

CK and LDH total activity (48% and 66% of contracting cultures, $p < 0.05$). GP and PGAM were not affected. Analysis of isoenzymatic pattern in paralysed versus contracting cultures, showed a slight decrease of CK and LDH MSI (85% and 94%, $p < 0.05$), and no difference in GP and PGAM MSI. MSI of all enzymes were higher in innervated versus non-innervated cultures ($p < 0.05$). We conclude that, in innervated cultured human muscle, MSI accumulation is regulated mainly by neuronal factors and in different ways for the various enzymes. Total enzymatic activity of CK and LDH is regulated mainly by muscle contractile activity.

Morphological changes induced by continuous low frequency electro-stimulation in rat EDL muscle

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Skeletal muscle undergoes pronounced physiological and biochemical changes when subjected to chronical low frequency electro-stimulation. By light and electron microscopy we studied the modification induced in EDL muscle of adult rats by indirect electro-stimulation at 10 Hz. Qualitative and quantitative analyses of neuromuscular junctions and muscle fibres were performed after 4, 11 and 34 days of continuous stimulation. The increase in capillary density and in mitochondrial volume observed by the second week indicates that the experimental muscles have been subjected to increased use. At neuromuscular junctions morphological aspects of remodelling are observed together with a trend of synaptic vesicles to decrease in number. In the muscle fibres signs of focal degeneration are rare and scanty even at early times, when damage should be expected in fast fatigable EDL fibres due to the sudden change in activity. When the 10 Hz pattern of electro-stimulation was directly applied to denervated EDL, a population of small round muscle fibres was found one month after continuous stimulation. Internalized or centrally placed nuclei are seen in the majority of the muscle fibres which also display subsarcolemmal accumulations of mitochondria. The presence of a double-layered basal lamina around clusters of myofibres demonstrates that regenerative events occurred and that in denervated stimulated muscle at least a part of the population was replaced by generation of new muscle fibres.

Stimulation-induced changes in activity and protein levels of hexokinase II in rat fast-twitch muscle

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Chronic low-frequency stimulation (10 Hz, 10 h/d) induced in rat fast-twitch muscle rapid increases in hexokinase (HK) activity with a 14 fold increment after 7 days of stimulation. We were interested to investigate whether this increase resulted from an enzyme modification or changes in enzyme protein or isozyme composition. For this purpose, the HKII isozyme was purified by affinity chromatography to raise monoclonal and polyclonal antibodies. Immunoblotting and sandwich ELISA with the use of these antibodies showed that the rise in HK activity closely correlated with an increase in the HKII protein. This correlation also applied to the decrease in HK activity which occurred with stimulation periods longer than 18 days. In view of the reversible binding of HKII to the outer mitochondrial membrane, we were interested to examine to what extent the increase in HK related to its bound and free forms. Fractional extraction studies revealed that the percentage of bound HK increased from 65% to 85% of the total cellular activity. The change in intracellular distribution was most remarkable during short-term (2 days) stimulation when the bound

form increased 5 fold, but the free form only 2 fold. The transient increases in HK activity seems to be related to an increased glucose utilization during that period when the aerobic-oxidative capacity of the muscle is still insufficient to meet the increased energy demand.

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Force-velocity relationship of regenerating rat triceps surae

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Rat triceps surae were minced and orthotopically autografted. The force-velocity relationship was determined by the isovelocity release method at 30 ($n = 15$), 60 ($n = 9$), 90 ($n = 5$) and 120 days ($n = 6$) after surgery. The data were analysed using the exponential relation of Fenn-Marsh-Aubert. At 30 days a single exponential could be fitted in 8 cases, with a slow velocity constant of $0.50 \pm 0.05 \text{ L}_0 \text{ s}^{-1}$, but a double exponential was required in the 7 remaining cases, with a slow velocity constant of $0.20 \pm 0.03 \text{ L}_0 \text{ s}^{-1}$, and a fast velocity constant of $1.74 \pm 0.20 \text{ L}_0 \text{ s}^{-1}$. At the later stages of the regeneration, all the force-velocity relationships required double exponentials, with slightly higher constants, 0.34 ± 0.07 and $2.75 \pm 0.45 \text{ L}_0 \text{ s}^{-1}$. The fractional contribution of the fast component varied between 0.5 and 0.8, and did not appear to depend on the duration of the regeneration. Since fetal isomyosins are still abundant at 30 days, but have disappeared at 60 days Maréchal, G., Schwartz, K., Beckers-Bleukx, G. & Ghins Et. *Eur. J. Biochem.*, 138, (1984), 421-8, it appears that the force-velocity follows the same pattern, being 'mixed' (neonatal or adult) at 30 days, and adult at 60 days and later. This is in strong contrast with the maximal isometric force which steadily increases from $290 \pm 51 \text{ mN}$ at 30 days to $1143 \pm 216 \text{ mN}$ at 120 days. Thus, the hypertrophic growth during regeneration occurs at constant force-velocity parameters.

Muscular plasticity and reinnervation capacity after reinnervation by a foreign nerve

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Following partial denervation, motor units can increase (by self-reinnervation) as much as 4-5 times their normal size. To investigate the still unknown quantitative capacity of a motor nerve in the case of foreign-reinnervation, in adult male rats, the denervated sternomastoid muscle was either self-reinnervated by its original nerve or foreign-reinnervated by the omohyoid nerve. By this the omohyoid nerve had to reinnervate a threefold amount of muscle fibres and six times the amount of muscle mass. After survival times of 7, 8, 9 or 10 months, nerves and muscles were investigated histochemically and immunohistochemically. The omohyoid nerve could fully reinnervate the sternomastoid muscle but at 7 and 8 months this muscle still revealed nearly the same proportions of IIA and IIB fibres as were seen in the self-reinnervated sternomastoid at all stages. However, in the following 2 months a shift of the fibre pattern towards that of the normal omohyoid was observed as evidenced by a strong increase of the type IIB fibres from 24% to 62% at the expense of type IIA fibres. These findings are in contrast to those after foreign-(cross-) reinnervation of leg muscles where the fibre transformation (according to the foreign motor input) occurs in parallel with the reinnervation process during the first 2-3 months. The delayed fibre transformation observed could be the consequence of the highly enlarged peripheral field of the omohyoid motoneuron pool or merely reflect a general difference between limb and neck muscles. Whatever the case may be, in the present experiments the