

Rat tibialis anterior muscles from the hind limbs were surgically denervated in aseptic conditions. Rats rapidly recovered and were stabulated in standard conditions before sacrifice. The muscles were removed from age-matched control rats and from rats that had undergone denervation. RNA was extracted by Tryzol® and quality controlled, then retrotranscribed and evaluated by Real Time PCR using SybrGreen dye. MCK RNA, a housekeeping gene, was used for standardization purposes. The following mRNAs were evaluated: Myogenin, MyoD, Mrf4, PGC-1alpha, embryonal myosin chain-3 (Myh3), metalloproteinase-2, calsequestrin-2, HSP70, VEGF, VEGF-R2 (KDR).

Main results can be summarized as follows: i) all examined mRNAs were found to be upregulated after 2-3 month-denervation in respect to controls; ii) the amount of expression widely varied among different mRNAs, with muscle-specific transcription factors and Myh3 mRNAs being the higher expressed; iii) for such genes, as well as for HSP70, VEGF and KDR, upregulation was maintained also after long-term denervation. This observation may be of clinical interest in humans2, where rehabilitation care is supplied months and even years from denervation.

1-Kostrominova T Y et al., Physiol Genomics. 2005; 22:227-43.
2-Carraro U et al., Artif Organs 2005; 29:187-91.

MHCS GENE CLUSTER ORGANISATION AND THEIR MRNA EXPRESSION IN DOG SKELETAL MUSCLES

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Myosin heavy chains (MHCs) are actin-based motor proteins that play a key role in determining muscle contraction speed. MHC isoforms expressed in mammalian muscles are encoded by a multigene family. This MHC gene superfamily presents a highly conserved cluster organisation in the human and mouse genomes (Weiss et al., 1999). In this work we used computer–based programs to identify and characterise dog MHC sequences in dog genome from NCBI Dog Genome Resources web site. Moreover, we analysed the expression pattern of the identified dog MHC isoforms by RT-PCR in the trunk, limb and specialised (masticatory, intrinsic laryngeal and extraocular) muscles.

BLAST searches suggest that the genes coding for the fast dog isoforms (embryonic, 2A, 2X, 2B, neonatal and extraocular) cluster on chromosome 5; the two "cardiac" genes, coding for MHC-β (slow) and MHC-α (cardiac), are arranged tandemly on chromosome 8; the gene for masticatory isoform (2M MHC, characteristic of masticatory muscles of dog) has been located on chromosome 6. The phylogenetic analysis confirmed the position of the dog orthologous isoforms within each specific MHC group with high similarity values (94-98%). The limb and trunk muscles express 1, 2A and 2X MHC, although a fourth cDNA, corresponding to 2B MHC, has been found in semimembranosus but not in longissimus dorsi. In contrast, in specialised muscles (retractor bulbi, rectus lateralis and in some laryngeal muscles) the 2B MHC mRNA was constantly present. Our results confirm that the fourth band identified by Wu et al. (2000) in thyroarytenoideus muscle correspond to the 2B MHC isoform.

TWO INDEPENDENT MECHANICAL EVENTS IN THE INTERACTION CYCLE OF SKELETAL MUSCLE MYOSIN WITH ACTIN

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The rate of the cross-bridge cycle and its dependence on the load, defining shortening velocity and energy consumption at the molecular level, vary widely among skeletal fibres expressing different isoforms of myosin II. The underlying mechanisms remain poorly understood. Here we apply a novel single molecule approach to rapidly (~300 μs) and precisely (~0.1 nm) detect acto-myosin interactions of two myosin isoforms (type 1 from rat and type 2B from mice) having large differences in shortening velocity. We show for the first time that skeletal myosin propels actin filaments performing its conformational change (working stroke) in two steps. The first step (~3.4–5.2 nm) occurs immediately after myosin binding, is followed by a smaller step (~1.0-1.3 nm) and then by dissociation of the acto-myosin complex. The two steps have independent durations ton1 and ton2. The rate of the first step (1/ton1) is much higher in the fast (type 2B) myosin isoform (1156±396 s-1) than in the slow (type1) one (42.8±5.9 s-1), independently of ATP concentration. On the other hand, the rate of the second step (1/ton2) depends linearly on ATP concentration and is similar in the two isoforms. These findings provide a simple model of the regulation, in different skeletal fibres type, of shortening velocity and its load sensitivity.

MMCM ENHANCED THE PROLIFERATION OF A SUB-POPULATION OF MUSCLE-DERIVED CELLS OBTAINED BY SERIAL PLATINGS

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Several methods have been developed to isolate stem cells from muscles; one of them is Huard's (serial) preplate protocol, with which Muscle Derived Stem Cells can be isolated from murine adult muscle.

Having already demonstrated that growth factors present in a murine macrophage conditioned medium (mMCM) increase myoblast proliferation, we set out to verify if mMCM could also affect mouse muscle stem cells. To this aim we designed a modified version of Huard`s protocol, starting from either (a) neonatal, primary muscle cultures or (b) the cellular fraction remaining in single fiber preparations after all fibers had already been removed. In both cases, cells were prepared from GFP transgenic animals and were continuously supplied with mMCM.

When using protocol a, in the presence of mMCM after the sixth plating passage we observed many round-shaped, desmine –ve, proliferating cells, growing in suspension to form floating clusters. On the other hand, no more cells were left in control plates after passage six. At each plating passage, a certain percentage of these round cells tended to adhere to the bottom of the plate and spread out; still, they did not fuse and continued to slowly proliferate. Importantly, both adhering and suspended cells could fuse with pre-existing fibers when injected in regenerating muscles.

With protocol b, control cells were still present after passage six, although not nearly as many as with mMCM. Again, cells were round-shaped but, as opposed to protocol a, most of them grew attached to the plates and tended to fuse as soon as they touched each other.

MURINE MACROPHAGE CONDITIONED MEDIUM (MMCM) IMPROVES IN VIVO MUSCLE REGENERATION AFTER MASSIVE TISSUE ABLATION

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After muscle injury, satellite cells proliferation is associated to inflammatory processes in which macrophages play an important role.

We have recently shown that factors present in a murine macrophage conditioned medium (mMCM) enhance myogenic properties and proliferation of cultured muscle-derived rat cells. Here we report data on mMCM myogenic properties in vivo. In our experimental model we surgically removed approximately 50% the Tibialis Anterior (TA) muscles in adult Wistar rats and replaced it with a bio-degradable filler.

We operated 15 animals: the left limb of each animal was injected with mMCM, while right limbs received just fresh medium (DMEM). In this experimental model, muscle ability to regenerate was per se insufficient to compensate the induced damage. In fact, no improvement of control leg weight values were observed 21 days after the surgery. In contrast, the weight of mMCM-treated muscles reached on average almost 80% of the initial mass. Histological analyses confirmed that the newly formed biomass was mostly composed of regenerating myofibers, associated to a modest amount of connective tissue. The latter was less abundant than what found in controls. After 38 days, most fibers in mMCM-treated muscles had a larger section and showed peripheral nuclei, revealing a higher maturation degree.

These findings clearly showed that mMCM administration could greatly enhance in vivo muscle regeneration. They also suggest that mMCM could interact with the complex network of signals involved in the interplay between inflammation and muscle regeneration in vivo.

CASPASES REGULATE SKELETAL MUSCLE REGENERATION

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With the aim to identify novel regulators of skeletal muscle regeneration, we studied this process in injured murine Tibialis anterior, injected with TNF-a or AVP, a negative and a positive regulator of myogenesis in vitro, respectively. Histological analysis revealed that TNF-a determined a robust reduction in the number and size of regenerating (centronucleated) fibers as compared to control (PBS-injected), AVP increased the number of regenerating fibers as compared to control, while a combination of TNF-a and AVP resulted in a regeneration level similar to controls. We have previously demonstrated the importance of caspases in regulating muscle differentiation in vitro, suggesting a possible involvement of these proteins in muscle regeneration. Indeed, caspase activity was detected in many interstitial cells and was upregulated in the TNF-a treated muscles in the absence of apoptosis, while AVP abrogates such upregulation. Moreover, a pan-caspase inhibitor mimicked AVP having a positive effect on both the number of myogenic stem cells and on the number of regenerating fibers, in the absence or in the presence of TNF-a. To study the molecular mechanism underlying the opposite effects of AVP and TNF-a on caspase activation, we studied the expression of Hsp 70, a chaperone known to inhibit caspases, in our experimental condition. Our data showed that TNF-a negatively affects Hsp 70 expression and AVP blocks such TNF-a-mediated downregulation. In addition, the regeneration extent of Hsp70-electroporated muscles was similar to that occurring in AVP-treated muscles, in the absence or in the presence of TNF-a. We suggest that Hsp70-regulated caspase activity controls muscle regeneration and accounts for the opposite effects of AVP and TNF-a on both caspase activity and muscle regeneration.

PLD ACTIVITY REGULATION AT THE EARLY STAGES OF MYOGENESIS

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We showed that that PLD participates in myogenesis through PA- and PIP2-dependent actin fibres formation, in line with the recognized central role of cytoskeleton rearrangement in myogenesis. Because PLD is a recognized target of ceramide and this sphingolipid messenger is involved in the pathophysiology of muscle tissue, we investigated the role of ceramide in the control of PLD and differentiation in myogenic cells We first evaluated ceramide levels in L6 myoblasts induced to differentiate by AVP. Bi-phasic changes were observed, a short-lived lowering being followed by a long-lasting elevation, attributable to the de novo pathway of synthesis because it was totally prevented by inhibitors of this pathway fumonisin B1 (FB1) and myriocin, and unaffected by the sphingomyelinase inhibitor desipramine. Both de novo synthesis inhibitors increased the AVP-induced expression and nuclear accumulation of myogenin, cell fusion rate, and expression of creatine kinase, whereas short-chain ceramides inhibited these responses. This strongly suggests that

endogenous ceramide mediates a negative control of myogenic differentiation, and that inhibitors of de novo ceramide synthesis promoted myogenesis by removing this control. FB1 induced an increase in PLD activity, whereas C6-ceramide decreased it. Furthermore, the expression of PLD1 isoform mRNA transcripts was selectively increased by de novo synthesis inhibitors and decreased by C6-ceramide, as observed in RT-PCR experiments. We hypothesize that ceramide regulates myogenesis at least in part through down-regulation of PLD1 expression. Taken together these data enlight the role of PLD as a regulator of myogenic differentiation, acting through the control of cytoskeleton.

CLINICAL AND FUNCTIONAL CHARACTERIZATION OF COMPOUND HETEROZYGOUS CASQ2 MUTATIONS ASSOCIATED WITH CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA (CPVT)

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The human cardiac calsequestrin gene (CASQ2) is implicated in the autosomal recessive variant of CPVT. So far four CASQ2 mutations have been reported in the literature. Here, we report a homozygous 16bp deletion (CASQ2G112+5X) that was found in a child with stress-induced ventricular tachycardia (VT) and cardiac arrest. The same deletion was also identified in association with a novel point mutation (CASQ2L167H) in another symptomatic CPVT child who is the first CPVT patient carrier of compound heterozygous CASQ2 mutations so far reported. Both probands had a history of stress-induced syncope and exercise-induced bidirectional or polymorphic VT. Family history was negative for premature sudden death. 13 heterozygous carriers in both families (11 with the deletion and 2 with the missense mutation) showed no clinical sign of CPVT. Functional studies revealed that CASQ2G112+5X did not bind Ca2+ at all, whereas CASQ2L167H had normal calcium binding properties in vitro; both mutations decreased the sarcoplasmic reticulum Ca2+ storing capacity and reduce the amplitude of macroscopic ICa-induced Ca2+ transients, in patch-clamped myocytes; myocytes developed isoproterenol-induced delayed afterdepolarizations in CASQ2G112+5X but not in CASQ2WT and CASQ2L167H isolated myocytes.

Our findings indicate: 1) compound heterozygous CASQ2 mutations cause CPVT; 2) despite complete knock-out of CASQ2 function, the clinical phenotype in the homozygous deletion carrier is similar to that of the prototypical CPVT patient suggesting that the CASQ2 function may be at least partially compensated by other mechanisms; 3) only CASQ2G112+5X displays altered calcium binding properties and delayed afterdepolarizations, whereas both CASQ2L167H and CASQ2G112+5X alter CASQ2 function in cardiac myocytes. Overall the functional properties of the mutants indicate that mutations identified in CPVT patients create the substrate for the development of triggered arrhythmias.

SYSTEMIC DELIVERY OF AAV VECTOR EXPRESSING ANTISENSE-U1 SNRNA RESTORES DYSTROPHIN EXPRESSION AND FIBRES FUNCTION IN SKELETAL MUSCLES OF MDX MICE

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Most mutations in the dystrophin gene determine a frameshift or a premature stop in mRNA and cause Duchenne muscular dystrophy. These mutations can be corrected by preventing the inclusion of mutated exon in the mature mRNA. Exon skipping can be obtained by expressing antisense sequences against the splice junctions of mutated exon. We studied the effects of systemic delivery, of AAV vector co-expressing antisense-U1 snRNAs against exon 23 as well as GFP, on dystrophin expression and fibre function in mdx mice. CK serum levels reached intermediate values between control C57/Bl6 and untreated mdx. WB and immunostaining showed that GFP-dystrophin expression prevails in the gluteus, gastrocnemius and vastus, while it is more diffuse in the diaphragm. The correct expression and localization of a and b sarcoglycans revealed a partial reconstitution of the dystrophin-sarcoglycan complex. To evaluate whether dystrophin expression supports function recovery, specific force (Po/CSA) of single fibres from the vastus of control, untreated mdx and GFP positive and negative bundles from treated mdx mice was measured. Results showed a significant lower Po/CSA in mdx and GFP negative fibres vs. GFP positive fibres; the functional recovery of treated mice was confirmed by treadmill exhaustion tests. We conclude that systemic delivery of AAV/antisense-U1 induces exon skipping of the mutated exon 23 followed by dystrophin rescue and functional recovery in mdx mice.

LACK OF SKELETAL CALSEQUESTRIN DETERMINES A CHANGE IN THE ULTRASTRUCTURE OF CALCIUM RELEASE UNITS

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Calcium release units (CRUs) in adult skeletal muscle fibers are usually named triads because they are constituted by three elements: two sarcoplasmic reticulum terminal cisternae (junctional SR) closely apposed to a central transverse tubule (TT). The lumen of the SR terminal cisternae contains calsequestrin (CSQ), a high-capacity Ca2+-binding protein located at the junctional face of the SR terminal cisternae (jSR) near the sites of Ca2+ release, the ryanodine receptors (RyRs). Two isoforms, skCSQ and caCSQ, are differentially expressed in adult skeletal muscle fibers: esclusively skCSQ in fast twitch fibers, while in slow twitch about 25% of the total amount is caCSQ. To better define the role of CSQ in the excitation-contraction coupling apparatus, we have created a knock-out model, null for the skCSQ isoform. Western blot analysis of skCSQ-null limb muscles confirms the absence of skCSQ, while caCSQ is still present. Mice are usually smaller in size and preliminary hand grip strength test values are significantly lower of 30-40% compared to control mice values. We have analyzed skCSQ-null calcium release units (CRUs) in the EDL and SOLEUS using electron microscopy and our results show a striking ultrastructural effect of the mutation. jSR contain caCSQ: the EDL shows several features peculiar to cardiac jSR, while in the SOLEUS the ultrastructure is not greatly altered. However, in both muscles the overall shape of the jSR is flat and CSQ is condensed into dense bands. Differently from SOLEUS, in EDL an increased assembly of CRUs is evident. Our results suggest that skCSQ is an important determinant of spatial orientation and ultrastructure of skeletal muscle CRUs.

LEVELS OF MRNA GROWTH FACTORS IN FOETAL AND NEWBORN PIGLETS

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Porcine muscles sampled at postnatal phases of development show new fibres which express myoD and neonatal myosin heavy chain (MHC-Neo). Muscle hyperplasia after birth is quite rare event in mammals. So far, this phenomenon has been described in detail only in few species although foetal and postnatal myogenic factor mRNAs have not been compared yet. To study these factors during different hyperplastic phases of development we used expression methods (standard RT-PCR, quantitative Real Time PCR, Western blotting and Immunohistochemistry) together with cell culture experiments. Initial experiments indicated that: - high levels of myostatin mRNA are found in muscles with a peculiar myosin heavy chain composition such as extraocular eye muscles; - myostatin mRNA level is higher during gestation than after birth and another peak is reached at 30 days post partum; - myostatin protein is present in its mature form especially in postnatal stages (and very weakly in foetus); - myogenin mRNA levels raised at 50 days post partum while Pax7 and CD34 showed a decreasing trend from birth. The biological significance of this expression pattern, responsible of muscle hyperplasia, is due to the development of tertiary myotubes. It is likely that the latter are committed not only during pregnancy but also throughout initial postnatal phases of growth, since at birth we detected a high percentage of proliferative satellite cells.

ROLE OF INFLAMMATION IN MUSCLE REGENERATION

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Muscle regeneration is a multiphasic process associated with the infiltration of inflammatory cells.

Emerging data suggest that macrophages release several factors, including chemokines, that modulate the muscle regeneration both activating migration, proliferation and differentiation of satellite cells (sc) and inducing mobilization of hematopoietic stem cells (HSCs) from bone marrow to periferal blood and then to injured muscle. We recently reported that regenerating MLC/mIgf-1 transgenic muscles are able to increase the proliferation of sc and the recruitment of bone marrow cells to site of tissue damage. In addition we studied the role of inflammation in the modulation of muscle regeneration. Semiquantitative RT-MPCR and proteomic analysis indicate that mIGF-1 modulates the expression of inflammatory factors, accelerating the "switching off" of inflammatory process and consequentially the timing of regeneration process.

These results suggest that mIGF1 generates a more qualitative environment improving the muscle regeneration, activation of satellite cells and recruitment of HSCs.

SATELLITE CELLS DERIVED FROM VASTUS LATERALIS MUSCLE OF CFS PATIENTS: FIRST DATA

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Chronic Fatigue Syndrome (CFS) is a debilitating and complex disorder characterized by profound fatigue that is not improved by bed rest and that may be worsened by physical or mental activity. Patients with CFS report various nonspecific symptoms, including weakness, muscle pain, impaired memory and/or mental concentration, insomnia, and post-exertional fatigue lasting more than 24 hours. In some cases, CFS can be reversible. The cause or causes of CFS have not been identified, and no specific diagnostic tests are available (Natelson et al. 2002, Environ Health Perspect. 110: 673-7).

In previous study we demonstrated functional alterations of muscular biopsies from patients with CFS, also related to the oxidative damage (Fulle et al. 2000, Free Rad. Biol. Med. 29:1252-9; Fulle et al. 2003, Neuromuscular Disorders 13:479-84).

In the present work we used satellite cells (SC) obtained from 10 CFS patients to investigate some functional activities and capacity to differentiate. What we noticed is that the proliferative life span SC from CFS patients is quite different from the controls (age-matched), indeed the proliferative potential is increased. Moreover with the increase of in vitro life span we noticed an increase of myogenic purity, evaluated by desmin expression. On the contrary a decrease of differentiation efficiency was observed; those data were also confirmed by videoimaging experiments. To check if CFS affects the excitation-contration coupling mechanism in myotubes, we tested the Ca2+ channels functionality during several differentiation phases. The data derived from those in vitro experiments seem to indicate a uncorrected E-C coupling, at least at the end of differentiative process. In conclusion satellite cells from CFS patients showed a dysfunctional behavior in their proliferative and differentiative capacity.

MYOTUBES AND FIBERS CULTURES ON TRANSISTOR MICROCHIPS: INNOVATIVE WETWARE DEVICES FOR SINGLE CELL AND NON-INVASIVE STIMULATION, REGISTRATION AND TRANSFECTION

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The aim of this study was to develop an artificial silicon-muscle junction to allow the control of single cells or fibers with in vitro long term contraction patterns, based on software and instrumental to muscular tissue engineering, new experimental approaches, and muscular regeneration models.

Two distinct biological models were employed: 1) Satellite primary mouse cells were cultured on different silicon chip devices and induced to differentiate to myotubes developing a muscle-silicon junction on stimulation spots integrated in the chip wire. This was used to elicit single myotubes excitation, EC coupling and contraction. 2) Fully differentiated muscle fibers were dissociated from one month mice and cultured on chip devices for the same purposes and for non-invasive registration through Transducive-Extracellular-Potential (TEP) induced in the cleft junction between surface chip and fiber membrane as a result of evoked action potential.

The development of this model has allowed us to replace the diffuse field stimulation of a cell population with the control of single cells with the high spatial resolution given by capacitive stimulation. Single cells can be stimulated and monitored in a precise and targeted way and cells exposed to different conditions can be compared.

CROSS-BRIDGE RECRUITMENT BY STRETCH OCCURS IN THE SUBMILLISECOND TIMESCALE

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We investigated the speed of cross-bridge recruitment during forcible lengthening of active muscle (linari et al., j. physiol. 526, 589-96, 2000) by collecting mechanical data at various times during and after a step stretch of ~4 nm per half sarcomere (hs) and x-ray interference data after stretches of various amplitudes, superimposed on isometrically contracting intact fibres from frog muscle (4°c and 2.1 mm sarcomere length). at 0.8 ms after the 4 nm/hs stretch, the stiffness was 1.23 ± 0.06 (mean ± sem, 3 fibres) times the isometric value, which was recovered several tens of ms after the stretch. changes in the x-ray fine structure of the myosin-based 3rd order reflection following stretches of 2-6 nm/hs could not be explained only by the stretch-induced axial movement of the attached myosin heads, and indicate an extra-mass moving together. these results can be explained if attached myosin heads act as strain sensors that induce a submillisecond recruitment of the second head during stretch.

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FUNCTIONAL AMELIORATION OF DYSTROPHIC PHENOTYPE IN A DOG MODEL OF DUCHENNE MUSCULAR DISTROPHY THROUGH INTRA-ARTERIAL DELIVERY OF MESOANGIOBLASTS

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We studied the effects of intra-arterial delivery of wild-type mesoangioblasts, a class of vessel-associated stem cells, in golden retriever dogs affected by a muscular dystrophy (GRMD) due to dystrophin absence. Untreated dogs and healthy control dogs were also studied for comparison. Three injections of 100.106 mesoagioblasts each were performed in the femoral artery of GRMD dogs. At the end of the treatment, biopsy samples were taken from muscles of the treated and untreated leg. Immunohistochemical and western blot analyses indicated dystrophin expression in a large percentage (~50%) of muscle fibres in the muscles downstream of the injection. Tibialis anterior muscles in the treated leg showed a significant recovery of tetanic force in vivo. The CSA and force of single muscle fibres, dissected from the biopsy samples, was determined in vitro. Fibers were classified on the basis of myosin heavy chain (MHC) isoform composition. Moreover, all fibres from treated dogs used for force measurements were immunostained with anti-dystrophin antibodies. Fibres could, therefore, be grouped in positive, containing dystrophin, or negative, without the protein. Data following heterologous delivery of mesangioblasts to dystrophic dogs suggest a recovery of force towards normal values in the type 2A fibers containing dystrophin in comparison with type 2A fibres from untreated dogs and with fibres of the same treated muscle, but lacking dystrophin.

MECHANICAL PROPERTIES OF FAST AND SLOW SKELETAL MUSCLE IN MLC/MIGF-1 TRANSGENIC MICE: AN IN VITRO APPROACH

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Since the first years of the last century, different protocols have been developed to characterize the mechanical properties of muscle tissue. In all of them, usually divided in isometric and isotonic test, the muscle is electrically stimulated and the induced displacement and force are acquired. Furthermore, during repeated contractions, muscles exhaust and fail to maintain the required force; therefore fatigue measurement, both in isometric and isotonic condition, becomes essential to fully characterize muscle tissue.

In this work we developed an isometric/isotonic protocol to define skeletal muscle properties of (TG) transgenic MLC/mIgf-1 mice. The MLC/mIgf-1 mice are a model of persistent, functional myocyte hypertrophy generated using a tissue-restricted transgene, encoding a locally acting isoform of insulin-like growth factor-1 that is expressed in skeletal muscle (mIgf-1).

In particular we examined mechanical properties of slow (soleus) and fast (EDL) muscles of TG mice compared to wild type muscles.

Our protocol consists of two experimental parts: in the first one the muscle is stimulated isometrically to characterize the response to a single twitch and its tetanic condition. Subsequently, the specimen is submitted to electrical stimulation in isotonic condition, evaluating Hill's equation, power generated by the muscle and isotonic fatigue.

Preliminary results confirm the presence of functional hypertrophy: the tetanic force (Fmax) in TG muscles is about 20% greater than wild type, while there is no change in the specific force (Fmax/weight).

In addition, TG muscles seem to generate mechanical power about 30% greater than wild type muscles, and no differences seem to arise in the fatigue.

GENETIC SCREENING OF THE ENTIRE RYR1 GENE AND EXPRESSION OF MUTATED PROTEINS

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Mutations in the RYR1 gene are linked to three muscle genetic disorders, malignant hyperthermia (MH), central core disease (CCD) and multi-minicore disease (MmD). MH is an autosomal-dominant inherited disorder that causes spasm tachycardia and hyperthermia in susceptible patients when exposed to volatile anaesthetics and muscle relaxants. The causative defect for MH was proposed to lie in an altered release of Ca2+ through RyR1 channels that may lead to a rapid and high increase in the myoplasmic Ca2+ concentration. Mutations in the RyR1 gene are clustered to three main regions of the channel, including residues 35-614 (region 1), 2117-2787 (region 2) and residues 4136-4973 (region 3). In general, patients affected by MH in the absence of clinical myopathy are more likely to carry mutations in the N-terminus of the channel. By contrast, mutations in the C-terminal region of RyR1, that contains critical domains for the activity of RyRs, have been frequently detected in CCD. However, some discordance can be observed between occurrence of specific mutations and clinical phenotype, in part due to a non-complete molecular analysis of the RyR1

gene. Actually, routine analysis and sequencing procedures usually cover no more than 30% of the gene.

In order to assess a reliable estimate of the frequency and location of RYR1 mutations in MH individuals, we performed a systematic screen by DHPLC analysis of the entire coding region of the RYR1 gene in 50 unrelated MH probands. The results indicate that more than 80% of the analyzed patients present mutations in the RYR1 gene. Sequence of the entire RYR1 coding region of patients negative at the DHPLC analysis is being performed to complete the study. Interestingly, many of the novel mutations detected have been found outside the three hot-spot regions. Mutated RYR1 channels have been expressed in HEK293 cells in order to study their regulatory properties and to verify their effect on intracellular calcium homeostasis.

EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF) ON IN VITRO DIFFERENTIATION OF MUSCOLAR CELLS

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In recent years the possible relation between chronic exposure to extremely low frequency electromagnetic fields (ELF) and the development of diseases has stimulated the scientific debate. At present, even if no definitive conclusion has been reached by epidemiological studies, it is clear that ELF are able to affect biological systems. Experimental investigations of ELF influence on living matter are then becoming urgent. Since a possible biological target of ELF action is skeletal muscle, we used C2C12 myocytes as a model to study ELF effects on the myogenic process.

We exposed cell cultures to a 50 Hz ELF (0-1mT) generated by a solenoid or Helmholtz coils adapted to a confocal microscope. The effects of ELF exposition were investigated by: a) cell proliferation, calcium dynamic, ROS production and mitochondrial potential in undifferentiated cells; b) fusion index, gap-junctional intercellular communication (GJIC) and calcium waves during myogenesis.

Control and ELF-exposed cultures were always kept in parallel under identical conditions. The growth rate did not show any differences between treated and control cells. The exposition to ELF promoted the myogenic process in 0.5- and 1mT-exposed cells, probably through the modification of GJIC, a function needed for myocytes fusion. It is of note that the enhancement of the differentiation rate is characteristic of the first period of ELF exposition (2-4 days), whereas at the end of the myogenic process (7 days) no differences were found between treated and control cells. ELF were able to affect calcium waves in both myoblasts and myotubes, probably due to a modification of oxidative response: the presence of ELF influenced, in fact, ROS production and mitochondrial potential, according to field intensity.

In conclusion, our data show the capability of ELF to affect myogenesis; however this interaction is limited to the first period of exposition, possibly because of the adaptive response of this biological system to ELF.

MYOBLAST DIFFERENTIATION: RELEVANCE OF CYTOSKELETAL REMODELLING AND SACS ACTIVATION. I – MORPHOFUNCTIONAL REMARKS

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We have previously demonstrated that S1P-induced stress fibre formation generates a mechanical tension on the plasmamembrane which activates stretch-activated channels (SACs) opening and Ca2+ channel inward current in C2C12 myoblasts. In the present study, we further investigated the morphofunctional coupling between actin cytoskeleton and SACs and addressed a possible role for such interactions in myoblast differentiation. We first demonstrated that stress fibers (SF) formation and remodelling correlated with changes in SACs properties during myogenesis, and that the maximal channel conductance occurred concomitantly with the maximal expression of SF in differentiating C2C12 cells. The effects of drugs selectively affecting the cytoskeletal integrity and SACs activity on myoblast differentiation were also evaluated by confocal immunofluorescence, Western Blot and electrophysiological techniques. It was found that disruption of SF by treatment with dihydrocytochalasin B (DHCB) was effective to block both the morphological and molecular changes associated with C2C12 myoblasts development. Moreover, the inhibition of SACs activity by Gadolinium chloride (GdCl3) treatment profoundly affected SAC sensitivity as well as the expression of sarcomeric contractile proteins, including a-sarcomeric actin and skeletal myosin as well as myoblast-myotube transition, whereas had little or no effects on myogenin expression. Together, these results suggest that actin cytoskeleton may control skeletal muscle differentiation through distinct mechanisms, involving either signals reaching the nucleus via the cytoskeletal remodelling (i.e. SF formation) and SACs activation.

TROPONIN COMPOSITION AFFECTS MYOFILAMENT CALCIUM SENSITIVITY BUT NOT CROSS-BRIDGE CYCLE KINETICS

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It has been shown that incorporation of slow skeletal troponin I (ssTnI) into the cardiac sarcomere is associated

with enhancement of myofilament Ca2+ sensitivity; it has also been suggested that ssTnI may affect cross-bridge cycle kinetics (Kentish et al. Circ. Res. 88, 1059-1065, 2001). The present study was aimed to 1) test the hypothesis that ssTnI is sufficient to impart increased Ca2+ sensitivity in any striated muscle, and 2) to directly determine the impact of ssTnI on cross-bridge kinetics by means of rabbit psoas single myofibrils activated and relaxed by rapid solution switching. Myofibril native fast skeletal Tn (control) was replaced by recombinant whole cardiac Tn (cTn), or a Tn chimera composed of cTnC, cTnT, and ssTnI. The replacement was confirmed by SDS gel analysis. Myofibril steady state force-[Ca2+] relations were fit to the Hill equation yielding maximum force (P0), calcium sensitivity (EC50), and cooperativity (nH). P0 was not different among myofibril groups, while introduction of either cTn or the ssTnI chimera resulted in a significant decrease in nH. cTn induced a marked decrease in EC50; the presence of ssTnI further decreased EC50. Despite the marked impact of either cTn or the ssTnI chimera on Ca2+ sensitivity, we found no effect on either the rate of force development upon Ca2+ activation, the rate of force redevelopment following a quick release-restretch, or force relaxation kinetics upon rapid Ca2+ removal. Our data suggest that 1) the presence of ssTnI is sufficient to impart increased myofilament calcium sensitivity in any striated muscle type, 2) the Tn composition and related alterations in thin filament activation dynamics does not affect the intrinsic cross-bridge cycle kinetics.

CULTURE OF SATELLITE CELLS IN AN ELECTRICALLY-STIMULATED 3D COLLAGEN SCAFFOLD FOR THE CREATION OF ENGINEERED SKELETAL-MUSCLE GRAFTS

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The creation of skeletal muscle engineered grafts holds promise for the treatment of a variety of muscle diseases using regenerative medicine, reconstructive surgery and cell therapy.

In the present study, satellite cells (isolated from GFP+ve murine single muscle fibers) were cultured on three-dimensional, high-porosity collagen sponges and exposed to electrical square pulses that mimicked physiological contraction.

The presence of electrical stimulation greatly improved satellite cell differentiation. To investigate the molecular mechanisms we measured the NOx released in the culture medium and found that stimulated cells released 65% more NOx than controls.

Stimulated and non-stimulated sponges were also implanted in syngenic, GFP–ve mice and analyzed at different time points. In both cases, GFP positive cells were still present on the 3D scaffolds in long term in vivo implants. However, electrically stimulated scaffold showed higher cellular and myofibers density.

One of the major issue in Tissue Engineering is the colonization of the cells inside the polymer structure. In our study, we showed the possibility of improving cell growth inside the grafts in dynamic conditions. While cells in static culture were present only in the surface of the polymers, in dynamic conditions we observed an homogeneous polymer cell colonization.

Furthermore, we are presently trying to improve the survival of the implanted grafts by cell transfection with a VEGF-coding plasmid before implant. Our findings show that 3D satellite culture coupled with electrical stimulation can be a new strategy for the effective creation of a skeletal-muscle engineered graft.

MYOBLAST DIFFERENTIATION: RELEVANCE OF CYTOSKELETAL REMODELLING AND SACS ACTIVATION. II – ELECTROPHYSIOLOGICAL RESULTS

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It has been recently showed that stress fibres (SF) are able to apply an internal load on the plasmamembrane and, in turn, modulate transmembrane Ca2+ current through stretch-activated channels (SACs) in C2C12 myoblasts stimulated with S1P. In the present study we further investigated the morpho-functional interaction between SF and SACs in differentiating C2C12 cells. We show here that the development of SF and their anchorage to the plasma-membrane is necessary for modulating the conductive properties of SACs. The electrophysiological experiments were achieved by whole cell patch clamp in cells in differentiation medium for different times (12, 24, 48 and 72 hs) in the presence or absence of sphingosine 1-phosphate, S1P (1 mM).

After 24 hs in differentiating medium control cells show the maximal conductance Gm and under S1P treatment Gm was higher at any time respect to control being maximal at 12 hs. This well agrees with the entity of SF formation induced by S1P, that has been demonstrated to be maximal at 12 hs, and thus with the role of SF in modulating SACs opening. When the cytoskeleton was disrupted by 12 h pre-treatment with DHCB (1 mg/ml), cells shape changed from spindle to rounde form and S1P stimulation caused a membrane conductance lower then in basal S1P-stimulated cells. We thus suggest that DHCB treatment causes a reduction of cells differentiation and that the cytoskeleton integrity is essential for cell differentiation. Moreover, also the treatment with the SACs blocker GdCl3 (50 mM) prevented the S1P action. Taken

together, all these data suggest that cytoskeleton and SACs-mediated ions current integration may play an important role in the differentiation, i. e. regulation of contractile protein gene expression.

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MHC ISOFORM COMPOSITION IN TRUNK, LIMB AND SPECIALISED CANINE MUSCLES

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Dog limb and trunk muscles present three types of fibres corresponding to type 1, 2A and another type that has been named 2dog fibre (La Torre et al. 1993). Smerdu et al.(2005) confirmed the presence of 3 MHC isoforms suggesting that the 2dog fibres contain 2X MHC. We report a study of histo-immunohistochemistry and SDS PAGE on dog muscles and single muscle fibres together with a gene expression analysis of MHC.

mAbs provided equivocal results concerning the presence of the 2X and 2B isoforms. This fact might be due to cross-reactivity of mAbs or to hybrid fibres 2A/X or 2X/B.

Electrophoresis on limb muscles identified 1, 2A, and 2X MHC isoforms. The comparison of these muscles with laryngeal muscles allowed the identification of the 2B MHC. The Eo band was identified comparing the retractor bulbi with rectus lateralis which was the only muscle that expresses 2B mRNA. A sixth band present in the retractor bulbi and rectus lateralis was identified as Neo MHC. 2M MHC was detected in masticatory muscles.

Expression data confirmed that: - trunk and limb muscles are usually composed of fibres expressing 1, 2A and 2X isoforms, - in laryngeal and extraocular muscles, also the 2B-similar MHC is present, - extraocular muscles, the rectus lateralis and retractor bulbi express additionally the Neo isoform but only the rectus lateralis expresses the Eo one. Masticatory muscles abundantly express a peculiar MHC isoform called 2M. Single fibres experiments confirmed the presence of pure and hybrid fibres.

Among limb muscles, the semimembranosus expresses also the 2B MHC mRNA although the corresponding protein has to be confirmed. The 2B isoform expression might be linked to the phasic function of this muscle or to the breed.

ANTISENSE OLIGONUCLEOTIDES AS A THERAPEUTIC TOOL FOR DYSTROPHIC MUSCLE: IN VIVO EXPERIMENTING IN THE MURINE MDX MODEL

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Recent studies have shown that the induction of AO-mediated exon skipping could potentially be used for the treatment of most of the DMD patients. Positive results (i.e., production of shortened but functional dystrophin, as in the milder BMD phenotype in man) have been obtained both in vitro and in vivo, in the mdx murine dystrophic model. Most of the results, however, have been so far obtained via intra-muscular injection of AO, associated with delivery systems based on cationic lipids or hydrophilic polymers. Research is now moving towards the creation of clinically relevant delivery systems, based onto systemic delivery. In this regard, we had previously shown that intra-arterial delivery (in the femoral artery) of DNA/liposome complexes could lead to good expression levels in regenerating muscles in rats. We have now applied a similar approach to the delivery of an exon-specific AO in mdx mice, using the F127 block co-polymer as a carrier to deliver a morpholino antisense oligo directed against the donor site of intron 23. We have used very old animals (>14 months), as their severe dystrophic phenotype resembles more closely the human disease. In these conditions, one single injection of morpholino oligos led to the expression of dystrophin in up tp 8% of muscle fibers. The results we obtained with two serial injections in the same animal were even more significant, as this protocol led to the production of dystrophin in up to 25% of the fibers in the EDL muscles of the treated limbs. This in turns translated into a remarkable 50% improvement of the muscles' strength and resilience.

SEARCHING FOR NEW PROTEINS PREFERENTIALLY EXPRESSED IN SKELETAL MUSCLE

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The purpose of this project is to identify novel membrane proteins preferentially expressed in skeletal muscle. One of the most challenging aspect of the post genomic era is to analyse the large amount of available transcriptional profiling data present in databases. Computational analysis of this information may provide a more complete picture of tissue-specific gene expression.

From several databases that report the tissue-expression pattern of novel genes, we have obtained a list of genes highly expressed in skeletal muscle. All potentially interesting genes have been cross-checked in different database to validate the

information. The following level of evaluation was to select only genes encoding proteins which contain putative membrane-targeting region by using the TMPRED software. Protein domains and protein similarities were studied using PROSITE and ExPasy Blast software's. This analysis showed that out of 235 initially selected cDNAs, 33 had putative TM region according to the prediction by TMPRED software. The expression pattern of these genes was tested by RT-PCR. Of the 33 cDNAs that were considered for experimental validation, 14 were confirmed to be muscle specific and 7 were shown to be expressed also in other tissues. The analysis of the 14 remaining genes still needs to be validated. The next step of this study is to verify the localization of the selected proteins in skeletal muscle cells.

SPHINGOLIPID SIGNALING IN DENERVATED SKELETAL MUSCLE

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Present work was aimed at studying the effects of sphingosine-1 phosphate (S1P), a natural sphingomyelin derivative, on the trophism of slow-twitch skeletal muscle. The action of S1P was investigated during muscle denervation, an experimental model characterized by a marked reduction of muscle mass. First, we examined the possible trophic action of S1P by reducing the level of the circulating lipid by treating denervated mice with an antibody specific for S1P (three doses of 10 μg/g of body weight injected intra-peritoneum). Denervation was performed by cutting monolaterally the sciatic nerve. The effect S1P removal was evaluated on muscle mass and fiber CSA. After 7 days, atrophy was significantly higher in animals without circulating S1P with respect to untreated animals. The action of S1P was also investigated by the exogenous supplementation of the lipid. In this case, denervation was performed in adult rats by the bilateral cutting of the sciatic nerve at the level of trochanter. Sphingosine (SPH, a precursor of S1P) or S1P were continuously released over soleus muscle by a mini-osmotic pump implanted subcutaneously in the scapular region and connected through a catheter to the muscle of the left leg. In general, both lipids decreased the development of atrophy in 7 and 14-day denervated soleus muscles, with SPH being the most effective. To verify the presence of S1P specific receptors at muscle fiber surface, the expression of S1P1 receptor and SCaMPER was investigated. The localization of these two receptors was demonstrated, for the first time, by immunofluorescence and western blot analyses, both in control and denervated skeletal muscles. Interestingly, the expression level of the receptors appears to be reduced during denervation. Present data seem to indicate that S1P signaling has trophic functions in skeletal muscle.

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