Functional Electrical Stimulation of Denervated Muscles: An Experimental Evaluation

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Abstract: For many years there has been a dearth of effective treatment options for the severe wasting and secondary consequences of motor nerve injury. In recent years, however, an intensive regime of electrical stimulation has been shown to have considerable therapeutic benefits. This article reviews the results of an extensive study designed to address the clinically relevant issues in an appropriate animal model. The study reveals both the benefits and the limitations of the technique, but strongly endorses the therapeutic advantages of introducing a program of stimulation during the initial, nondegenerative phase of the muscle response to nerve or root injury. **Key Words:** Functional electrical stimulation—Denervated muscle—Flaccid paralysis—Rehabilitation.

Denervation of limbs occurs when there is trauma to peripheral nerves and roots; examples are radial and peroneal nerve injuries, brachial plexus injury, and spinal cord injury with concomitant root damage. The flaccid paralysis that results from these lower motor neuron injuries has more serious consequences than the spastic paralysis caused by an upper motor neuron injury (1). The muscles lose mass rapidly, and much of the cross section becomes occupied by noncontractile tissues, notably collagen and fat. As a result, the force that can be elicited by electrical stimulation quickly declines. This phase of atrophy is followed, after about 1 year to 18 months, by progressive necrosis of muscle fibers. Although some muscle regeneration does occur (2), it is at a level insufficient to replace the degenerative loss.

Such a denervating injury has secondary consequences over and above the loss of mobility. When it involves the lower limbs, a lack of cushioning over bony prominences, combined with a loss of skin condition, greatly increases the risk of developing pressure sores. Bones become osteoporotic. The severely wasted appearance of the affected limbs is a source of distress to the patients.

The potential therapeutic value of using electrical stimulation to restore a degree of contractile activity to denervated muscles has not been overlooked in the past. It has not, however, been a feasible proposition. Direct stimulation of muscle fibers, in the absence of intramuscular motor nerve branches, requires stimulus pulses with an amplitude and duration that are beyond the capacity of conventional stimulators. Recently, however, equipment was designed specifically for this purpose, and permission was obtained from the EU to use it in experimental trials.

The clinical results were striking. Electrical stimulation improved the excitability of the denervated tissues, reversed much of the loss of mass, and in some cases restored the contractile function of the quadriceps muscles sufficiently to enable the patient to rise from a wheelchair and to stand between parallel bars (3–5).

On the strength of these results, a 4-year multi-center trial was initiated, with funding from the European Commission (Project "RISE"). In addition to patient-centered aspects of the program, the project supported the development of new instruments and electrode systems for stimulation, new techniques for evaluating the results, and a program of animal experimentation designed to investigate what could

be achieved by stimulating denervated muscles under closely controlled and highly reproducible conditions. This article is concerned with the results of the experimental program undertaken in rabbits. The initial findings were summarized earlier (6). With the completion of the study, we can now present a more complete picture of the results.

RATIONALE FOR EXPERIMENTAL DESIGN

It emerged very clearly from the clinical trials that a successful outcome was related to the time allowed to elapse between the denervating injury and the commencement of stimulation. The shorter this time was, the greater were the improvements seen in the size and functionality of the stimulated-denervated muscles. The best results were obtained when stimulation was initiated within the first 18 months post injury, a period that corresponds to the atrophic phase of the denervation response. This suggests that atrophy is more readily recoverable than the necrosis which dominates the later stages of the response.

The animal model

Much of the literature on the effects of long-term denervation of mammalian skeletal muscle has focused on experimental studies of total sciatic section in the rat (7–10). However, rat hind limb muscles enter the degenerative phase of the denervation response within a few months of injury (11,12). The rat is therefore an unsuitable model for the longer-lasting atrophic phase in humans. In the rabbit, however, we found that selective motor denervation of the foot dorsiflexors resulted in a nondegenerative atrophy that was stable from 10 to at least 51 weeks post injury (13). This was therefore a much more suitable vehicle in which to study the effects of stimulation on muscles during the purely atrophic phase of the denervation response.

Experimental design

Microdissection of the common peroneal nerve, followed by selective section of motor branches, left sensation in the lower limb largely intact, and avoided entirely the problems of self-harm associated with complete sensorimotor denervation in the rabbit. Moreover, the body size was sufficient to accommodate an implantable device designed specifically for these experiments (14). This had to be larger than those used to stimulate innervated muscles (15) because of the much greater charge delivery required.

In animal studies, stimulation has usually been initiated soon after denervation. However, such an experimental design hardly reflects clinical reality: stimulation would not normally be initiated until the patient has been stabilized and various assessments performed, a process that could take several months. There is no possibility, therefore, of preventing denervation atrophy; the requirement is rather for a technique that will reverse established denervation atrophy. In our experiments, the nerve was divided and stimulation was initiated only after 10 weeks had elapsed; by this time, muscle mass had declined to about 40% of normal (13).

The patterns

In the clinical part of the "RISE" study, the protocol was tailored to the condition and progress of individual patients, but typically delivered up to 18 000 impulses/day. The protocol used for the experimental animals was a similar, and therefore clinically relevant, pattern, consisting of rectangular bipolar constant-current pulses, 20 ms per phase, at 20 Hz, with a duty cycle of 1s On/2s Off, delivered automatically once a day in an hour-long session, a total of 24 000 impulses/day (Pattern 1). The response to this pattern was studied in some detail after 2, 6, and 10 weeks of stimulation (16). A separate group was denervated for 39 weeks and stimulated for 12 weeks, with similar results (16).

We went on to examine the effects of departing from this pattern, with a view to answering the following questions:

1 Is it beneficial to double the number of impulses delivered per day? This was achieved by changing the duty cycle to 2s On /1s Off (Pattern 2).
2 Is there any advantage to be gained by distributing the stimulation over more than one daily session? This was addressed by delivering Pattern 1 in two sessions, each of 30-min duration (Pattern 3).
3 Is there an upper safe limit to the amount of stimulation that can be delivered? We had already established that the temperature rise under the electrodes was less than 1°C. Here we examined the possibility of stimulation-induced damage other than thermal damage. The pattern consisted of 40 Hz bursts, 2s On/1s Off, for 5 h/day, delivering a total of 480 000 impulses/day (Pattern 4).
4 Can the same daily number of impulses be delivered in a shorter span of time? This pattern consisted of 40 Hz bursts, 1s On/1s Off, for 20 min/day, and like Pattern 1 it delivered 24 000 impulses/day (Pattern 5).

As much of the response to stimulation had taken place by 6 weeks (16), this period was used for

Patterns 2–4. Pattern 5 was continued for 10 weeks, because of the short daily duration of stimulation.

Procedures

All animal experiments were conducted with appropriate sterile precautions, anesthesia, antibiotic prophylaxis, and postoperative analgesia, and in strict accordance with national legislation. At the end of each experiment, the muscles were subjected to a detailed physiological characterization. The animals were then sacrificed, and the muscles removed and frozen for subsequent morphological examination and point-counting morphometry.

Statistical analysis

Conventional statistical comparisons were made between the stimulated-denervated muscles and denervated or innervated controls. Where the effects of the various patterns of stimulation were not statistically different, the results were pooled to provide an enlarged data set for testing the effects of stimulation per se. In many cases, these combined results form the basis of the conclusions summarized in this article.

MORPHOLOGY AND MORPHOMETRY

Stimulation markedly improved the morphological appearance of the denervated muscles at the light microscopic level (16,17). Although the changes were not uniform throughout the cross section, fibers tended to be larger and packed more closely together, with a corresponding reduction in endomysial and perimysial connective tissue. Unbiased morphometric analysis confirmed an increase in the proportion of the muscle cross section occupied by muscle fibers, and a concomitant decrease in fibrotic tissue. There was some indication that this trend would have been maintained with more prolonged stimulation, although this was not feasible for the animal experiments. Such a trend would, however, be consistent with biopsy results obtained from patients stimulated for several years, in which the proportion of muscle tissue appeared to be close to normal (18).

There was no change in the total number of fibers in the cross section, further evidence that degenerative/regenerative processes were not occurring to any significant extent in these muscles.

MASS, CROSS-SECTIONAL AREA (CSA), AND MAXIMUM ISOMETRIC FORCE (P_0)

The wet weight, CSA, and maximum isometric force (P_0) of the stimulated-denervated muscles was greater than that of denervated controls (Fig. 1), the difference for the pooled data being 66.5% ($P < 0.0001$), 46.1% ($P < 0.003$), and 72.5% ($P < 0.001$), respectively. This was consistent with the observation that stimulation, particularly with patterns containing a high-frequency component, up-regulates protein synthesis in both the presence and the absence of the nerve (19–23).

The recovery of CSA was not quite commensurate with wet weight because of a change of muscle shape, the denervated and stimulated-denervated muscles tapering less rapidly toward the tendinous insertion than the innervated controls.

Maximum force followed changes in the CSA occupied by muscle tissue, and despite the considerable improvement over denervated muscle, attained little more than half that generated by the innervated muscles.
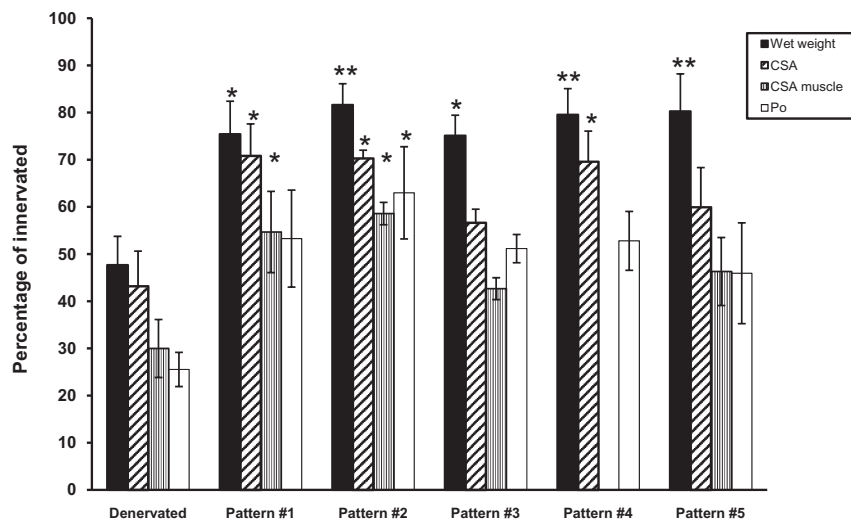

FIG. 1. Wet weight (solid bars), CSA (diagonally hatched bars), CSA occupied by muscle tissue (vertically hatched bars), and maximum tetanic force (P_0, open bars) of denervated and denervated-stimulated tibialis anterior muscles expressed as a percentage of the values for the contralateral innervated muscles. Data for the CSA of muscle tissue was not available for Pattern #4. Significant differences between denervated-stimulated muscles and muscles subjected to denervation alone are denoted *, **, for $P < 0.05$, $P < 0.01$, respectively.
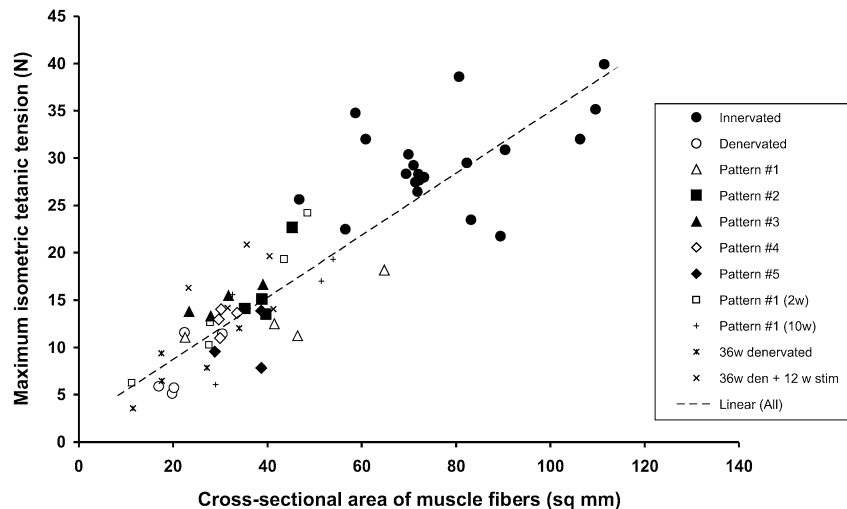
FIG. 2. Relationship between the maximum tetanic force (P_0) and the CSA occupied by muscle fibers, plotted for all experimental and control muscles. $r^2 = 0.75$, $P < 2.7 \times 10^{-20}$ for the linear regression.

In Fig. 2, the maximum isometric force is plotted against the CSA occupied by muscle tissue for innervated and denervated control groups, and for all patterns and durations. The linear regression is significant at $P < 2.7 \times 10^{-20}$, with an r^2 of 0.75. This means that maximally activated fibers in both denervated and denervated-stimulated muscles were capable of generating the same specific tension as their innervated counterparts.

CONTRACTILE SPEED

The denervated muscles contracted much more slowly than their innervated counterparts. Initial observations suggested that stimulation had no effect on this slow contractile speed [6,16]. This result was confirmed in the subsequent experiments, where it appeared to be independent of the pattern of stimulation (17).

However, when the data for denervated-stimulated muscles were pooled, an interesting contrast emerged. The isometric twitch kinetics of the denervated muscles (measured by contraction time, time to half-relaxation, and the twitch : tetanus ratio) were indeed unaffected by stimulation, but variables that were related to isotonic shortening (maximum shortening velocity, velocity for maximum power, and maximum power) increased significantly. This increase in speed was not due to changes in myosin heavy chain composition, because the denervated muscles consisted entirely of type 1 and type 2A fibers, and this composition was not altered by stimulation (16).

We will interpret these findings in relation to the electron microscopic observations below.

FATIGUE RESISTANCE

Denervated muscles exhibited a higher mitochondrial density, both in sections stained histochemically for NADH tetrazolium reductase, and in ultrathin sections prepared for, and viewed under, the electron microscope (13). Such a concentration of mitochondria would normally be associated with a well-developed oxidative metabolism and would be expected to render the muscles highly resistant to fatigue. This was not the case for denervated muscles. The post-tetanic phase of the fatigue test resembled that of slow muscles, but the fatigue resistance was not greater than normal.

Stimulation of the denervated muscles did not result in an improvement in fatigue resistance; indeed, in many cases it made the fatigue behavior worse. There could be a number of reasons for this. Oxidative metabolism may have been rate-limited by the blood supply to the muscle or oxygen transport to the fibers, or by a lack of coordination in the response of different steps in the relevant biochemical pathways. Mitochondrial function may have been disrupted by the loss of their normal distribution and structural associations within the fibers, as revealed by electron microscopic examination (see below). The fatigue curves may have reflected a progressive failure of excitation-contraction coupling. The phenomenon is worthy of further study.

ELECTRON MICROSCOPY

Denervated muscles had an abnormal ultrastructural appearance (13). Myofibrils were thinner and fewer in number, and they were often discontinuous. Sarcomeres lacked M-lines or showed streaming of Z-lines, and the registration normally seen across the fiber was lost. Wide spaces between the myofibrils were filled with amorphous cytoskeletal material, mitochondria, and fragments of the sarcoplasmic reticulum (SR) and T-system. Mitochondria occurred

in rows between myofibrils rather than in their usual specific association with the I-bands of the sarcomeres. Sarcotubular elements, normally seen as transversely orientated junctional complexes (triads) adjacent to the A-I junctions, became longitudinally orientated or more seriously disrupted.

Stimulation resulted in a marked structural recovery. Myofibrils were larger, and were packed more tightly and in registration across the fibers. Although there was variation between fibers in the degree of restoration, a semiquantitative classification of ultrastructural appearance revealed a clear overall improvement (16).

The stimulated-denervated muscles continued, nonetheless, to show some abnormal features: mitochondria were still seen in longitudinal rows, and T-tubules retained their abnormal longitudinal orientation.

These ultrastructural observations provide additional insights into the physiological findings we noted earlier. The transition, induced by stimulation, from filamentous disorder to correctly assembled and orientated myofibrils, was probably responsible for the observed increase in shortening velocity. The fact that this was not matched by changes in twitch kinetics is probably the result of slow activation. Intracellular Ca^{2+} release would normally take place adjacent to the A-I junctions. The lack of triads in this position, and the predominantly longitudinal orientation of T-tubular networks, would increase the diffusion distance for Ca^{2+} and account for the persistent slowness of isometric contraction in the denervated-stimulated muscles. Although slow, activation was evidently complete in both denervated and denervated-stimulated muscle fibers because Fig. 2 shows that they did not differ in specific tension-generating capacity during maximal tetanic contraction. Thus, slowness was probably due to disorder, rather than actual loss, of sarcotubular networks.

As mentioned above, the abnormal location of mitochondria may have been related to the lack of improvement in fatigue resistance. Recent evidence suggests that, at least for a subpopulation of mitochondria, the generation of adenosine triphosphate is linked to activation by a Ca^{2+} flux that depends on the structural proximity of these organelles to the SR (24–26).

In the embryological and neonatal differentiation of muscle, T-tubules and SR are initially in a longitudinal orientation before adopting a transverse orientation in the adult muscle (27,28), and only after triads are formed do they establish their association with the A-I junctions (29). During the corresponding period, there is an increase in isometric contractile speed (30). In this respect, the effects of stimulation on the electron microscopic appearance of the denervated muscles appeared to recapitulate part of a normal developmental sequence.

The same sequence was observed in biopsy samples of human denervated muscles that were stimulated in the clinical study within "RISE." Whereas stimulation in the animal experiments had to be limited to 6 or 10 weeks, it was of much longer duration (2.4–9.3 years) in the human studies. At the end of this time, both myofibrillar and sarcotubular organization had reverted to normal, although the muscles remained completely denervated (18). On this basis we would expect some normalization of isometric contractile speed, and possibly mitochondrial function, in the longer term.

EXCITABILITY

In the past, a major obstacle to the clinical use of electrical stimulation of denervated muscle has been the very high charge delivery needed to elicit an adequate contraction. The situation should improve with the prospective commercial availability of equipment based on that developed within "RISE," coupled with changes to the EU regulations governing such stimulation. Such equipment is capable of generating the high stimulus currents required, but the prolonged duration of the stimulus pulses still places a limit on their repetition frequency, and hence on the tetanic force that can be generated. It was therefore an important finding that daily activation of human denervated muscles appeared to result in an increase in excitability after about 4 months, enabling the stimulation regime to be switched from low-frequency twitch contractions to higher-frequency tetanic contractions (5).

We investigated this phenomenon in the animal model by recording complete strength-duration curves as part of the terminal physiological procedure. The threshold for activation of the pooled stimulated-denervated muscles showed a small, but significant reduction. This could have been a consequence of the increase in mean fiber size (16). There was no firm basis for postulating a contribution from changes in the structure or composition of the muscle fiber membrane.

The changes in excitability that we observed in the rabbit were not of the same order as the marked changes observed clinically. In the latter situation, stimulation is delivered via electrodes placed on the surface of the skin. After several months of stimulation there is a reduction in the thickness of the layer of fat and connective tissue lying between muscle and

skin, as demonstrated by computed tomography scans of the whole thigh (5,31). The reduction in threshold may therefore be explained at least partly by the resultant changes in the distribution, depth of penetration, and rate of rise of current within the muscle cross section.

Many of the patients recruited into the study had already progressed into the necrotic phase of the response to stimulation. It is also possible, therefore, that stimulation has a more marked effect on the size or membrane properties, and therefore overall excitability, of severely atrophic, degenerating, or regenerating muscle fibers.

SUMMARY OF EFFECTS

In the animal studies, stimulation of denervated muscles resulted in significant increases in wet weight, CSA, tetanic tension, shortening velocity, and power. The muscle fibers were larger in diameter and more closely packed, with a corresponding reduction in endomysial and perimysial tissue. There was no evidence of loss of fibers by necrosis, nor of extensive proliferative activity. A small increase in excitability was seen, which was probably secondary to changes in fiber diameter. The kinetics of isometric contraction and relaxation, and related measures such as twitch : tetanus ratio, remained slow and there was no improvement in fatigue resistance. None of these findings was materially altered by varying the actual pattern of stimulation.

THERAPEUTIC BENEFITS

The use of an animal model enabled us to investigate these phenomena with a well-defined denervation injury and reproducible stimulation conditions, and to apply methods of assessment that would be impossible in a clinical study. Nonetheless, where there was a basis for comparing the study with results obtained clinically, there was remarkable agreement: examples are measurements of CSA, fiber size, and ultrastructure.

Although the clinical findings were qualitatively similar, many of the measures reflected the more extensive changes that could be obtained with the much longer stimulation periods (several years and more) possible with human patients. Examples of this are the closer approach to normality in the proportions of muscle and connective tissue, and the more advanced state of recovery of ultrastructure (18). Nonetheless, the stimulated-denervated muscles appear to remain abnormal in terms of excitability, force-generation capacity, fatigue resistance, isometric contractile speed, and fiber-type composition.

Despite these limitations, the improvement is sufficient in many cases to enable a patient to rise from a wheelchair to a standing position, and to take steps between parallel bars.

Electrical stimulation can address some of the most serious secondary effects of muscle denervation, including the poor appearance of the wasted muscles, and the high incidence of pressure sores. The risk of the latter seems to be greatly reduced by improvements in the general condition and circulation of the skin, combined with the better cushioning that results from an increase in muscle mass. In those cases where the patient achieves standing, there are indications of improvements in bone density, as well as the improved cardiovascular fitness and heightened patient self-esteem that come from short daily periods spent in an upright, weight-bearing posture.

If stimulation is discontinued, the muscles revert to their former wasted condition. It is therefore inherent in a treatment of this kind that it calls for an ongoing commitment from the patient. Thus, the actual benefits depend less on stimulation pattern than on the motivation of the subjects, and the availability of home-based equipment. Further work could, with advantage, focus more closely on protocols that produce the major benefits with the least intrusion into patients' activities of daily living. The experimental results reviewed here take a first step in this direction, indicating that there is no advantage to be gained from stimulation regimes that are more energetically demanding than those in current use, or that deliver the stimulation in more than one daily session.

A crucial factor is the need to introduce stimulation during the initial nondegenerative phase post injury. Many physicians are reluctant to commence such a treatment during the first year post injury for fear that it may reduce the potential for reinnervation (32,33). The evidence for such a contention is far from secure; indeed, some groups report that reinnervation is either unaffected or actually proceeds more rapidly under these conditions (34–37). The clinical experience of our colleagues in Project "RISE" suggests that nerve regrowth is not inhibited; they have observed sensory or motor reinnervation, or both, during the application of stimulation therapy 2 to 5 years after injury (3,38). The balance of benefit, therefore, appears to be with the earliest possible introduction of a therapeutic regime of stimulation.

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