Induced Myogenesis in Long-Term Permanent Denervation: Perspective Role in Functional Electrical Stimulation of Denervated Legs in Humans

Ugo Carraro, Katia Rossini, Maria Elena Zanin, Corrado Rizzi[(1)], Winfried Mayr[(2)], Helmut Kern[(3)]

C.N.R. Institute of Neuroscience, Unit for Muscle Biology and Physiopathology, Department of Biomedical Sciences, Padua Medical School, University of Padova, Padova, (1) Scientific and Technologic Department, University of Verona, Verona, Italy, (2) Department of Biomedical Engineering and Physics, University of Vienna Medical School and (3) L. Boltzmann Institute for Electrostimulation, Department of Physical Medicine and Rehabilitation, Vienna, Austria

Abstract

Long-term permanent denervation (LT-PD) induces severe atrophy of skeletal muscle accompanied by apoptotic loss of myonuclei. Morphologic characteristics of the long-term denervated muscle suggest that the original fibers are lost and those seen are the results of repeated cycles of cell death and regeneration. Myoblasts and myotubes express peculiar myosins. Light and heavy chains of embryonic myosin are sensitive indicators of myogenic events in adult muscles. Electrical stimulation of permanent denervated muscle increases the mean size of the myofibers, maintains the sarcomeres and possibly prevents secondary degeneration and apoptosis/necrosis. Mechanisms underlying the recruitment of satellite cells for regenerative or hypertrophic processes have not been established, but cytokine interactions among macrophages and satellite cells seem to be essential. Here we show that long-term denervated muscles increase in size and change their myosin content after full regeneration is induced by bupivacaine treatment. After transient expression of embryonic myosin, fast myosins prevail in all the experimental muscles. In spite of a severe atrophy (around 90%), ten days after injury the regenerated muscles have twice the area at the muscle belly of the respective long-term denervated muscles. Since satellite cells activity is required for extreme hypertrophy of overloaded adult muscles or eutrophy after severe atrophy in recovering muscles, positive regulation of activation, division and fusion of myoblasts could be an important tool to understand limits of recovery of neurogenic muscle myopathies by functional electrical stimulation (FES). Pilot studies in humans show that standing up with denervated muscles using functional electrical stimulation is a reality in permanent complete denervation.

Key words: embryonic myosin, FES, LCemb, long-term permanent denervation, myogenic stem cells, myosin heavy chains, regeneration, satellite cell, SDS PAGE, spinal cord damage, therapeutic functional electrical stimulation.

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Long-term permanent denervation (LT-PD) induces severe atrophy of skeletal muscle accompanied by apoptotic loss of myonuclei. After the fully differentiated pattern of fast and slow myosins has been established in normal adult skeletal muscles, acute denervation is of very little influence on the type of contractile proteins synthesized in early atrophying muscle fibers. Preferential atrophy of fast fibers followed by atrophy of slow fibers appears to be the typical feature of the early phases of denervation, producing only a small unbalance in fiber typing (reviewed in [57]). However during several months of permanent denervation there is an almost complete transformation of rat mixed muscles into almost pure fast muscles [16, 19, 20], the residual slow myosin being present with fast myosin in single myofibers [22, 58]. Gel electrophoresis of myosin light and heavy chains are unable to reveal the presence of embryonic or neonatal isoforms in early muscle denervation.

In long-term permanent denervation (LT-PD) necrotic and apoptotic cell death is known to occur at significant

rate [7, 23, 42, 51, 59, 61]. Permanent denervation does not prevent induced muscle regeneration [43] and a long-term retention after denervation of this capability has been also demonstrated [6, 8, 9, 30, 38]. Light and electron microscopy shows that the long term denervated muscle maintains a steady-state atrophy for the animal's life span, some morphological features indicating that events of aneural regeneration occur continuously [22]. Spontaneous myofiber regeneration in LT-PD has been quantified and shown to be non-compensatory and to result in reduction of satellite cell pools [6, 8, 9, 26, 35, 36, 62]. Satellite cell proliferation and myofiber regeneration is enhanced in LT-PD muscles subjected to electrical stimulation [2, 3, 5, 14, 15, 27, 29, 44, 47-50, 53].

Finally, it is well known that in eccentric treadmill running satellite cell activation is far greater than required to repair the small number (4%) of necrotic/regenerating myofibers identifiable at the light microscopic level, suggesting that molecules released trough subcellular lesions are the activating factors [25]. Myoblasts and myotubes express peculiar myosins, so that light and heavy chains of embryonic myosin are sensitive indicators of myogenic events in adult muscles. Therefore, one can answer the question if the expression of the embryonic myosin is also related to cell regeneration in long-term permanent denervated muscle, since both fast and slow muscles regenerating in absence of the nerve accumulate fast myosins after a transient expression of the embryonic isoforms [21].

Satellite cells are small mononucleated skeletal muscle stem cells located between the basal lamina of the muscle and the sarcolemma of myofibers [63]. Satellite cells are mobilized in response to increased loading conditions or after injury of the myofibers (see for review [52]).

The initial event after satellite cell activation is a proliferative response in which some or all of the activated satellite cells undergo at least one mitotic cycle. After this initial phase, some of the activated cells and/or their progeny differentiate into myoblast-like cells. In regenerating muscle, these myoblasts fuse with each other to form new myofibers or become incorporated into existing myofibers. In the case of the hypertrophy response, satellite cell-derived myoblasts are thought to fuse with existing myofibers, thereby providing additional myonuclei. The mechanisms underlying the recruitment of satellite cells for regenerative or hypertrophic processes have not been fully established (see for review [28]), but interactions through cytokines among resident phagocytes, inflammatory cells (macrophages) and satellite cells/myoblasts seem to be essential [9-12, 53].

We here show that long-term permanent denervated fast and slow rat muscles change their myosin content and fully regenerate after an injury induced by bupivacaine treatment. After transient expression of embryonic myosin, fast myosins prevail in all the experimental muscles and slow myosin almost disappears in regenerated soleus muscle. These observations further support the hypothesis that fiber regeneration participates in the maintenance and contributes to the increasing uniformity of the myosin gene expression of chronically denervated muscles. The transient relative hypertrophy of the early regenerating muscles could have important implications in functional recovery of flaccid muscles by means of electrical stimulation in long-term permanent denervated patients. Indeed restoration of degenerated long-term denervated muscles in humans has been demonstrated [34]. These clinical results are in accordance with experimental studies showing that electrical stimulation changes membrane properties and contraction characteristics of denervated muscles and that these restorative effects are reproducible in repeated sequences of degeneration – regeneration periods (atrophy-eutrophy cycles) [37]. What is still missing is an explanation for the obviously enormous number of myofibers needed to achieve the clinically observed level of restoration of muscle mass and force output [34].

Materials and Methods

Adult male Wistar rats were sciatectomized at both legs as described in [15] to reduce spontaneous reinnervation. Four months later extensor digitorum longus (EDL) and soleus of the right limbs were treated with bupivacaine as described in [21]. Either four or ten days after surgery the rats were killed. EDL and soleus of both legs were separately chilled in liquid nitrogen. After histological examination of transverse sections at the muscle belly, the muscles in which denervation was unquestionable, were pooled. Myosin was isolated as described in [18, 22]. Two-dimensional gel electrophoresis of myosin light chains (MLC) was as reported in [15]. The one dimensional SDS PAGE of myosin heavy chains was essentially performed as described in [18], but with the following modifications. The gel electrophoresis was carried out in 6% polyacrylamide in the separating gel and 4% polyacrylamide in the stacking gel. Both the stacking and the separating gel contain 37.5% (v/v) glycerol. The run was performed at constant current, setting the value corresponding to a voltage of 50 V. In these conditions the run lasted about 16 hours, the voltage increasing to about 80 V during the first hour and then to about 200 V during the overnight run. About 0.5 µg or 0.05 µg of myosin were loaded when the slab was stained with Coomassie blue or silver stain respectively [17, 18].

Results

Gross appearance

Four months after sciatectomy the EDL and soleus were surgically exposed to induce the bupivacaine injury. Their severe atrophy suggested that reinnervation did not occur. Four and ten days after injury they appeared increased in size in comparison to the contralateral denervated muscles. Figure 1, which shows the replica of cryostat sections at the muscle belly of the experimental muscles and of normal muscles from rats of comparable

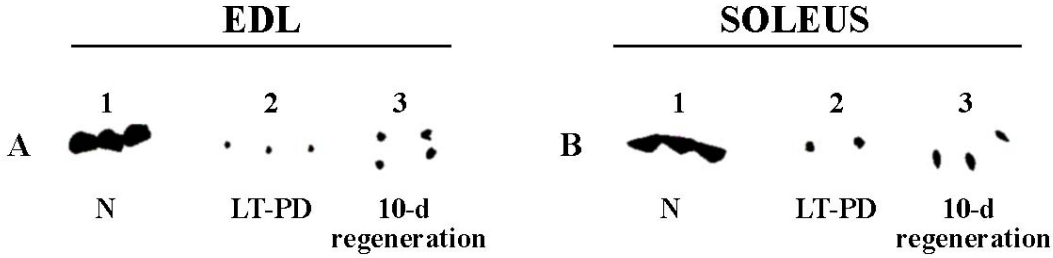

Figure 1. Long-term denervation and regeneration capability of denervated rat muscles. Actual size of the transverse sections at the muscle belly of EDL (A), and soleus (B). Normal muscles (A 1), (B 1); four month denervation (A 2), (B 2); four month denervated muscles at day ten after regeneration (A 3), (B 3).

age and weight, allows a qualitative evaluation of the extent of changes occurred in experimental muscles.

Microscopic appearance

Panels (a) and (c) of Figure 2 show that four months after sciatectomy the EDL and soleus are composed of angulated fibers showing different extents of severe atrophy. Only one of the 24 sciatectomized muscles showed groups of round, large fibers resulting from partial reinnervation (result not shown). This muscle was excluded from the muscle pool used for myosin studies.

Histochemical characterization of myosin ATPase showed that the denervated EDL maintained its characteristic alkali resistance and acid lability (Fig. 2 e and i). On the other hand the denervated soleus lost its acid resistance and acquired alkali resistance (Fig. 2 g and k), therefore showing an ATPase pattern similar to that of a fast muscle. The mosaic pattern due to the few slow fibers present in normal EDL or fast fibers in normal soleus is barely discernible in the long term denervated muscles.

Ten days after bupivacaine injury few muscle fibers are angulated and severely atrophic, several have centrally located nuclei (Fig. 2 b and d). The regenerated muscles have alkali resistant and acid labile ATPase staining. The microscopic appearance of the bupivacaine-injured muscles therefore confirms that the muscle has undergone necrosis, and after satellite cells' multiplication and fusion the regenerated muscle fibers have reached the stage of adult myofibers before showing the effect of the lack of reinnervation. These results are in full agreement with our previous data showing that maturation of myofibers, dystrophic changes and continuous production of fibers is not prevented by the lack of reinnervation in skeletal muscle regenerating in the absence of the nerve [43]. ATPase stainings show that the long-term denervated EDL and soleus muscles regenerated in absence of the nerve acquire fast-like characteristics, a result in agreement with our previous reports [21].

Myosin Light Chains

The presence of the embryonic isoform of myosin light chain in injured muscles is direct evidence that the myofibers underwent regeneration and then dystrophic changes due to lack of innervation during the last ten days of the experiment (panel c and f in Fig. 3). The two-dimensional analysis of myosin light chains from experimental muscles give further information suggesting that the changes occurring in chronically denervated muscles at myosin level are not only the consequence of a higher loss of slow myosin in slow myofibers.

Fig. 3, b shows that LC3F is underexpressed in denervated EDL; in the denervated soleus while LC2F almost fully substitutes LC2S, LC1'S and LC1S (in the ratio of normal soleus) are present in amounts larger than LC1F (Fig 3, e). Besides the presence of LCemb the regenerated EDL is characterized by LC1F and LC2F and by trace amounts of LC3F and LC1'S, but not LC1S (Fig. 3, c). The regenerated soleus though dominate by LCIF and LC2F remains discernible from the EDL because of the relative abundance of LC1'S and LCIS, which are present in similar amounts (Fig. 3, f). The smallness of LCemb spot in the regenerated muscle is the consequence of two mechanisms. The lower content of myosin in myotubes in comparison with myofibers (in myotubes the central area of fibers occupied by the nuclei is much larger than the cortical layer containing the myofilaments). The regenerative process proceeded to the myofiber step in the few days of the experiment in spite of the lack of reinnervation (Fig. 2).

Myosin Heavy Chains

By SDS-polyacrylamide gel electrophoresis in appropriate conditions of polyacrylamide concentration, electric field, buffer system and protein amount, four myosin heavy chains are separated in adult rat muscles, only one of them being peculiar to slow fibers. The presence of three MHC in fast fibers of rat muscles has been previously identified by immunohistochemical approaches [54, 55] and then separated by gel electrophoresis [4]. The third component has been indicated either as 2X-MHC [54] or MHC-IID [4]. Figure 4 shows the gel electrophoretic analysis of MHC from adult rat diaphragm (lanes 1-3), EDL (lanes 4-6), tongue (lanes 7-9) and soleus (lanes 10-12). According to their decreasing electrophoretic mobility and content in different muscles the bands are indicated as MHC-1, MHC-2B, MHC-2A and MHC-2S.

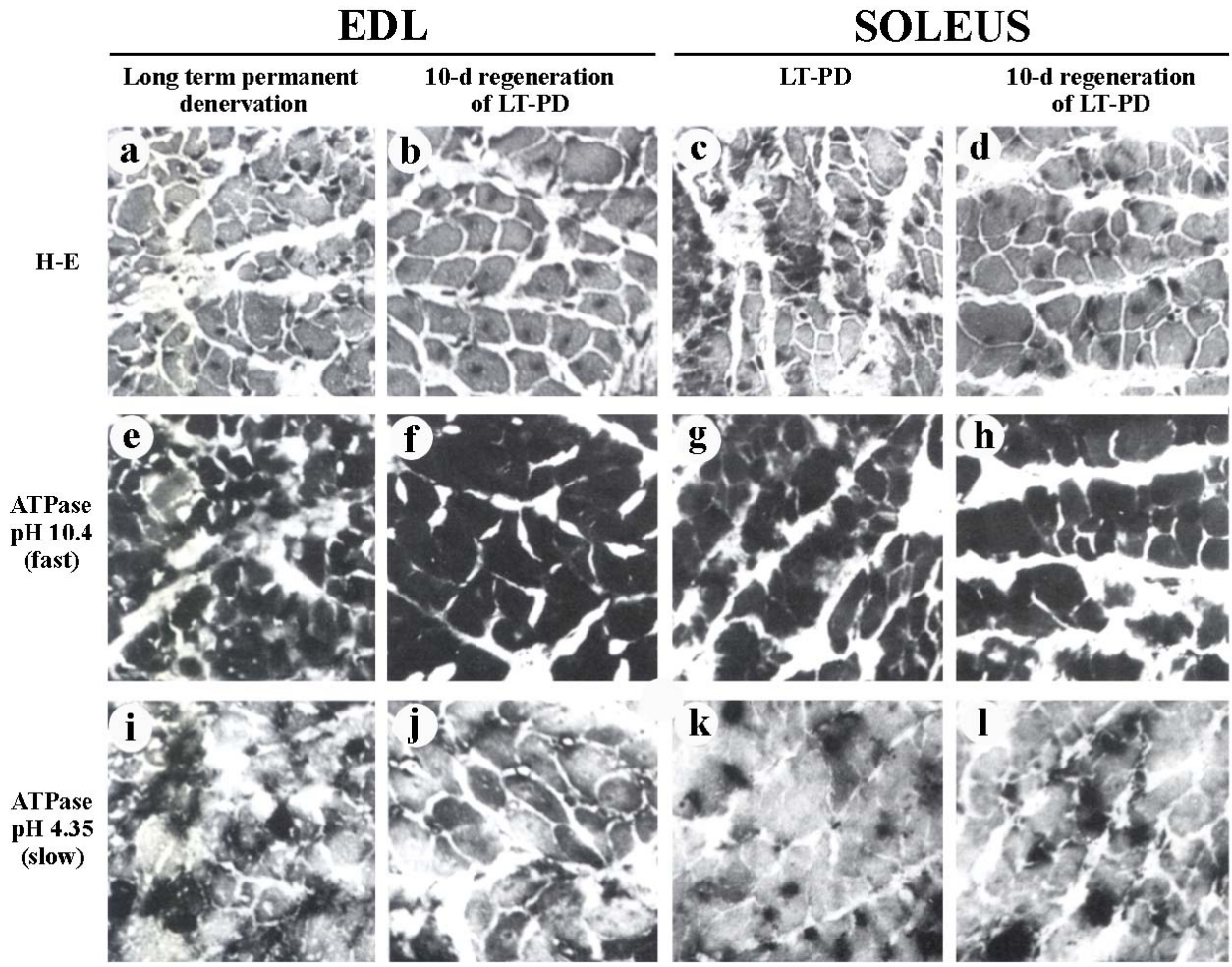

Figure 2. Regeneration of long-term denervated EDL and soleus rat muscles. Hematoxylin-eosin (a), (b), (e), (d); Myosin ATPase after preincubation at pH 10.4 (e), (f), (g), (h); Myosin ATPase after preincubation at pH 4.35 (i), (j), (k), (1). Four-month denervated EDL (a), (e), (i). Four month denervated EDL at day 10 after injury (b), (f), (j). Four month denervated soleus (c), (g), (k). Four month denervated soleus at day 10 after injury (d), (h), (1).

Two of four MHC are easily identified on the ground of their electrophoretic mobility and relative amount in EDL and soleus. MHC-1 is recognized as the myosin heavy chain of slow fibers, due to its large prevalence in the soleus muscle. Due to its larger amount in the EDL, MHC-2B is identified as the heavy chain of fast-glycolytic fibers. The heavy chain indicated as MHC-2A, the prevailing isoform of tongue muscle, is also present in both diaphragm and EDL, but absent in soleus. On the grounds of histochemical classification of the fast fibers of the EDL and of the relative abundance in respect to the accompanying MHC-2S, we indicate as MHC-2A the component with a slightly faster electrophoretic mobility. We prefer the initials MHC-2S for the fourth component to stress that this MHC is the only fast component present in the rat soleus muscle, and therefore peculiar to a well confined subset of fast-oxydative fibers. The fact that the soleus contains the two bands which run faster and slower in gel electrophoresis explains why it has been the first mammalian muscle in which separation of MHC isoforms by SDS PAGE has been attained [18, 22].

Myosin heavy chains of experimental muscles analyzed by SDS 6% PAGE are shown in Figure 5. In the long-term denervated EDL the two slower migrating bands (MHC-2S and MHC-2A) considerably increase at the expense of MHC-2B, the MHC-1 became a distinct band, and a faint new band is present behind MHC-2B (Fig. 5A, lane 2).

In the long-term denervated soleus MHC-1 disappears substituted by bands in the region of MHC-2S, MHC-2A and MHC-2B (Fig. 5A, lane 6). Four days after injury in both muscles the pattern is dominated by a prominent band which spans the position of MHC-2S and MHC-2A and by a faint band in the region of MHC-2B (Fig. 5A, lanes 3 and 7). The bands are poorly defined because of a high background probably due to coextraction of glycoproteins, which we found in MHC gel electrophoresis of crude myosins from embryonic

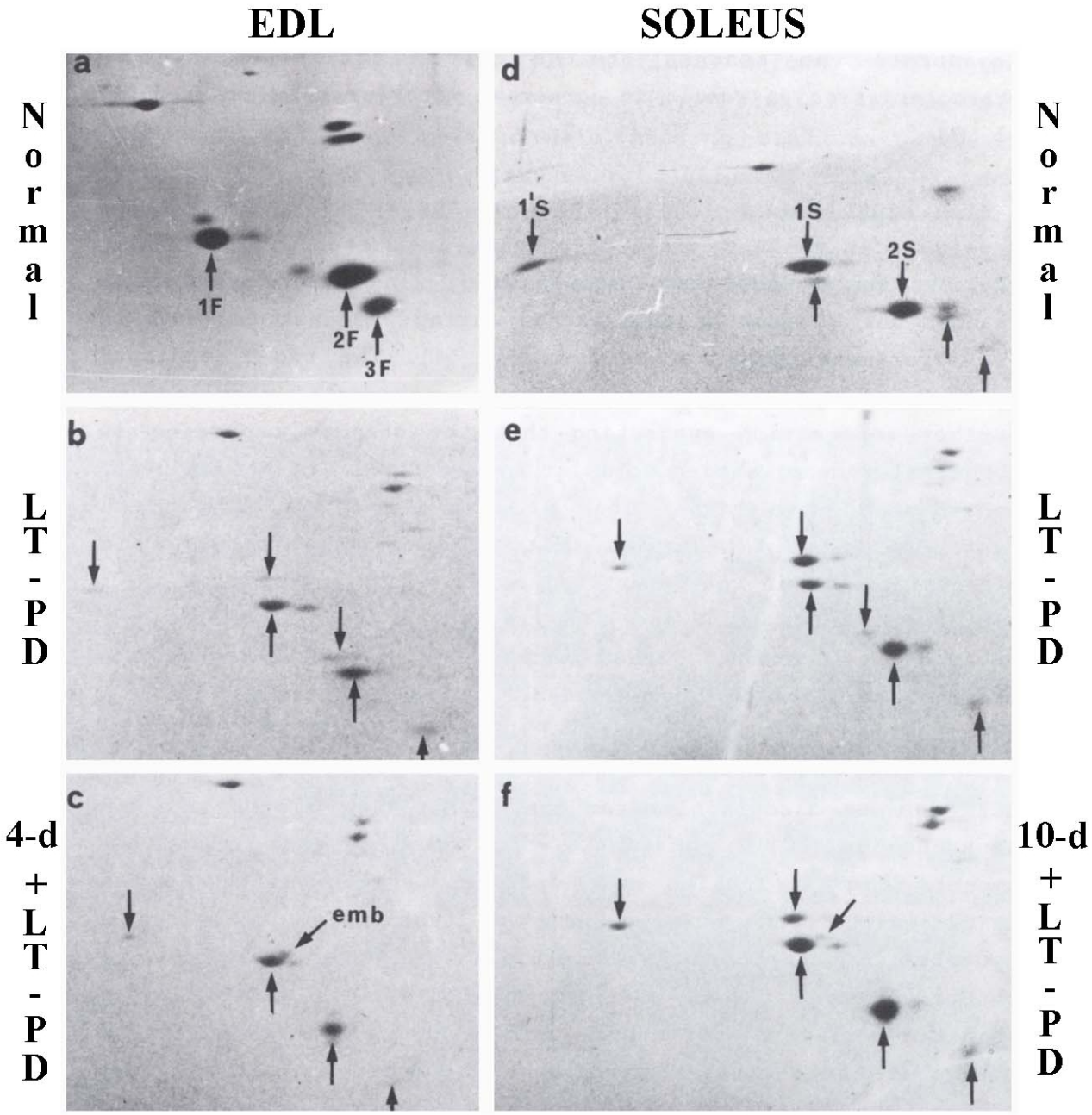

Figure 3. Long-term denervation and regeneration capability of long-term denervated rat muscles. Two-dimensional gel electrophoresis of myosin light chains of (a) normal EDL, (b) four month denervated EDL, (e) four month denervated EDL at day four after injury, (d) normal soleus, (e) four month denervated soleus, (f) four month denervated soleus at day ten after injury. The position of the light chains (IF, 2F, 3F, 1'S, 1S, 2S and emb) is indicated. Upward vertical arrows indicate fast-type light chains, downward vertical arrows indicate slow-type light chains, diagonally downward arrows indicate the light chain characteristic of the embryonic muscle.

muscle, muscle cell cultures and early regenerating muscle four-day after injury. Ten days after injury the long-term denervated soleus shows two bands: the more abundant with the electrophoretic mobility of MHC-2A, the second one in the region of MHC-2B (Fig. 5A, lane 8). Ten days after injury the long-term denervated EDL shows a clear four-band pattern: two bands in the region of MHC-2S and MHC-2A and two bands in the region of MHC-2B (Fig. 5A, lane 4). If present, the MHC-1 does not represent a distinct band discernible from the background material in the regenerating muscles.

To determine the identity of the four bands present in the ten-day regenerating EDL, we analyzed this extract mixed with either myosin from normal adult EDL (MHC-2S, MHC-2A and MHC-2B), from 7-day newborn EDL (neonatal MHC) or from bulk skeletal muscles of 18-day

rat embryos (embryonic MHC). Panels B and C of Fig. 5 show the results of these analyses. When the normal adult myosin is added to the regenerating EDL, three of the four bands are identified as MHC-2s, MHC-2A and MHC-2B (Fig. 5B, lanes 1 and 2). When 7-day-old EDL myosin is analyzed alone the major band runs with slightly less mobility than MHC-2B: indeed it comigrates with the unknown band of the regenerating EDL (Fig. 5B, lanes 3 and 4). This identification is reinforced by the results of the coelectrophoresis of adult EDL with either 7-day-old EDL or long-term denervated EDL (Fig. 5B, lanes 5 and 6). The neonatal heavy chain is therefore present not only, as expected, in regenerating denervated EDL but also in EDL after four months of sciatectomy. When the myosin from bulk skeletal muscles of 18-day rat embryos (embryonic MHC) is analyzed alone two bands appear: MHC-1 and a band in the region of MHC-2A (Fig. 5 C, lane 3). Unfortunately the resolution of our electrophoretic system does not allow us to separate MHC-2A from MHC-emb in the coelectrophoresis of embryonic myosin with either normal adult (not shown) or regenerating EDL (Fig. 5C, lanes 1 and 2).

Discussion

The high dynamic state of skeletal muscle fibers is well exemplified by their ability to accommodate to different amounts of contractile activity and/or hormonal stimuli by: i) continuously adjusting their total mass (atrophy, eutrophy and hypertrophy); ii) responding to damage with fiber regeneration; and iii) switching among a relatively broad range of genes' pools, so that myofibers tune their metabolic and contractile characteristics to changing demands. We here report: i) the striking increase of some of the adult fast myosins in both long-term denervated EDL and soleus rat muscles, ii) the maintenance of muscle regeneration ability after an induced damage in spite of long-term permanent denervation, and iii) the initial expression in these regenerated aneural fibers of imma-

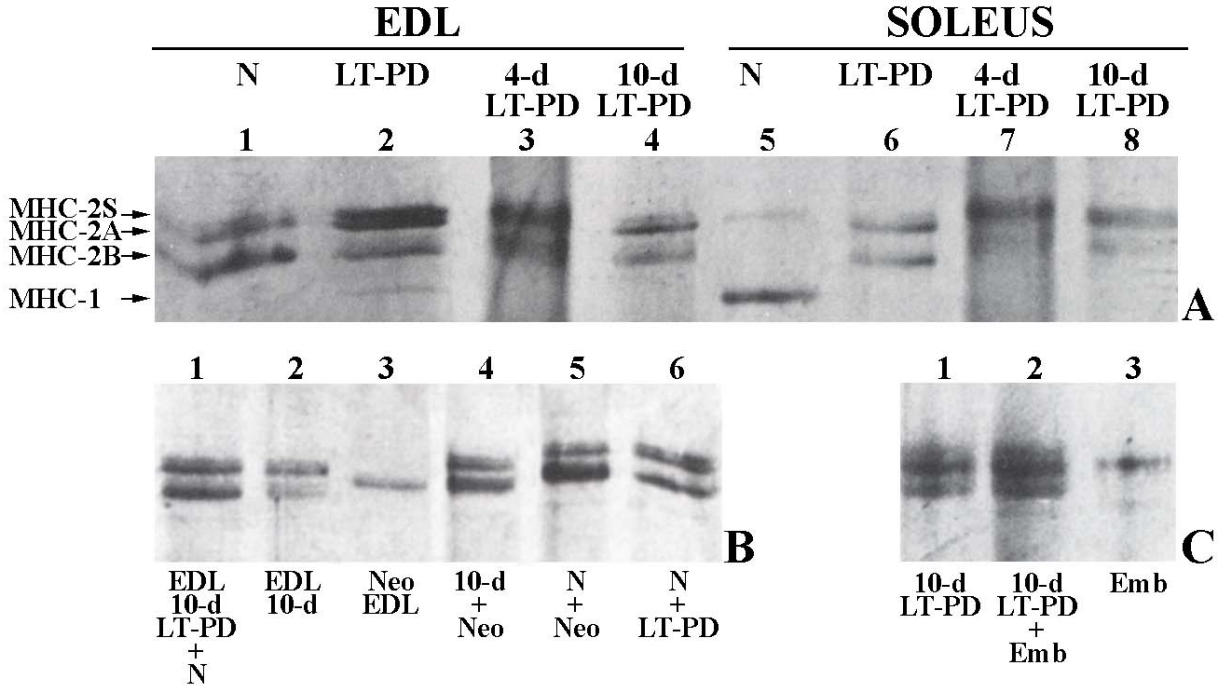

Figure 5. Long-term denervation and regeneration capability of long-term denervated rat muscles. SDS-6%PAGE of myosin heavy chains of (A) normal EDL (1), four month denervated EDL (2), four month denervated EDL at day four after injury (3), four month denervated EDL at day ten after injury (4), normal soleus (5), four month denervated soleus (6), four month denervated soleus at day four after injury (7), four month denervated soleus at day ten after injury (8); (B) four month denervated EDL at day ten after injury + normal EDL (1), four month denervated EDL at day ten after injury (2), 7-day-old EDL (3), four month denervated EDL at day ten after injury + 7-day-old EDL (4), 7-day-old EDL + normal EDL (5), normal EDL + four month denervated EDL (6); (C) four 'month denervated EDL at day ten after injury (1), bulk skeletal muscles of 18-day-old embryo + four month denervated EDL at day ten after injury (2), bulk skeletal muscles of 18-day-old embryo (3). MHC-25, the unique myosin heavy chain of the fast fibers of the soleus; MHC-2A, myosin heavy chain of the prevailing subpopulation of fast- oxidative fibers in mixed fast muscles; MHC-2B, myosin heavy chain of fast-glycolytic fibers; MHC-1, myosin heavy chain of slow fibers; MHC-neo, myosin heavy chain of neonatal fibers; MHC-emb, myosin heavy chain of muscle fibers in embryo.

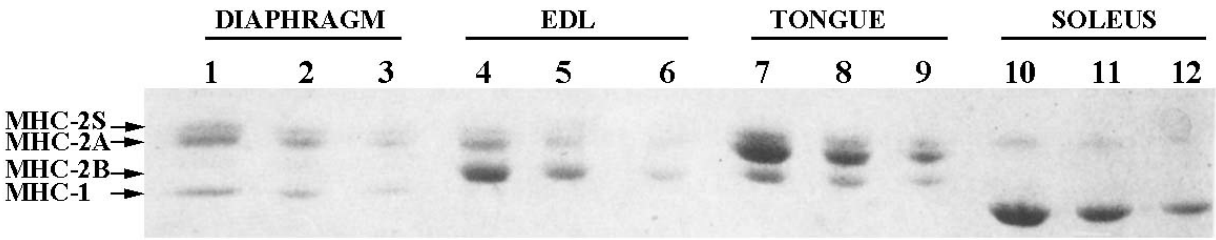

Figure 4. SDS-polyacrylamide gel electrophoresis of rat myosin from (1, 2, 3) diaphragm, (4, 5, 6) EDL, (7, 8, 9) tongue, (10, 11, 12) soleus. In (1), (4), (7), (10) 1 μg, (2), (5), (8), (11) 0.5μg, (3), (6), (9), (12) 0.25 μg of myosin were loaded. MHC-2S, the unique myosin heavy chain of the fast fibers of the soleus; MHC-2A, myosin heavy chain of the prevailing subpopulation of fast-oxidative fibers in mixed fast muscles; MHC-2B, myosin heavy chain of fast-glycolytic fibers; MHC-1, myosin heavy chain of slow fibers.

ture myosins and then of those adult fast isoforms which accumulate in denervated muscles.

Hematoxylin-Eosin and ATPase stainings showed that: i) the severely atrophic, angulated fibers of long-term denervated muscles had lost the segregation of ATPase stainings peculiar of adult muscles; ii) the 10-day regenerated muscles displayed a more homogeneous population of small round fibers, mostly with central nuclei and all with fast-like ATPase stainings; iii) transverse sizes of both muscles and regenerated myofibers at ten-day regeneration are larger than that of four-month denervated muscles and myofibers.

By SDS-glycerol-low-current-6%-PAGE we separate six sarcomeric MHC in mixtures of immature and mature rat skeletal muscles. According to their increasing electrophoretic mobility they are: MHC-2S (the only fast-type MHC of soleus which accounts for 10- 20% of total MHC in mixed and fast-twitch muscles), MHC-2A, MHC-emb, MHC-neo, MHC-2B and MHC-1.

In four month denervated muscles MHC-2B and MHC-1 substantially decrease in EDL and soleus respectively, so that both MHC patterns become dominate by MHC-2S, MHC-2A and MHC-2B at an almost 1:2:1 ratio. Four days after bupivacaine injury the adult type MHC isoforms were absent, thus confirming the effectiveness of the bupivacaine treatment; six days later (10-day regeneration) on the other hand the adult fast MHC isoforms are re-expressed in both EDL and soleus (MHC-2S, MHC-2A, and MHC- 2B). In the absence of nerve MHC-1 is missing in both regenerated EDL and soleus. Direct molecular evidence of myofiber regeneration are: i) presence of the embryonic light chain in the regenerated muscles, ii) presence of the neonatal isoform of the myosin heavy chain in both denervated and regenerated EDL.

These results strongly confirm the evidence first reported by us that myosin expression switches to a fast type in long-term denervated [16, 19-22] and regenerating-denervated [21] muscles when the fibers are deprived of main modulating influence of nerve (activity and possibly neurochemical factors) and that fiber regeneration may continuously occur in long-term denervated muscles [6, 8, 9, 13, 30, 43]. They also are indirect evidence that induced activity is the dominant factor of slow myosin accumulation in denervated fast muscles continuously electrostimulated at low frequency [3, 5, 14, 15, 17, 24, 32, 39, 40, 44, 47-50, 52, 56].

The results of myosin light and heavy chain analyses help to clarify previous discrepancies in the effects of denervation in different muscle types. Though the loss of the most peculiar characteristics of fast and slow muscles (MHC-2B and MHC-1, respectively) is the common effect of denervation, different muscles remain recognizable, not only after long-term denervation, but also more surprisingly after full aneural regeneration. Clear example is the high expression of 1'S and 1S light chains in the soleus regenerating in absence of the nerve, while its other slow myosin subunits are virtually absent. On the other hand, a barely visible LC1S light chain is the only slow myosin subunit present in trace amount in the EDL regenerating in absence of the nerve. These observations imply the presence of satellite cells with different gene expression potentials in fast and slow myofibers or the presence of differential extracellular factors in EDL and soleus. The present results are very similar, but not identical to those we have obtained studying in EDL and soleus aneural muscle regeneration induced by bupivacaine injury at the time of sciatectomy, suggesting that long-term pre-denervation may influence the expression of myosin genes in the regenerated myofibers. Indeed, not only the long-term denervated soleus and EDL are composed of myofibers with patterns of myosin expressions different from those of the innervated muscles, but they might be also different in extracellular factors such as interstitial molecules or extent of vascularization/perfusion, which may play a role in driving to slight, but qualitative differences of myosin expression in muscle fibers after damage. In any case, there is no room for doubting that the long-term denervated myofibers and those regenerated in permanent absence of the nerve offer examples of unusual myosin subunit composition which may help to fill the gap among the known myosins and the hundreds of combinations which are theoretically possible combining all the known light and heavy myosin subunits [46]. As the analytical methods become more sensitive and discriminating skeletal muscle tissue appears composed

not by a mixture of few stereotypic rigid types, but by a cell population that, taking into account pathologic and physiopathologic states, is better described as a continuum (though probably not fully reversible) of fibers.

Taken together, all our results confirm that the dynamic state of the muscle fibers survives denervation at both cellular and molecular levels and that this plasticity of the unused muscle could be the basis of its potential for recovering function.

Perspectives

Over the last 30 years there has been a good deal of interest in the use of FES to restore posture and movement of the limbs of patients immobilized by spinal-cord injury (upper motor neuron lesion, spastic paralysis). A number of research programs, including several funded by the EU, have demonstrated the potential of these techniques for restoring hand grasp, respiration, standing and walking. Secondary benefits include improved cardiovascular fitness, relief of pressure sores, increased independence and improved self-esteem.

There is, however, another group of patients whose problems are more severe and more difficult to treat: those in whom injury has also resulted in irreversible loss of the nerve supply to some or all of the affected limbs (flaccid paralysis due to lower motor neuron lesion). The condition is more severe because of the marked atrophy of denervated muscles and the associated loss of bone mass and skin atrophy causing severe secondary medical problems. It is technically more difficult to treat these patients because direct stimulation of muscles requires more electrical energy than commercially available stimulation devices can deliver. The absence of functional nerve fibers makes it more difficult to recruit a sufficient population of myofibers (i.e., parts of muscle or muscles distant from surface electrodes) to regain functional movements at an acceptable force level, as control on distribution of the electrical field in the muscle is very limited using electrodes at the skin surface. Despite these difficulties two pilot studies on functional clinical application of FES on denervated muscles have been published. One has demonstrated gait correction via direct FES of the denervated tibialis anterior muscle [60], the second, concerning pilot work in the clinic of one of us, has shown that, contrary to widely accepted opinion, electrical stimulation in such patients can restore muscle mass, force production and movement even after long-standing complete denervation. Electrical stimulation reversed much of the deterioration undergone by the denervated tissues, and muscle function in the lower extremities was restored sufficiently to support standing up, standing, and even a few steps [34]. In addition to this functional restoration, improvements were observed in the condition of associated tissues (cartilage, tendons, bones, joints and skin) within the denervated area. The work showed that restoration of denervated muscles and partly restoration of extremity movements could be solved in principle, but it has also highlighted some of the problems, e.g. efficient training protocols and safety limits, that must be addressed systematically before the technique could be widely used in clinical practice [33]. The associated stimulation equipment proofed to be effective for home-based training in a limited number of patients, but it is far from the developmental level required for commercial utilization and general availability [31].

Both systematic investigation of the mentioned open questions and development of a set of equipment in an applicable development stage are main objectives of the EU research project RISE that started in November 2001 and focuses on rehabilitation of patients with degenerated muscles due to complete denervation of the lower extremities [41].

The aim of our work is to place this therapeutic application of electrical stimulation on a firm scientific foundation and to use the resulting knowledge to design protocols for clinical use that provide the maximum beneficial effects with the minimum intrusion into the patient's normal daily activities.

Since satellite cell activity is required for both maximum hypertrophy by overloading and regain of mass from atrophy by relative overloading [1], positive regulation of activation, division and fusion of myoblasts may be an important tool to understand limits of recovery by functional electrical stimulation in neurogenic muscle myopathies [33, 45].

A major outcome of the work will be a set of innovative technical products, that are designed to deliver therapeutic stimulation in a safe and effective way and suitable for use by the patients in their own homes. Not at least a revision of existing EU regulations governing the use of electrical stimulation, which currently limit the stimulation parameters to levels that exclude an efficient therapy of the targeted patient group, is indispensable for a later commercialization of the developed equipment. Our incoming studies will provide all the treatment parameters, training protocols and supervision guidelines needed for routine clinical application.

Patients suffering from flaccid paraplegia (denervation of lower extremity muscles, conus cauda syndrome) are especially good candidates for these approaches. On the basis of the pilot studies, it is anticipated that improvements in mobility will be associated with substantial reductions in the risk and the severity of secondary medical problems, resulting in less frequent hospitalization and a reduced burden on public health services and careers. This group of patients could thus look forward to improved health, independence and quality of life, and the prospect of better professional and social integration.

In the long-term we may consider the development and application of implantable solutions alternatively to the actual approaches based on surface electrodes. In extensive studies on sheep crycoarytenoid muscles chronic respiration-synchronized stimulation could be demonstrated for periods up to 18 months using implantable

electrodes [18, 64]. Functional maintenance of the muscle through the whole investigation period without observing major signs of muscle damage in multiple animals may encourage further work on technologically complex implantable solutions for various applications, which promise more comfort in equipment handling and substantially improved selectivity in activating single denervated muscles under avoidance of affecting intact neural structures in the closer vicinity of the muscle. A much achievable goal will be to apply our results to spastic paralysis.

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Address correspondence to:

Prof. Ugo Carraro, Dept. Biomedical Science, Viale G. Colombo, 3, I-35121 Padova, Italy, phone +39 0498276030, fax +39 0498276040, Email bam@bio.unipd.it.

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