

# EFFECT OF NERVE STIMULATION ON RAT SKELETAL MUSCLE. A STUDY OF PLASMA MEMBRANE

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## SUMMARY

Acute effects of electrical stimulation of the sciatic nerve are mediated via a plasma membrane change, are dependent on the strength as well as the duration of the current, and are reversible. These changes may be of significance in explaining the observed beneficial effects of chronic electrical stimulation.

## STATE OF THE ART

The chronic effects of direct electrical stimulation on denervated rat skeletal muscle have been studied extensively /1-2/. However, there are no systematic studies of the immediate effects of electrical stimulation of nerve on normal skeletal muscle, especially with reference to plasma membrane changes. This investigation reports on the acute effects of electrical stimulation of sciatic nerve on the morphology, histochemistry, histometry, and ultrastructure of rat gastrocnemius muscle. The state of the muscle plasma membrane was studied by lectin binding techniques.

## MATERIALS AND METHODS

Sprague-Dawley rats weighing up to 250 grams were used in this study. In all the experiments, rats were anesthetized with Nembutal and the sciatic nerve of the right leg was exposed and stimulated by a monopolar microelectrode (150  $\mu$ s, 10 Hz). The indifferent electrode was placed on the dorsum of the right foot. Six groups of rats with five rats in each group were used for six different time-current studies /1/. Groups I and II received a 5-mA current for 30 and 60 minutes respectively while groups III and IV received a 10-mA current for a period of 30 and 60 minutes. In addition, dosages of 10 mA for 200 minutes (group V) and 15 mA for 5 minutes (group VI) were selected for the last two groups to determine the effects of longer time and higher current strength respectively. Gastrocnemius muscle was removed at the end of the stimulation period in all groups. Muscle biopsies from another similar set of six groups were taken following a 60 minute rest period at the end of stimulation to investigate the reversibility of changes. The sciatic nerve of the left leg in all the experiments was not stimulated and served as a control. The sciatic nerve from the stimulated as well as the nonstimulated leg was removed in all cases and observed for morphological changes at light and electron microscopic (EM) levels. The experimental and the control muscle was immediately frozen and processed for routine histology and histochemistry /3/. Histometric studies were carried out with respect to mean fiber diameter and percentage of two fiber types using an MOP-3 Image Analyzer System (Carl Zeiss, Inc.). A part of the fresh sample was fixed in glutaraldehyde and processed for routine EM studies /4/.

Sciatic nerve was processed for electron microscopy in a similar manner. Part of the muscle tissue was processed for ultrastructural study of plasma membrane state using peroxidase loading /5/, peroxidase labeled lectin (Concanavalin A) binding /6/, and the ferritin-conjugated Concanavalin A (Con A) technique /7/.

## RESULTS

In all six groups, the muscle morphology was preserved. Since the normal rat skeletal muscle does show ragged red fibers, the percentage of these fibers was determined in the control as well as the experimental side of all the groups (Table 1). As seen in Table 1, this increase was 3.1 and 2.56 times of control in groups IV and V respectively while the remaining groups showed an increase of 1.06 to 1.55 times of control. This increase did not persist following the rest period.

Table 1. Effects of various dosages on number of ragged red fibers.

Muscle	Group I	Group II	Group III	Group IV	Group V	Group VI
Control	23.20%	23.30%	29.80%	15.40%	20.80%	20.30%
Experimental	32.60%x	36.20%	43.10%	47.80%	53.30%	21.60%
X increase - Experimental group	1.40x	1.55x	1.44x	3.10x	2.56	1.06

Histometry. Percentage of fibers. The high percentage of type II fibers in the gastrocnemius reflected its composition. The fiber type ratio (Type I/Type II) of the experimental side (range 0.09 to 0.44) was not significantly different from that of the control side (range 0.17 to 0.36) in all the groups. In all six groups the mean fiber diameter of type I and type II fibers in the stimulated muscle was not significantly different from the control (nonstimulated) muscle and ranged from 25 to 40  $\mu\text{m}$ . In groups I, II, III, and VI, the muscle fibers showed no ultrastructural alterations. The group IV and V rats receiving the current of 10 mA for 60 and 200 minutes respectively showed mitochondrial and structural changes. The muscle of these groups showed large aggregates of mitochondria in the subsarcolemma. The mitochondria were swollen and occurred in a variety of shapes. Mitochondrial cristae were convoluted and partially destroyed. Focal disruption of myofibrillary architecture was seen in some fibers. In many areas, myofibrils were rarefied and sarcotubules dilated. The sciatic nerve of the stimulated side showed no morphological changes when compared to the control in all six groups. In order to guard against drawing incorrect conclusions, the state of the plasma membrane was carefully checked in the serial sections of all the blocks in six groups, and we made sure that all cytochemical methods /5-7/ gave consistent results in each group. In groups I, II, III and VI, the plasma membrane was intact and showed no abnormality. The membrane integrity was first checked using phase contrast microscopy with the peroxidase loading /5/ and Con A-peroxidase /6/ techniques. Epon sections, 1  $\mu\text{m}$  thick, showed uniform density around muscle fibers and no penetration of peroxidase by the Con A-peroxidase method. At the ultrastructural level, the plasma membrane showed a dense reaction all along the cell surface with the peroxidase loading and Con A-peroxidase binding. Ferritin-Con A labeling was seen as Con A binding external to the plasma membrane and ferritin granules distributed along the basement membrane. However, groups IV and V showed plasma membrane abnormalities. With peroxidase loading and Con A-peroxidase techniques, the population of fibers showed focal alterations on the cell surface in phase contrast microscopy.

The focal lesions in these fibers appeared as wedge-shaped, and the sarcomeres were highly contracted. At the ultra-structural level, the breaks in the membrane were evident by the absence of the reaction product in focal areas along the membrane. Ferritin-Con A labeling also showed an absence of Con A binding and ferritin granules where the membrane was not intact.

The subcellular abnormalities were marked in the areas where the membrane was indistinct and showed focal breaks. Myofibrillary dissolution, aggregation of glycogen and abnormal mitochondria were evident in this region. Following the 60 minute rest period, muscle in all groups showed no pathological changes. Biopsies from groups IV and V were particularly investigated to detect mitochondrial and plasma membrane alterations showed no lesions.

## DISCUSSION

The direct electrical stimulation of muscle has shown beneficial effects in retarding atrophy in the denervated rat muscle /1-2/. This technique has also been tried clinically as a therapeutic measure to reduce spasticity, develop muscle force in paraplegics /8/ and affect ambulation in spinal cord injury patients /3/. In the present study, we observed the acute effects of sciatic nerve stimulation using various current strengths and duration on the skeletal muscle. The results of histometric measurements on fiber size and ratio showed that the experimental and control values were not significantly different in all the groups. This indicated that unlike denervated rat muscle, the normal rat muscle was not effected in terms of the muscle fiber size and percentage distribution of fiber types by the strength and duration of the current applied to the sciatic nerve. The nerve stimulation with the current strengths of 5 mA for 30 and 60 minutes, 10 mA for 30 minutes, and 15 mA for 5 minutes (groups I, II, III and VI) did not exert any morphological changes or influence the state of plasma membrane. The failure of a 15 mA current employed for a short duration (5 minutes) to cause any morphologic change, showed that in addition to the current strength the duration of nerve stimulation was also an important factor in causing muscle abnormalities. The increase in the number of ragged red fibers and mitochondrial abnormalities with a 10 mA current applied for 60 and 200 minutes (groups IV and V) was noteworthy. Walter et al /9/ also showed similar mitochondrial changes after 60 minutes of stimulation with 5V and a frequency of 10 Hz. As in Walter's experiment /9/, the mitochondrial abnormalities in our study were not permanent and were absent after a 60 minute rest period. Green and Harris /10/ have attributed such mitochondrial changes to a variety of energized states. The muscle fibers in these groups did not show any inflammatory response or muscle necrosis. The mitochondrial changes, therefore, are more likely to represent an adaptation to an altered energy state. The muscle in groups IV and V also showed plasma membrane alterations following the nerve stimulation. They were characterized by the penetration of peroxidase into the affected fibers, observed under phase contrast microscopy, and focal breaks in the plasma membrane at the ultrastructural level. Similar plasma membrane defects have been shown in dystrophic muscle using these techniques. Based in our observations in groups IV and V, we suggest that the electrical stimulation of the nerve in these groups was of "supramaximal" strength and resulted in the muscle contraction and subsequent mitochondrial and plasma membrane changes. These changes were, however, reversible as seen by their absence following a 60 minute rest period. The reversible nature of the alterations suggests that this is a physiological rather than a pathological response.

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