Sarcoplasmic Reticulum Function of Rat Diaphragm Following Five-day Inactivity.

Hirofumi MIYATA[1] and Masanobu WADA[2]

[1] *Dept. of Biological Sciences, Faculty of Agriculture, Yamaguchi University, Japan*
[2] *Dept. of Exercise physiology, Faculty of Integrated Arts and Science, Hiroshima University, Japan*

Abstract

MIYATA, H. and WADA, M., Sarcoplasmic Reticulum Function of Rat Diaphragm Following Five-day Inactivity. Adv. Exerc. Sports Physiol., Vol.7, No.1 pp.27-32, 2001. We hypothesize that the adaptation of sarcoplasmic reticulum (SR) function in muscle fiber to inactivity is affected not only by muscle activity itself but also by some trophic factors. We compared two hemidiaphragm models in rats; 1) Spinal cord hemisection (SPH) at cervical second level; 2) Denervation (DNV) in phrenic nerve. After 5 days, contraction time (CT) and half relaxation time (1/2RT), and SR Ca^{2+}-ATPase activity were measured. There were no significant changes in CT and 1/2RT in the SPH, but significant alterations in the DNV. As compared to the control (CTL) group, SR Ca^{2+}-ATPase activity decreased in the DNV but was unchanged in the SPH group. No differences in the population and cross-sectional area of each muscle fiber type were found among the SPH, DNV and CTL groups. Our results suggest that the rapid adaptations of SR function to inactivity were not attributable to the changes in muscle fiber types, and that some neurotrophic factors from phrenic motoneurons influence the adaptation.

Key words: Rat, Diaphragm, Inactivity, Sarcoplasmic reticulum, Trophic factor

Introduction

The effects of inactivity have been studied primarily in inactivity models of hindlimb muscle and marked changes in physiological (13, 22), histochemical (21, 28) and biochemical (27) properties were reported. In some studies, a transection of the spinal cord at the thoracic level was used to induce inactivity of hindlimb muscle (2, 26). The model of muscle inactivity differs from the denervation model in that the axoplasmic flow of the neurotrophic substance anterogradely carried by axons from motoneurons and the myotrophic substance produced in muscle is preserved. Therefore, the neurotrophic influence on muscle can be assessed by the degree of changes in muscle induced by denervation and spinal cord transection. Based on this concept, a number of studies demonstrated that some trophic factors from motoneurons or muscle have a significant effect on the muscle structural (3) and contractile (33) properties. Recently, Takekura et al. (31) suggested that morphological adaptation of the membrane system including sarcoplasmic reticulum (SR), transverse tubules and triads was affected by trophic factors, and that muscle activity was not always necessary for a new membrane system to form in disused rat hindlimb muscles. To date, however, relatively little attention has been paid to the trophic influence on SR function in muscle fibers.

In this kind of study, it is important to assess the prior activation history of the experimental muscle in order to know the impact of activity imposed by each treatment. However, the activation history of hindlimb muscle is quite varied and difficult to quantify. In contrast, the diaphragm muscle is continuously and stereotypically activated throughout life to maintain ventilation. Furthermore the activity in diaphragm is much higher than that in hindlimb muscle. For example, the duty cycle of the rat diaphragm is about 40 % (24), while in rat hindlimb muscles, duty cycles range from as low as 2% (extensor digitorum longus muscle) to 14% (soleus muscle) (19). Therefore, the diaphragm may be especially responsive to decreases in activity.

We hypothesize that the SR function in diaphragm muscle fiber is regulated not only by muscle activity itself but also by the neurotrophic factor from phrenic motoneurons. In this study, to verify our hypothesis, we examined the functional adaptation of rat diaphragm to inactivity in two models of hemidiaphragm inactivity: spinal cord hemisection (SPH) at the cervical second (C2) level and denervation (DNV) in phrenic nerve.

Methods

General procedures

Male Wistar rats (body weight on the final day of the experiments 250-310g, Kyudo, Kumamoto) were divided into SPH (n=5), DNV (n=5) and sham control (CTL, n=5) groups. All animals were anesthetized with ketamine (60 mg/kg) and xylazin (2.5 mg/kg) and prepared for aseptic surgery. Throughout the surgery, rectal temperature and electrocardiogram were monitored in some rats. The core temperature and heart rate remained stable, and were unaffected by surgery. After ensuring that there was no bleeding in the operating field, the layers of muscles and skin were sutured separately. The wound was cleaned and disinfected with betadine and the animals received an injection of antibiotic which was repeated for 2 days following. All procedures used in this study were approved by the Animal Use Committee at Yamaguchi University which followed

Correspondence: Hirofumi Miyata, Ph. D.
Dept. of Biological Science, Faculty of Agriculture, Yamaguchi University Yoshida 1677-1, Yamaguchi 753-8515, Japan
Phone: (+81) 83-933-5866, Fax: (+81) 83-933-5866
E-mail: hiro@po.cc.yamaguchi-u.ac.jp

the Japanese Physiological Society Animal Care Guidelines and the Policy Statement of the American College of Sports Medicine on Research with experimental Animals.

Inactivity models of the diaphragm

SPH and CTL: The rat was placed in the prone position on the operating table. A midline incision (less than 4cm) was made on the back of neck, and superficial and paravertebral muscles were separated by blunt dissection. Under a surgical microscope, a C2 dorsal laminectomy was performed and then the cervical spinal cord was exposed by cutting and retracting the dura. The right spinal cord was sectioned using a micro-knife near the entrance of the C2 dorsal root. Precautions were taken to avoid cutting blood vessels of the spinal cord in this region. The entire lateral and ventral funiculus of the right spinal cord was transected without section of the dorsal funiculus. In CTL group, the same surgery without section of the spinal cord was performed.

DNV: The rat was placed in the supine position on the operating table. A midline incision (less than 3cm) was made on the neck and superficial muscles. Under a surgical microscope, the main trunk of the right phrenic nerve was dissected. A 10 to 20 mm length of the nerve was removed to avoid possible reinnervation of the muscle and effects of trophic substances in the nerve.

The efficacy of surgery was evaluated by monitoring EMG in the right hemidiaphragm under anesthesia during surgery and in the terminal experiments. The EMG was recorded by a pair of fine-wire electrodes (stranded stainless steel wires of 0.05 mm diameter: A-M systems Inc., WA USA) implanted into the mid-costal regions of the right hemidiaphragm. These electric signals were amplified, and viewed on a storage oscilloscope (DCS-7020, Kenwood, Yokohama).

In vitro measurements of physiological properties

A part of the excised diaphragm was mounted vertically in a glass chamber containing Ringer solution (137 mM Na^+, 5 mM K^+, 2 mM Ca^{2+}, 2 mM Mg^{2+}, 121 mM Cl^-, 20 mM HCO_3^-, 1.9 mM HPO_4^{2-}, 11mM Glucose and 0.012 mM d-tubocurarine). The solution was aerated with 95% O_2-5% CO_2, which maintained pH at 7.4 and the temperature was kept at 25℃. The central tendon of the muscle was attached in series to a calibrated force transducer. The other muscle clamp was fixed to a micromanipulator. Muscle fiber length was adjusted using the micromanipulator until maximal isometric twitch responses were obtained (i.e., optimal fiber length). The muscle was stimulated directly with a stainless electrode placed in contact with the entire width of the muscle on one side. The anodal current pulses were referred to a large Ag-Cl-coated plate electrode (1×1.5 cm) placed 5 mm from the muscle. Stimulus intensity was increased until maximal twitch tension responses were obtained and then set at 125 % of this value (i.e., supramaximal intensity). Contraction time (CT) and half relaxation time (1/2RT) were determined from a contraction induced by single pulse stimuli. The evoked isometric force responses were displayed on the digital storage oscilloscope.

SR Ca^{2+}-ATPase activity

Another part of the mid-costal diaphragm was used for the biochemical assessment of SR Ca^{2+}-ATPase activity. Muscle pieces of ~ 100 mg wet weight were washed in ice-cold homogenizing buffer (pH 7.4) consisting of 40 mM Tris-HCl and 300 mM sucrose until the buffer appeared free of blood. Samples were diluted 1:10 (mass/vol.) in cold homogenizing buffer after having been weighed and homogenized for 3×30 s at 1000 rpm. SR Ca^{2+}-ATPase activity in the muscle homogenate was measured spectrophotometrically according to the methods of Simonides and van Hardeveld (29) with some modifications where the concentration of NADH in the assay mixture was increased as follows to avoid depletion of this reagent during measurement. The assay mixture (pH 7.5) was composed of 20 mM N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 0.005% (vol./vol.) Triton X-100, 1 mM EGTA, 200 mM KCl, 15 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM sodium azide, 0.4 mM NADH, 10 mM phosphoenolpyruvate, 18 U/ml pyruvate kinase and 18 U/ml lactate dehydrogenase. The free Ca^{2+} concentration in the assay mixture is estimated to be 10 μM, the value necessary to measure maximal activity (29). After the addition of a 20 μl aliquot of the homogenate, the assay mixture was preincubated for 2 min. The reaction was started by adding ATP to give a final concentration of 4 mM. The absorbance signal was recorded until the slope was constant, usually within 3 min. Finally the $CaCl_2$ concentration was increased to 20 mM in order to selectively inhibit SR Ca^{2+}-ATPase activity. The remaining activity was defined as the background ATPase activity. The activity of SR Ca^{2+}-ATPase was calculated as the difference between total ATPase and the background ATPase activities. During this procedure, the assay mixture was continually stirred and temperature was maintained at 37 ℃.

Muscle Fiber Histochemistry

Another part of the mid-costal diaphragm was frozen at estimated optimal length (Lo). This was accomplished by measuring the relaxed excised length of a muscle bundle directly and pinning the muscle on cork at 1.5X this length (estimated Lo) before immersion in isopentane cooled to its melting point by liquid nitrogen. Serial cross sections of muscle fibers were histochemically stained for myofibrillar ATPase after preincubation at different pHs (4.3, 4.6 and 10.3) to classify fiber types. Populations and cross sectional areas (CSA) of each muscle fiber type were

determined based on measurements of 300 to 600 fibers in each animal. The relative contribution of each muscle fiber type to total CSA was calculated from the proportions and CSAs of each muscle fiber type.

Statistical analysis

The results obtained in this study were analyzed by a one-way ANOVA for all data with experimental groups as grouping variables. Post hoc analysis was performed using a Scheffe's F method. In all cases, statistical significance was established at $p<0.05$. All values are shown as means ±standard deviation (SD).

Results

Verification for inactivity models

Although the body weights in the SPH group decreased by approximately 10-15% initially after surgery, the body weights were returning to normal levels by 3 days after the surgery. On the final day of the experiment, the animal body weights in SPH (273±21g) and DNV (285±15g) were comparable to those in the CTL group (282±18g). The DNV rats showed no obvious behavioral changes after surgery, except for the paradoxical movements of the rib cage and abdomen associated with hemidiaphragm paralysis. By the third day after surgery, the SPH rats displayed normal ambulation, with only minor defections in fine motor control, e.g., during grooming behaviors. Hemidiaphragm paralysis in SPH rats was also associated with paradoxical movements of the rib cage and abdomen during inspiration. The spontaneous activity disappeared immediately after surgery (figure 1) and the end of experiments. From these results, we judged that the activity of the right hemidiaphragm muscle disappeared during 5 days.

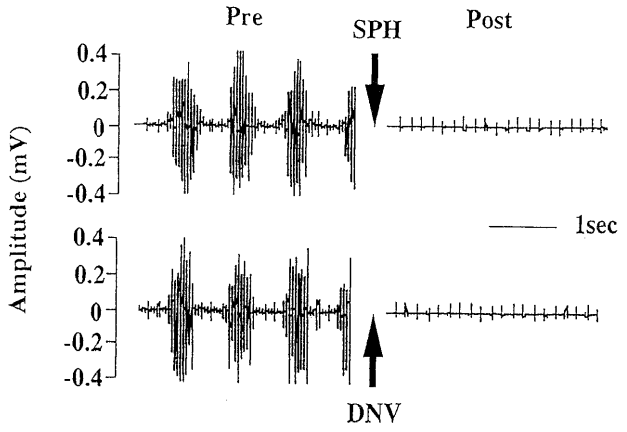

Fig. 1 Electrical activity taken from the right diaphragm muscle of rats subjected to a right spinal hemisection (SPH) at cervical level 2, and a denervation (DNV) of right phrenic nerve, respectively. Left and right recordings were made before and after surgery, respectively. In both cases, the spontaneous activity disappeared completely immediately and 5 days after surgery (end of experiments).

Muscle contractile properties

The mean values of CT and 1/2RT in each group are shown in Figure 2a and b, respectively. The CT and 1/2RT in DNV were significantly longer than those in CTL and SPH groups. No significant difference was found between CTL and SPH groups.

SR Ca^{2+}-ATPase activity

The mean values of SR Ca^{2+}-ATPase activity in each group are shown in Figure 3. The SR Ca^{2+}-ATPase activity in DNV was significantly lower than those in CTL and

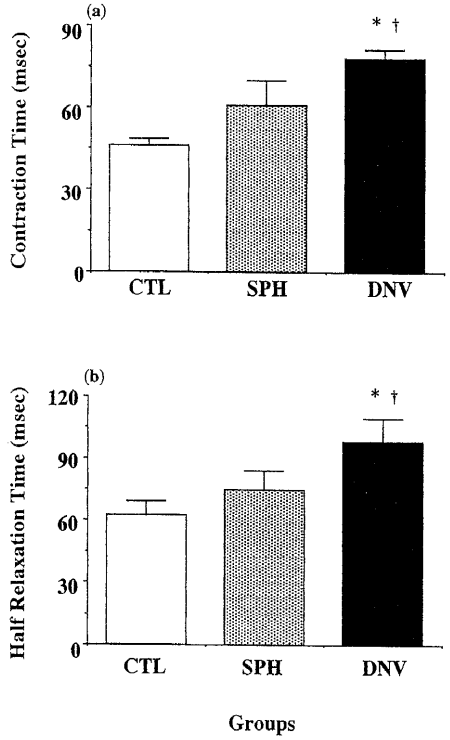

Fig. 2 Changes in contraction time (a) and half relaxation time (b) of the diaphragm muscle after 5-day inactivity induced by DNV and SPH.
*Significant difference ($P<0.05$) from the control (CTL) group;
† significant difference ($P<0.05$) from SPH group.

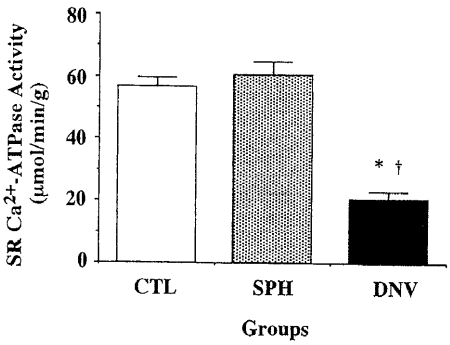

Fig. 3 Changes in SR Ca^{2+}-ATPase activity of the diaphragm muscle after 5 days inactivity induced by DNV and SPH.
*Significant difference ($P<0.05$) from the CTL group;
† significant difference ($P<0.05$) from SPH group.

SPH groups. While, there was no significant difference in SR Ca^{2+}+-ATPase activity between CTL and SPH groups.

Muscle fiber type population and cross-sectional area

The mean values of population, CSA, and relative contribution to total CSA of each muscle fiber type are shown in Table 1. No significant difference in the population, CSA or relative contribution to total CSA of each muscle fiber type was found among three groups.

Discussion

In this study, we compared two models of hemidiaphragm inactivity for 5 days: 1) SPH at C2, where communication between motoneurons and muscle remained intact: 2) DNV in phrenic nerve, where communication between motoneurons and muscle fibers was disrupted. Therefore, the neurotrophic effect on muscle can be assessed by comparison between SPH and DNV groups.

Although most mammalian muscles begin to atrophy after loosing their innervation and/or neurotrophic influence, the denervated hemidiaphragm of the rat undergoes selective hypertrophy of type I muscle fiber and atrophy of type IIb muscle fiber (20, 24, 32). This unique adaptation of denervated hemidaiaphragm has been attributed to repetitive stretch resulting from rhythmic contractions of the intact, contralateral hemidiaphragm. From these pilot studies (20, 24, 32) and our preliminary study, we judged that 5 days after denervation is an optimal period to examine rapid functional changes in the SR without marked selective changes in the population and cross sectional area of each muscle fiber type. Our results indicated that the 1/2RT and SR Ca^{2+}-ATPase activity were changed in DNV, but not in SPH as compared to those in CTL group, and that these changes were not attributable to the changes in the population or CSA of each muscle fiber type.

In denervated hindlimb muscles, typical changes are atrophy of muscle and prolongation of isometric twitch. The atrophy of the denervated muscle seems to occur within a week. For example, Gundersen (16) demonstrated that reduction in muscle mass was 12% in soleus and 18% in extensor digitorum longs (EDL) following 5-day denervation. The prolongation of the isometric twitch, that is, elongation of CT and 1/2RT, seems to occur within a few days (13, 16). The contractile process is regulated by the cyclic release of Ca^{2+} into the cytosol and its subsequent resequestration by SR (30). It is well known that ryanodine receptor/channel mediates the release of Ca^{2+} from the SR in both skeletal and cardiac muscle cells (25). In rat DNV muscles, Delbono and Chu (11) demonstrated that maximum activation of the ryanodine receptor/channel was shifted to higher Ca^{2+} concentrations (pCa 4) and the channel remained activated at mM Ca^{2+} concentrations. The alteration in response of the ryanodine receptor/channel for Ca^{2+} concentration might account for elongation of CT in the DNV muscle in our study. A decrease in SR Ca^{2+}-ATPase activity found in DNV reflects the dysfunction of the SR Ca^{2+} pump since a number of previous studies have indicated that SR Ca^{2+}-ATPase activity is positively correlated with the rate of Ca^{2+} uptake by SR (5, 6, 23). The elongation of 1/2RT in DNV was consistent with the depression in the catalytic activity of SR ATPase protein. In previous study (12, 17), it was demonstrated that the changes in SR functions and morphology were related to changes in the morphological properties of muscle fiber. The volume of SR in fast muscle fiber is much larger than that in slow twitch muscle fiber (8, 9). In this study, in spite of complete paralysis in the right diaphragm for 5 days, there were no significant difference in the proportion and CSA of each fiber type between eperimental groups and CTL. Although we can not specify a main reason from our results, alterations in quantity and/or quality of SR protein may be reasons for a marked decrease in SR Ca^{2+}-ATPase activity in DNV group. We speculate that the alteration in quality of SR protein is a major factor, as well as the altera-

Table 1 Mean values of population, cross sectional area (CSA) and relative contribution of each muscle fiber type to total area in control (CTL), denervation (DNV) and spinal hemisection (SPH) groups.

Groups	N	Muscle Fiber Type		
		I	IIa	IIb
		Population (%)		
CTL	5	25.2 ± 3.2	35.6 ± 2.6	39.2 ± 3.8
SPH	5	27.1 ± 1.4	33.4 ± 3.6	39.5 ± 2.8
DNV	5	26.2 ± 3.2	32.6 ± 3.3	41.2 ± 4.3
		Cross-sectional Area (μm^2)		
CTL	5	1183 ± 189	960 ± 112	1800 ± 225
SPH	5	1061 ± 96	1024 ± 180	1875 ± 216
DNV	5	1323 ± 141	1172 ± 155	1584 ± 240
		Relative Contribution (%)		
CTL	5	22.2 ± 1.4	25.4 ± 5.1	52.4 ± 4.3
SPH	5	21.0 ± 4.4	24.9 ± 6.5	54.1 ± 9.2
DNV	5	25.1 ± 2.0	27.7 ± 6.1	47.2 ± 6.5

tions in ryanodine receptor/channel for changes in CT.

A number of studies have shown that tropic factors released by motoneurons or muscle fibers exert an influence on each other. Brown and Ironton (4) demonstrated in partially denervated soleus muscle that intact axons produced both terminal and collateral sprouts which inhibited by direct stimulation of the denervated muscle. Czeh et al. (7) indicated that the electric physiological properties of motoneuron were maintained, at least in part, by trophic factors retrogradely carried by the axons. From these results, it is clear that the release of trophic factors by motoneurons and inactive muscle is important for the adaptation of muscle and motoneurons to inactivity. In addition, Davies et al. (10) demonstrated that in the embryonic chick trigeminal mesencephalic nucleus, two distinct neurotrophic factors, one in the central nervous system and the other in skeletal muscle, cooperated in regulating neuron survival during development. Previously, we speculated that when the levels of activation of motoneurons and muscle fibers are appropriately matched, even if this reflects inactivity of both, then disuse adaptations are avoided (24). These results indicate that cooperation of myotrophic and neurotrophic factors is one of important factors for the normal development of neuromuscular system. Concerning SR morphology, Heck and Davis (18) showed that administration of nerve extract including neurotrophic factors ameliorates the SR volume in denervated soleus and gastrocnemius muscles in mouse. Furthermore, Takekura et al. (31) suggested that morphological adaptation of the membrane system including SR is affected by some neurotrophic factors. Based on our results, in combination with the results in previous studies (18, 31), we suggest that the myoneural interaction between muscle and motoneurons plays important roles in regulating SR.

It has been reported that muscle fibers express several trophic factors, that is, brain-derived neurotrophic factor (BDNF), neurotrophin3 and neurotrophin4/5, and their receptors (14, 15). Funakoshi et al. (14, 15) demonstrated that sciatic nerve transection in rats resulted in increased muscle expression of BDNF, but decreased muscle expression of NT4/5. In addition, many growth factors may affect adaptation of SR function. Some myotrophic influences mentioned above may mediate SR adaptation to inactivity. To explain the regulation of the nerve ending density in a partial denervation model, Aguilar et al. (1) proposed the existence of a negative feed back control system: the muscle continually manufactures a substance that stimulates nerves to sprout, and that this effect is neutralized in some way by neurotrophic factors.The SR adaptations might be regulated by a similar mechanism in the nerve sprouting. We speculate that a myotrophic factor released by inactive muscle fiber is normally opposed by neurotrophic factors expressed by inactive motoneurons. This mechanism may explain why rapid adaptations of SR do not normally occur in fast motor units that are infrequently activated in the diaphragm muscle. Further studies concerning the expression of several trophic factors in muscle fibers and motoneurons are needed to confirm our speculation.

Acknowledgment

The research was supported by a grant from The Japanese Ministry of Education, Science and Culture (No.10780024).

References

1) Aguilar CE, Bisby MA, Cooper E, and Diamond J (1973) Evidence that axoplasmic transport of trophic factors is involved in the regulation of peripheral nerve fields in salamanders. J Physiol 234: 448-464
2) Bary JJ, Hubbard JI, and Mills RG (1979) The trophic influence of tetrodotoxine-inactive nerves on normal and reinnervated rat skeletal muscle. J Physiol 297: 479-491
3) Betz WJ, Caldwell JH, and Ribchester RR (1979) Sprouting of active nerve terminals in partially inactive muscles of the rat. J Physiol 303: 281-297
4) Brown MC, and Ironton R (1978) Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles. J Physiol 278: 325-348
5) Byrd SK, Bode AK, and Klug GA (1989) Effects of exercise of varying duration on sarcoplasmic reticulum function. J Appl Physiol 66: 1383-1389
6) Byrd SK, McCutcheo LJ, Hodgson DR, and Gollnick PD (1989) Altered sarcoplasmic reticulum function after high-intensity exercise. J Appl Physiol 67: 2072-2077
7) Czeh G, Gallego R, Kudo N, Kuno M (1978) Evidence for the maintenance of motoneurone properties by muscle activity. J Physiol 281: 239-252
8) Davey DF, and O'Brien GM (1978) The sarcoplasmic reticulum and T-system of rat extensor digitorum longus muscles exposed to hypertonic solutions. Aust J Exp Biol Med Sci 56: 409-19
9) Davey DF, and Wong S Y (1983) Morphometric analysis of rat extensor digitorum longus and soleus muscles. Aust J Exp Biol Med Sci 58: 213-230
10) Davies AM, Thoenen H, and Barde Y-A (1985) Different factors from the central nervoussystem and periphery regulate the survival of sensory neurones. Nature 319: 497-499
11) Delbono O, and Chu A (1995) Ca^{2+} release channels in rat denervated skeletal muscles. Exp Physiol 80: 561-574
12) Dux L (1993) Muscle relaxation and sarcoplasmic reticulum function in different muscle types. Rev Physiol Biochem Pharmacol 122: 70-147
13) Finol HJ, Lewis DM, and Owens R (1981) The effects of denervation on contractile properties of rat skeletal muscle. J Physiol 319: 81-92
14) Funakoshi H, Belluardo N, Arenas E, Yamamoto Y, Casabona A, Persson H, and Ibanez CF (1995) Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neurons. Science 268: 1495-1499
15) Funakoshi H, Frisen J, Barbany G, Timmusk T, Zachrisson O, Verge VM, and Persson H (1993) Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. J Cell Biol 123: 455-65
16) Gundersen K (1985) Early effects of denervation on isometric and isotonic contractile properties of rat skeletal muscles. Acta physiol Scand 124: 549-555
17) Hamalainen N, and Pette D (1997) Coordinated fast-to-slow transitions of myosin and SERCA isoforms in chronically stimulated muscles of euthyroid and hyperthyroid rabbits. J Muscle Res Cell Motil 18: 545-554
18) Heck CS, and Davis HL (1988) Effects of denervation and nerve extract on ultrastructure of muscle. Exp Neurol 100: 139-153
19) Hensbergen E, and Kernell D (1997) Daily durations of spontaneous activity in cat's ankle muscles. Exp Brain Res 115: 325-332
20) Hopkins D, Mancheste KL, and Gregory M (1983) Histochemical and biochemical characteristics of the transient hypertrophy of the denervated rat hemidiaphragm. Exp Neurol 81: 279-293
21) Kappati G, and Engel WK (1968) Correlative histochemical study of skeletal muscle after suprasegmental denervation, peripheral nerve section, and skeletal fixation. Neurol 18: 681-692
22) Kotsias BA and Muchnik S (1987) Mechanical and electrical properties of denervated rat skeletal muscle. Exp Neurol 97: 516-528
23) Leberer EK, Hartner T, and Pette D (1987) Reversible inhibition of sarcoplasmic reticulum Ca-ATPase by altered neurom uscular activity in rabbit fast-

twitch muscle. Eur J Biochem. 162: 555-561

24) Miyata H, Zhan WZ, Prakash YS, and Sieck GC (1995) Myoneural interactions affect muscle adaptations to inactievity. J Appl Physiol 79: 1640-1649

25) Ogawa Y, Kurebayashi N, and Murayama T (1999) Ryanodine receptor isoforms in excitation-contraction coupling. Adv Biophys 36: 27-64

26) Pierotti DJ, Roy RR, Bodine-Fowler SC, Hodgson JA, and Edgerton VR (1991) Mechanical and morophological properties of chronically inactive cat tibialis anterior motor units. J Physiol 444: 175-192

27) Schiaffino S, Ausoni S, Gorza L, Gundersen SK, and Lomo T (1988) Myosin heavy chain isoforms and velocity of shortening of type 2 skeletal muscle fibers. Acta Physiol Scand 134: 575-576

28) Schiaffino S, Gorza L, Pitton G, Saggin L, Ausoni S, Sartore S, and Lomo T (1988) Embryonic and neonatal myosin heavy chain in denervated and paralyzed rat skeletal muscle. Dev Biol 127: 1-11

29) Simonides WS, and van Hardeveld C (1990) An assay for sarcoplasmic reticulum Ca^{2+}-ATPase activity in muscle homogenates. Anal Biochem 191: 321-331

30) Sreter FA, Lopez JR, Alamo L, Mabuchi K, and Gergely J (1987) Changes in intracellular ionized Ca concentration associated with muscle fiber type transformation. Am J Physiol 253: C296-300

31) Takekura H, Kasuga N, Kitada K, and Yoshioka T (1996) Morphological changes in the triads and sarcoplasmic reticulum of rat slow and fast muscle fibres following denervation and immobilization. J Muscle Res Cell Motil 17: 391-400

32) Yellin H (1974) Changes in fiber types of the hypertrophing denervated hemidiaphragm. Exp Neurol 42: 412-428

33) Zhan WZ, and GC Sieck (1992) Adaptation of diaphragm and medial gastrocnemius muscle to inactivity. J Appl Physiol 72: 1445-1453

(Received 7 August, and in revised form 17 November, accepted 27 November 2000)