occurred. There was a significant difference between the peak decrease in HR in the two animal groups. In contrast, i.v. injection of L-enk in monkeys resulted in a significant rise in HR so that 60 s after the injection the change in HR was 10 ± 3 beats/min. There was a significant difference between the time course of changes in HR in monkeys and in the other two animal groups as illustrated in the lower panel of the figure. Naloxone prevented cardiovascular responses to L-enk in each animal group as shown in table 3.

Discussion. The results of this study showed that L-enk produced depressor effects in anesthetized rabbits, dogs and monkeys; however, the depressor response to L-enk in rabbits was significantly shorter in total time than that in monkeys and dogs. Additionally, in monkeys, L-enk caused a significant rise in HR in contrast to a significant fall in HR in rabbits and in dogs. These results suggest that the species of animal may be important in determining the cardiovascular action of such opioid peptides as L-enk. Inhibition of cardiovascular responses to L-enk by naloxone, as shown in the present study, indicates that the response is evoked by opioid receptors. Recently, it was reported that enkephalins and enkephalin analogs causes inhibition of the reflexly elicited decrease in heart rate evoked by pressor agents in conscious cats[14]. Therefore, the inconsistencies in the reported cardiovascular effects of the peptides may have resulted from different sensitivities of the arterial baroreceptor reflexes in different animal species used. The present study demonstrated that the pressor response to BCO was significantly greater in monkeys than in rabbits and dogs. It has been indicated also that there are species differences in the passive mechanical properties of carotid artery[3], and of carotid sinus baroreceptor sensitivity[17]. The results of the present study may explain why HR responses to L-enk in monkeys differ from those in rabbits and dogs.

Various enkephalins and enkephalin analogs caused a brief reduction in the systemic blood pressure and heart rate in anesthetized rats[1,11,18], cats[9,15,16] and dogs[7,13]. It has been reported that L-enk (35 μg/kg, i.v.) produces decreases in heart rate and systemic blood pressure in the pentobarbital anesthetized dog; however, it has been reported that in conscious animals these reductions of heart rate and systemic blood pressure are reversed and increase[12–14,19]. Therefore, the effects of anesthesia would appear to account for many of the inconsistencies in the reported action of the opioid peptides. It has since been demonstrated that administration of enkephalin analogs into the central nervous system caused increases in systemic blood pressure and heart rate in anesthetized rats[2,4,11]. These investigations suggest that the use of different routes for the administration of opioid peptides may also have been a cause of the inconsistensies in the reported cardiovascular effects of the opioid peptides, in addition to anesthetic agents and species differences.

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*Present address: Kagawa Medical School, Department of Physiology, Miki, Kita, Kagawa 761-07 (Japan).

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Effect of nerve stimulation on rat skeletal muscle. A study of plasma membrane

A. Shah, F. Nagao, V. Sahgal and H. Singh

Neuromuscular Studies Unit, Rehabilitation Institute of Chicago, 345 E. Superior Street, Chicago (Illinois 60611, USA), 26 October 1984

Summary. The gastrocnemius muscle of the rat showed no morphological, histometric or plasma membrane changes, after sciatic nerve stimulation with a 5 mA current for 30 to 60 min, 10 mA for 30 min and 15 mA for 5 min. However, 10 mA for 60 and 200 min gave rise to mitochondrial and plasma membrane abnormalities. These changes were absent after a rest period. The results indicated that the sciatic nerve stimulation at 10 mA for 60 and 200 min caused reversible changes in the rat skeletal muscle mitochondria and plasma membrane.

Key words. Electrical stimulation; membrane; morphometry, muscle, ultrastructure.

There have been a number of reports on the chronic effects of direct electrical stimulation on denervated rat skeletal muscle[1–10]. 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. In the present investigation, we report on the immediate (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 g were used in this study. Electrical stimulation. 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. 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. Stimulus duration of 150 μs and frequency of 10 Hz was maintained in all six groups.

Groups I and II received a 5-mA current for 30 and 60 min respectively while groups III and IV received a 10-mA current for a period of 30 and 60 min respectively. In addition, dosages of 10 mA for 200 min (group V) and 15 mA for 5 min (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-min 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 also removed in all cases and observed for morphological changes at light and electron microscopic (EM) levels.

Methods. In both the experimental and the control muscle, part of the fresh specimen was immediately frozen and processed for routine histology and histochemistry[11]. 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[12]. 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[13], peroxidase labeled lectin (Concanavalin A) binding[14], and the ferritin-conjugated Concanavalin A (Con A) technique[15].

Results. Light microscopy. In all six groups, there was no significant variation in fiber size, increase in centralized nuclei or muscle necrosis in the stimulated muscle as compared to the control muscle. Since the normal rat skeletal muscle does show ragged red fibers, the percentages of these fibers were determined in the control as well as the experimental side of all the groups (table). Due to the variation in the percentage increase of ragged red fibers in both the control and the experimental group, the extent of increase in relation to the corresponding control group was determined. As seen in the table, this increase was 3.1 and 2.56 times of control in groups IV and V respectively while the remaining groups showed this increase from 1.06 to 1.55 times of control. This increase did not persist following the rest period.

Histometry. Percentage of fibers. The high percentage of type II and low percentage of type I fibers in the control groups reflected the gastrocnemius muscle composition. The experimental muscle in all six groups did not show any variation in the composition of two fiber types as compared to the control. Consequently 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).

Mean fiber diameter. 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 μm. The exception was type II fibers in group VI (stimulated with 15 mA for 5 min) which showed 62.0 μm mean diameter on the experimental side.

Electron microscopy. In groups I, II, III and VI which received 5 mA for 30 and 60 min, 10 mA for 30 min and 15 mA for 5 min respectively, the muscle fibers showed no ultrastructural alterations. The mitochondria were not increased in size and showed no distortion in structure. The myofilaments were intact, with a normal myofibrillary pattern.

The group IV and V rats receiving the current of 10 mA for 60 and 200 min respectively showed mitochondrial and structural changes. The muscle of these groups showed large aggregates of mitochondria and occasional glycogen deposits 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 6 groups.

Ultrastructural cytochemistry (plasma membrane state). 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 6 groups, and we made sure that all cytochemical methods[13–15] 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 in phase contrast microscopy when the peroxidase loading[13] and Con A-peroxidase[14] techniques were used. 1 μm thick

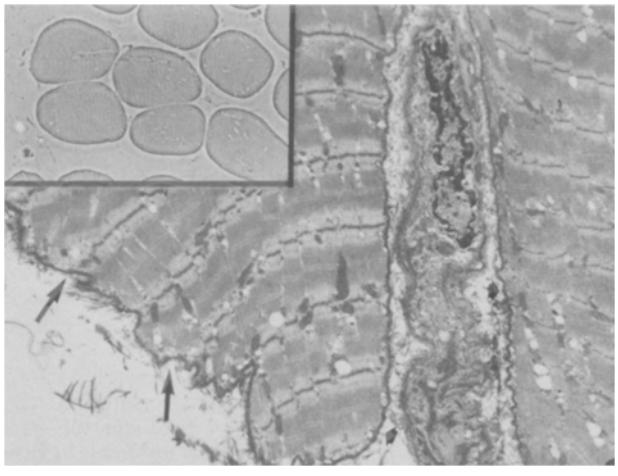

Figure 1. Muscle fibers from group III show dense staining all along the membrane (arrows) with Con A-peroxidase technique (× 7350). Inset: Semithin sections of the same block show fibers with intense staining around the membrane in phase contrast microscopy (× 270).

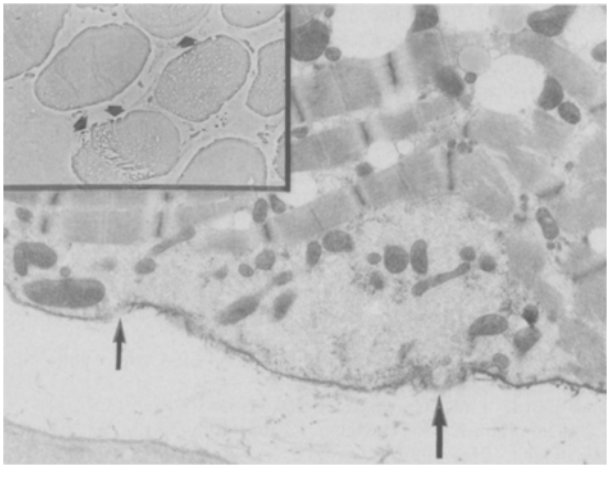

Figure 2. The muscle fiber from group IV shows breaks in the plasma membrane (arrows) with Con A-peroxidase technique. Note the abnormal mitochondria,glycogen deposits, myofibrillary dissolution under the membrane (× 12,600). Inset: Phase contrast microscopy of the same block shows focal areas of myofibrillary rarefaction and peroxidase penetration (arrows) (× 270).

Effects of various dosages on the 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%	36.20%	43.10%	47.80%	53.30%	21.60%
X increase in experimental group	1.40	1.55	1.44	3.10	2.56	1.06

Epon sections showed uniform density around muscle fibers and no penetration of peroxidase (as seen in fig. 1 inset) 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 (fig. 1). 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 (fig. 2 inset) techniques, the population of fibers showed focal alterations on the cell surface in phase contrast microscopy. The focal lesions in these fibers appeared as areas of rarefaction, as wedge-shaped, and the sarcomeres were highly contracted. In ultrastructural investigations, the breaks in the membrane were evident by the absence of dense reaction in focal areas along the membrane by peroxidase loading and Con A-peroxidase (fig. 2) labeling. 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 (fig. 2).

Following the 60-min 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.

Discussion. The direct electrical stimulation of muscle has shown beneficial effects in retarding atrophy in the denervated rat muscle[1,2,4,7,8,10]. This technique has also been tried clinically as a therapeutic measure to reduce spasticity, develop muscle force in paraplegics[16] 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 min, 10 mA for 30 min and 15 mA for 5 min (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 min) 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 min (groups IV and V) was noteworthy. Walter et al.[17] also showed similar mitochondrial changes after 60 min of stimulation with 5 V and a frequency of 10 Hz. As in Walter's experiment[17], the mitochondrial abnormalities in our study were not permanent and were absent after a 60-min rest period. Green and Harris[18] 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[13–15].

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-min rest period. The reversible nature of the alterations suggests that this is a physiological rather than a pathological response.

We conclude from these findings that 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.

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