MyoD, myogenin, myf-5 and MRF4/myf6, the myogenic regulatory factors (MRF), constitute a four-member family of basic helix–loop–helix transcription factors that are very important for guiding the development from myoblasts into skeletal muscle fibres. Their role in development is well characterised, whereas their function in the adult muscle is much less clear. The promoter of many, if not all, muscle specific genes contains recognition sites, E-boxes, for the MRFs and it is believed that the MRFs are important for the basal expression of the muscle specific genes in the adult muscle. However, the distribution of the different MRFs is not uniform among the different muscle and fibres types suggesting that they also play a role in determining the metabolic and contractile properties of the fibres. Furthermore, stimuli that can alter the muscle phenotype like exercise and inactivity, also affects the expression level of the MRFs indicating that they are not only involved in maintaining basal expression. However, the investigation of the MRFs in adult muscle is complicated by the fact that the adult muscle contain quiescent myoblasts (satellite cells), which can be activated and undergo the developmental program to produce new myotubes or increase the number of myonuclei in existing fibres. During this process the satellite cells express far higher levels of the MRFs than the mature fibres thereby masking the signal from the mature fibres. On the other hand, the satellite cells may also play an important role in the determination of the fibre type. In this presentation, I will summarise our current knowledge of the role of MRFs in the adult muscle.

**VIII-6: Oral presentation 17 September, 16:00–16:15**

**Ca^{2+}**-transients trigger calcineurin/NFATc1-mediated fast-to-slow transformation in a primary skeletal muscle culture

J.D. MEISSNER, N. HANKE, H.-P. KUBIS, R.J. SCHEIBE and G. GROS
Zentrum Physiologie, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

The calcineurin/NFATc1 (nuclear factor of activated thymocytes) signal transduction pathway is involved in mediating fast-to-slow transformation of skeletal muscle cells in primary culture. Specifically, induction of slow myosin heavy chain I (MHC I) gene expression is correlated with the Ca^{2+}-dependent activation of calcineurin. In T-cells the calcineurin/NFATc1-pathway is activated by a small and sustained increase in intracellular resting Ca^{2+}-levels, whereas in muscle cells the type of Ca^{2+}-signal necessary for the activation of calcineurin is not yet known. We first investigated the Ca^{2+}-ionophore A23187-induced activation of the calcineurin/NFATc1-pathway in myotubes grown on microcarriers. Calcineurin-dependent nuclear translocation of NFATc1 was analysed by immunofluorescence. The Ca^{2+}-ionophore caused concentration-dependent translocation of NFATc1 from the cytoplasm to the nucleus. A concentration of 5 × 10^{-5} M A23187 was sufficient to induce complete nuclear import of NFATc1 in nearly 50% of the cells. We then electrostimulated myotubes grown on microcarriers with 1 Hz in 45 min stimulation cycles (15 min stimulation, followed by a pause of 30 min). The expression of slow MHC I and of fast MHC IId mRNAs was analyzed by Northern blot analysis, and resting intracellular Ca^{2+}-concentration ([Ca^{2+}]) was measured using the Fura-2 AM fluorescence method. After an electrostimulation period of 24 h we found the following: (1) increase in MHC I and decrease in MHC IId mRNA levels suggests that stimulation for only 24 h was sufficient to start the transformation process; (2) after 24 h of stimulation NFATc1 was highly accumulated in the nuclei, indicating the activation of the calcineurin pathway; (3) however, no increase in resting [Ca^{2+}], was found after 24 h of stimulation. We conclude from this that the calcineurin/NFATc1-mediated fast-to-slow transformation at the MHC I level is induced by the series of short, rapid calcium transients associated with excitation–contraction coupling, rather than by a continuous elevation of resting [Ca^{2+}]. Electrostimulation for 7 or 14 days led to an pronounced change in MHC mRNA expression, and in addition resulted in an increase in resting [Ca^{2+}], by around 50%. This delayed long-lasting elevation of resting Ca^{2+} observed after several days may serve to trigger other elements of fast-to-slow transformation such as metabolic adaptation.

**VIII-7: Oral presentation 17 September, 16:15–16:30**

Effects of deafferentation on soleus fiber size and MHC isoform distribution in rats exposed to hindlimb suspension

Y. VOLODKOVICH1, A. MUCHINA1, M. SAYAPINA1, Q. LARINA1, E. BRATCSEVA1, B.S. SHENKMAN2 and T.L. NEMIROVSKAYA1,2
1Lomonosov Moscow State University, RAS, Moscow, Russia; 2SRC Institute for Biomedical Problems, RAS, Moscow, Russia

The study was purposed to reveal the impact of afferent inputs in maintaining structure and myosin heavy chain (MHC) isoforms of soleus fibers subjected to simulated hindlimb unloading. Male Wistar rats were distributed between three groups, which included cage control (C, n = 7); hindlimb suspended intact rats (HS, n = 7) and hindlimb suspended deafferented rats (HSD, n = 6). Deafferentation was made by means of removal of 3–4 mm of dorsal roots including ganglion at levels L7, L6 and L5. After 14 days of exposure all the rats were sacrificed by means of Nembutal overdose. The significant reduction in muscle soleus weight and fiber CSA were revealed in all hindlimb suspended rats as compared to control. However, in HS rats the percentage of fibers expressed slow MHC was diminished and of those contained fast MHC increased as compared to C group (P < 0.05). The percentage of fibers with slow MHC in HSD rat fibers was higher than that in HS and C group (P < 0.05). The quantity of fibers which contained fast MHC in HSD rat tended to be also higher than in C group. It is concluded that reduction in afferent input from suspended extremity leads to increase in number of fibers, which contained slow MHC, and those, which contained fast MHC as well. Since the MHC expression in muscle fibers is controlled via neuromuscular activity patterns, we may suppose that those patterns in rats exposed to hindlimb unloading vs. reduction in afferent input are not similar.

**VIII-8: Oral presentation 17 September, 16:30–16:45**

Effects of strain and insulin on myonuclear z-skeletal actin, myogenin and MyoD mRNA content determined by in situ hybridisation in cultured single muscle fibres of Xenopus laevis

R.T. JASPERS1, H.M. FEENSTRA1,2, P.A. HUIJING1,3 and W.J. VAN DER LAARSE2
1Instituut voor Fundamentele en Klinische Bewegingswetenschappen, Vrije Universiteit, van der Boechorststraat 9, 1081 BT Amsterdam, The Netherlands; 2Department of Physiology, VU medical center, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands; 3Biomedical Technologic Instituut, Universiteit Twente, Postbus 217, 7500 AE Enschede, The Netherlands

Strain is an important factor in the regulation of the number of sarcomeres in series in vivo. We previously found that, at low and high fibre strain (Jaspers et al., 2001, J Physiol 533: 133P) the number of sarcomeres in series of mature, single Xenopus laevis muscle fibres was not changed during long-term culture (Lee-de Groot and van der Laarse, 1996, J Muscle Res Cell Motil 17: 439–448). We quantified mRNA content in serial sections of cultured muscle fibres at low strain in order to test (1) whether breakdown of sarcomeres is prevented by enhanced mRNA transcription due to insulin in the culture medium, and (2) whether high strain enhances mRNA expression. Fibres were cultured for 4 or 5 days at low strain (~15% below passive slack length) in low (50 pg/ml) and high (1 µg/ml) [insulin] culture medium,
as well as at high strain (~5% above passive slack length) in high [insulin] medium. After culture, in situ hybridisation using biotinlabelled oligonucleotide probes for α-skeletal actin, myogenin and myoD, followed by tyramide signal amplification, reveals high absorbance signals in and around the myonuclei. The hybridisation signals were quantified by determination of the spatially integrated myonuclear absorbance. The data were normalised by the α-skeletal actin hybridisation signal in control muscle sections. The myonuclear mRNA content for α-skeletal actin, myogenin and MyoD was either similar or smaller than the value in freshly frozen control muscle sections. High [insulin] increased the myonuclear α-skeletal actin mRNA content, whereas strain during culture had no effect on α-skeletal actin mRNA content. After culture at high strain the myonuclear myogenin mRNA content was similar to control, whereas at low fibre strain, myonuclear myogenin mRNA content was reduced in low- and high-insulin medium. Myonuclear MyoD mRNA content was not changed in culture at high [insulin] and strain. Although high strain and [insulin] may stimulate expression of mRNA, the mRNA levels of the muscle-specific mRNA’s tested did not exceed mRNA control levels. Therefore, enhanced mRNA expression leading to levels of the muscle-specific mRNA’s tested did not exceed mRNA control levels. Thus, additional factors, other than strain and [insulin], may partially inhibit the reduction of the number of sarcomeres during culture is not induced by high strain. At control levels. Therefore, enhanced mRNA expression leading to... 

VIII-9: Poster presentation

Identification of a novel stretch-sensitive SPRY domain-coding gene in skeletal muscle

G. MCKOY1, T. KEMP2, R. VELINENI1 and G.R. COULTON1

1Neuromuscular Research Group, Department of Biochemistry and Immunology and Department of Cardiological Sciences, St. George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK; 2Imperial College London, Cardiac Medicine, NHLI, Dovehouse Street, London SW3 6LY, UK

Skeletal muscle undergoes hypertrophy in response to mechanical stretch in vivo. This is accompanied by the induction of genes coding for ‘slow’ isoforms of a range of contractile proteins whilst the muscle adjust to its new length. Although several agonists including IGF-1 isoforms are known to activate muscle fibre hypertrophy our understanding of how hypertrophy and fibre type shift are independently regulated and interact is limited. In order to extend our knowledge of the key intracellular regulators of muscle fibre hypertrophy we used subtractive hybridisation to identify a panel of differentially expressed mRNAs in mouse tibialis anterior muscle subjected to stretch/immobilisation in vivo for 7 days. We have so far identified 18 novel and 13 known transcripts exhibiting significantly increased expression in response to stretch. One of these novel transcripts TA553 encodes a putative peptide containing a predicted SPRY domain. SPRY domains are found in over 300 proteins though its function is yet to be determined. The closest homologues of TA553 are the ‘midline’ and RET families of transcription factors. TA553 is expressed in a range of fast and slow skeletal muscles of as well as in tongue and heart. It is at extremely low levels in the liver. We hypothesise that TA553 has an important role in phenotypic adaptation of skeletal muscle in response to mechanical stretch in vivo and may also fulfil a similar function in other contractile tissues including the heart.

VIII-10: Poster presentation

Decreased myoglobin concentration in intercostal muscle fibres of rats suffering from monocrotaline-induced ventilatory insufficiency

M.J.C. SCHOORL, B.J. VAN BEEK-HARMSN and W.J. VAN DER LAARSE

Department of Physiology, Institute for Cardiovascular Research, Vrije Universiteit Medical Centre, van der Boechorststraat 7, NL-1081 BT Amsterdam, The Netherlands

Monocrotaline induces pulmonary hypertension, leading to right ventricular dysfunction and ventilatory insufficiency. We hypothesize that intercostal muscle fibres in monocrotaline-treated rats increase their myoglobin concentration to adapt to hypoxemia (Reynafarje, 1962, J Appl Physiol 17: 301–305; van der Laarse et al., in press, J Muscle Res Cell Motil). Two male Wistar rats (body weight 180 g) were injected subcutaneously with 40 mg monocrotaline per kg body weight. Two untreated rats served as age-matched controls. After 2 or 4 weeks the intercostal muscles were dissected. In individual fibres of the externus and internus muscle we determined cross-sectional area, succinate dehydrogenase activity (van der Laarse et al., 1989, J Muscle Res Cell Motil 10: 221–228), the number of capillaries per muscle fibre (Madsen and Holmskov, 1995, Eur J Appl Physiol 71: 472–474) and the myoglobin concentration (Lee-de Grooth et al., 1998, J Histochem Cytochem 46: 1077–1084). The succinate dehydrogenase activity was inversely related to the cross-sectional area of the fibres in all muscles, i.e. the product of cross-sectional area and enzyme activity was fairly constant in each muscle. A similar inverse relationship was found between the myoglobin concentration and cross-sectional area of the fibres in each muscle. Spatially integrated succinate dehydrogenase activity and myoglobin concentration were analysed using three-way ANOVA with replication. Analysis of succinate dehydrogenase activity (n = 8 fibres per muscle) detected significant first-order interactions for muscle × time, muscle × treatment and time × treatment (P < 0.01). Spatially integrated myoglobin concentration (n = 8 fibres per muscle) showed significant differences in the first-order interactions muscle × treatment and time × treatment (P < 0.002). The number of capillaries per fibre (n = 3 samples per muscle) differed between the external (mean 1.6 ± SEM 0.1 capillaries per fibre) and internal (1.8 ± 0.2) intercostal muscles (P < 0.05), but treatment or time effects were not present. The second-order interactions were not significant. The results confirm intercostal muscle wasting in monocrotaline-treated rats. Contrary to the expected increase in myoglobin concentration, we have found a decrease which is muscle and treatment-time dependent.

VIII-11: Poster presentation

Exploring the genetic basis of muscle weight in laboratory mice

A. LIONIKAS1,3, D.A. BLIZARD2, G.E. MCC-LEARN2 and L. LARSSON1,3

1Noll Physiological Research Center, 129 Noll Lab, University Park, PA, 16802, USA; 2Center for Developmental and Health Genetics, University Park, PA, 16802, USA; 3Department of Clinical Neurophysiology, Uppsala University, Sweden

Skeletal muscle size varies substantially between different mouse strains. For instance, hindlimb muscles such as the soleus, tibialis anterior (TA), extensor digitorum longus (EDL) and gastrocnemius of C57BL6J (B6) mice are 11–25% bigger in males and 14–33% bigger in females than in the corresponding gender from DBA/2 (D2) mice. The aim of this project is to improve our understanding of the genetic basis for the difference in muscle size. To address this, we have used two kinds of crosses of B6 and D2 mice: (1) 400 of F2 mice derived from B6 and D2 strains, where each mouse has a unique genetic pattern inherited from the parental strains, and (2) 21 recombinant inbred strains (BxD) including a total of 528 mice in total, each strain having a unique genetic combination from B6 and D2 progenitors. Soleus, TA, EDL and gastrocnemius muscles of the right hind limb were...