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**Time Course and Cellular Control of Muscle Fiber Transformation Following Chronic Stimulation**

Chronic electrical stimulation has long been used by basic scientists to study skeletal muscle adaptation since it induces a repeatable, quantifiable amount of "exercise." In this setting, a well-defined progression of muscular changes is observed that enables investigation of the mechanism and time course of muscle adaptation. Experimental observations of muscular changes following electrical stimulation may provide insights into other forms of muscle adaptation such as those that occur during immobilization, disease, or exercise.

**BACKGROUND**

**Skeletal Muscle Structure**

Because skeletal muscle is a classic biological example of a structure-function relationship, studies of muscle function are intimately tied to studies of muscle structure (1). A given muscle may take on almost any size or shape (Fig. 1). Variability in this gross structure does not result from different muscles being constructed of different components. In fact, all muscles are composed of single muscle fibers that are nearly identical in diameter. While the length and arrangement of these individual fibers may vary between muscles, the fibers themselves have a diameter of 20 to 80 μm in mammalian muscle. A whole muscle can thus be viewed as a number of muscle fibers arranged in series and in parallel (Fig. 1). The muscle fiber itself is composed of myofibrils arranged in parallel with diameters of about 1 μm. The myofibril is composed of many sarcomeres, which are 2 to 3 μm in length and about 1 μm in diameter, arranged in series. The sarcomere is composed of a number of interdigitating protein filaments (the so-called "thick" and "thin" filaments), which are responsible for the regulation and generation of muscle force. Thus, the sarcomere (actually the half-sarcomere due to sarcomere symmetry) is the functional unit of force generation in skeletal muscle. Morphologically, the sarcomere is bound by the Z-disk, further adding to the striated appearance of skeletal muscle (Fig. 1). Structural hierarchy is also demonstrated at the molecular level, as the individual thick filaments are composed of a number of myosin molecules arranged in an antiparallel fashion. The myosin molecule contains enzymes responsible for force generation. This occurs as a result of cyclic interaction between the actin and myosin filaments and utilizes adenosine triphosphate (ATP) as the immediate energy source. The thin filament is composed of a series of actin monomers arranged in a helical pattern.

**Skeletal Muscle Contraction**

The process by which actin and myosin filaments are activated to generate force is known as excitation–contraction coupling (2). Following nerve depolarization, an action potential propagates down the motorneuron and is transmitted to the muscle fiber at the neuromuscular junction by diffusion of the neurotransmitter acetylcholine. The resulting depolarization is conducted across the muscle via the surface membrane (sarcolemma), which is, itself, an excitable membrane. The action potential is then conducted deep within the muscle fibers via the transverse tubular system, or T system, which runs perpendicular (transverse) to the myofilaments. The action potential from the T system then signals the sarcoplasmic reticulum (SR) membrane system by an unknown mechanism to release calcium, causing force generation (3). Relaxation occurs as the calcium is pumped back into the SR by the calcium pump localized within the SR membrane.

**Muscle Fiber Types**

Because muscle shortening involves these two major processes, excitation and contraction, muscle "speed" can be modulated by at least two mechanisms. First, shortening speed can be altered by changing the attachment–detachment kinetics of the myosin cross-bridges. Second, "activation speed" can be altered by changing the relative amount or composition of the T and SR systems. In fact, normal mammalian skeletal muscle fibers vary in the types of myosin they contain and in the amount of T and SR membranes they contain, conferring on them different contractile properties. Muscle fibers can thus be separated into at least two categories, fast contracting and slow contracting. For an unknown reason, fast fibers have a narrow Z-disk while slow fibers have a wide Z-disk (4).
Skeletal muscle fibers can not only differ in their speed, but they can also differ in their endurance. Generally, a muscle can either generate energy without oxygen (anaerobically), which occurs during maximal efforts, or with oxygen (aerobically), which occurs during moderate intensity exercise. Generation of ATP occurs by glycolysis anaerobically by oxidizing glucose (which is stored in the muscle fiber as glycogen) to lactate. In aerobic metabolism, oxygen is used in steady state oxidative phosphorylation to oxidize glucose to carbon dioxide and water. In each case a certain complement of metabolic enzymes is required. Some muscle fibers have a large proportion of glycolytic enzymes, suggesting a preferential utilization of anaerobic metabolism, whereas others have a preponderance of oxidative enzymes, suggesting preferential use of aerobic metabolism.

It is clear, therefore, that skeletal muscle fibers can possess a spectrum of characteristics related to contractile speed, oxidative capacity, and glycolytic capacity. For convenience, a fiber type classification scheme has been suggested to separate fibers into three major categories (5): fast contracting fibers with high oxidative and high glycolytic capacity (known as type FOG fibers), fast contracting fibers with low oxidative and high glycolytic capacity (FG fibers), and slow contracting fibers with high oxidative and low glycolytic capacity (SO fibers). While this scheme artificially implies abrupt distinctions between fibers of different types, it is nevertheless useful for muscle fiber characterization in normal muscle and muscle which is changing its properties, for example, as a result of chronic stimulation.

**CURRENT STATUS**

The best-documented effects of electrical stimulation on skeletal muscle are those that occur after chronic, low-frequency stimulation of a predominantly “fast” muscle superimposed on normal muscle activity. In this setting,
a well-defined progression of changes is observed whereby the muscle first changes its metabolic and then its contractile properties to become a "slow" muscle (6-8). This transformation process has been documented in a number of different muscles and species so that the effects observed are probably not species or muscle specific. The fast-to-slow transformation that occurs is detectable by measurement of muscle contractile, ultrastructural, histochemical, biochemical, and morphological properties. In all cases, following transformation, the new slow fibers are completely indistinguishable from normal slow skeletal muscle fibers. It is also clear, on the basis of time-series studies (9,10) and single fiber biochemistry (11,12) that the changes which occur result from transformation at the level of the single fiber and not from fast fiber degeneration with subsequent slow fiber regeneration or proliferation.

**Time Course of Muscle Fiber Transformation**

If low-frequency stimulation is applied 24 h/day, the total transformation process requires only about 30 days. The earliest observed changes occur within a few hours after the onset of stimulation when the SR begins to swell (9) (Fig. 2a). Within the next 2 to 12 days, increases are measured in the volume percent of mitochondria, oxidative enzyme activity, the number of capillaries per square millimeter, total blood flow, and total oxygen consumption, reflecting the increased metabolic activity of the muscle (9,13-15) (Figs. 2b and c). Histochemically, this is reflected in an increased percentage of FOG fibers at the expense of FG fibers (14,16) (Fig. 2c). At this point, the width of the Z-band begins to increase toward the wider value observed for normal slow muscle (9) (Fig. 2d). The amount and activity of the calcium transport adenosine triphosphatase (ATPase) decreases and changes its particle distribution within the SR bilayer (17) (Fig. 2e). This decrease in the amount and activity of the calcium ATPase can be detected physiologically as a prolonged time-to-peak twitch tension and a prolonged relaxation time of a muscle twitch or as a decrease in the fusion frequency (18-20) (Fig. 3). The increase in oxidative enzymes and capillary density is manifested as a decrease in muscle fatigability (11, 20). Finally, after about 4 weeks of continuous stimulation, an alteration in the myosin light chain profile is observed whereby the normally fast muscle, containing only light chains LC1F, LC2F, and LC3F, now contains light chains characteristic of slow fibers, i.e., LC1S and LC2S (20) (Fig. 2f). By this time, muscle fiber cross-sectional area, maximum tetanic tension, and muscle weight have decreased significantly (4, 21) (Fig. 2g). The Z-band is now the full width of that normally observed in a slow fiber, and the density of the T system is greatly decreased (9) (Fig. 2h). The muscle is now indistinguishable from a normal slow skeletal muscle in every respect.

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**Figure 2** Schematic representation of the time course of muscle fiber transformation. (a) SR begins to swell after 3 h of stimulation. (b) After 2 to 12 days of chronic stimulation, an increase in the volume percent of mitochondria is observed. (c) After 2 - 12 days of chronic stimulation, an increase in capillary density and an increase in FOG (represented as "darkly" staining in this simulated SDH stain) fibers is observed. (d) After 14 days, the Z-band begins to increase in width. (e) After 14 days, a decrease in the amount and activity of calcium ATPase is observed. (f) After 28 days, the myosin light chain profile is altered (this figure is schematic and actual structural changes associated with differences in light chains are not known). (g) After 28 days, muscle mass and fiber area are decreased. (h) After 28 days, the Z-band is the full width of a normal slow contracting muscle and the density of the T system has increased. At this point, the transformed fast contracting muscle is indistinguishable from a normal slow contracting muscle.
We thus conclude from this typical time course of transformation that: 1) muscle metabolic enzymes, capillaries, SR, and T-system are much more easily changed than the contractile proteins, and 2) while chronic stimulation does increase muscle endurance capacity, it is not an effective means for strengthening normal muscle. The decrease in skeletal muscle mass and fiber area should not be viewed as an atrophic or degenerative response. Rather, it appears to represent a deliberate effort on the part of the muscle fiber to decrease diffusion distances from the muscle fiber to the interstitial spaces that contain the capillaries.

**Cause of Muscle Fiber Transformation**

At this point it should be noted that, while the time course of fast-to-slow fiber transformation is best documented for chronically stimulated muscle, fiber type transformation also occurs under other conditions. For example, it was documented in classic experiments by Buller et al. [23] that if the nerve which normally innervated a fast contracting muscle was surgically attached into a slow contracting muscle, the fast contracting muscle would transform to a slow contracting muscle, matching the properties of the new nerve. These experiments gave rise to the concept that something in the nerve (the “neurotrophic factor”) determined the properties of the innervated muscle fibers. Subsequent experiments revealed that, while the nerve certainly influenced muscular properties, it did not unilaterally determine them.

A second hypothesis put forth to explain the mechanism of muscle fiber transformation was based on the observation that nerves which innervated predominantly slow muscles were continually activated at relatively low frequencies (tonic activation) while nerves which innervated predominantly fast muscles were only occasionally activated in short bursts of high-frequency activity (phasic activation) [23]. It was thus hypothesized that muscle fibers responded to the nervous pattern of electrical activity and not to a neurotrophic factor. This hypothesis was later supported by novel experiments in which the denervated soleus muscle was transformed into a fast muscle or maintained as a slow muscle, depending on the pattern of electrical activity directly imposed on the muscle by using implanted electrodes [16]. However, it currently appears that this clear-cut result was peculiar to the denervated soleus and not generally applicable to innervated skeletal muscle. Current experiments, using a variety of models, are focused on differentiating between the activity pattern and total amount of activity in determining muscle properties.

**Cellular Regulation of Transformation**

The elegantly coordinated transformation sequence suggests that chronic stimulation induces a shift in the protein synthesis and degradation machinery within the muscle cell. Transformation occurs at slightly different rates along the length of individual fibers, suggesting that the nuclei of transforming fibers no longer act in a concerted manner [24]. However, alterations in the amount and type of proteins present in the cell can result from numerous different mechanisms, including alterations in DNA replication, transcription rates, translation rates, and degradation rates. Which of these several mechanisms operate within the transforming muscle cell? Do changes in different proteins occur by the same mechanism?

In an effort to address these questions, Williams et al. [25] measured the concentration of messenger RNA (mRNA) coding for a glycolytic enzyme (alcoholase) and mRNA coding for an enzyme involved in oxidative phosphorylation (cytochrome b) in muscles which had been chronically stimulated at 10 Hz for 5 or 21 days. They documented an asynchronous change in mRNA levels coding for the two proteins. After 21 days, alcoholase mRNA fell to one-fourth of control levels and cytochrome b mRNA increased five-fold, paralleling the observed decrease and increase of glycolytic and oxidative enzymes, respectively. However, after only 5 days of stimulation, alcoholase mRNA concentration had decreased significantly, but cytochrome b mRNA concentration remained unchanged. These data suggested that chronic stimulation resulted in reciprocal changes in the expression of the alcoholase and cytochrome b genes at the level of transcription. However, because of the different time courses, the transcriptional changes may have occurred by different regulatory mechanisms. Pette has reported a similar change for mRNA coding for the oxidative enzyme citrate synthase [26].

**FUTURE DIRECTIONS**

While the process of fiber type transformation has been well documented in a number of experimental models, active investigations continue in order to address such questions as: Exactly which factor does the muscle cell transduce when it transforms (i.e., electrical activity, tension, etc.)? How does the nerve influence muscle fiber properties during differentiation and transformation? How are transcriptional rates altered secondary to stimulation? How does the cell coordinate the numerous events that occur sequentially? Why do the nuclei along the length of a cell transform asynchronously? What is the signal which is transduced by the cell, causing it to initiate the transformation process? In which cell structure(s) does such transduction take place? Precisely what is muscle “activity”? Is it related to tension, electrical current, and transmembrane potentials? It is believed that answers to these questions may be applicable to other models of muscle adaptation, including exercise.
immobilization, and overload. In addition, future research may provide insights into optimal methods for eliciting muscle functional recovery following surgery, trauma, or disease.

**KEY CONTRIBUTORS**

The list below represents individuals who are involved primarily in basic science studies of neuromuscular adaptation.

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**GLOSSARY**

ATP: Adenosine triphosphate. The immediate energy source for muscle contraction. May be obtained via aerobic or anaerobic metabolism.

FES: Functional electrical stimulation. External stimulation of skeletal muscles for strengthening or increasing muscle endurance.

FOG: A muscle fiber that is fast contracting, with high oxidative and high glycolytic capacity.

FG: A muscle fiber that is fast contracting, with low oxidative and high glycolytic capacity.

SO: A muscle fiber that is slow contracting, with high oxidative and low glycolytic capacity.

SR: Sarcoplasmic reticulum. The membranous network within skeletal muscle that releases and sequesters calcium ions, causing contraction and relaxation, respectively.

T-system: Transverse tubular system. The membranous network within skeletal muscle that conducts the action potential from the surface membrane deep into the fiber.

Z-disk: The boundary of the sarcomere.

myosin: The major protein of the thick filament. Contains enzymes that cyclically interact with actin during active shortening.

sarcomere: The functional unit of force generation in muscle.

**Acknowledgments** The author wishes to thank medical illustrator Kurt Smolen for his brilliant visual representation of muscle fiber transformation, given these sketchy details. Support for research performed by the author is gratefully acknowledged from the Veterans Administration and National Institutes of Health grant AR35102.

**REFERENCES**


RESEARCH FRONT 86–3479

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