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Effect of Hind-Limb Immobilization on Contractile and Histochemical Properties of Skeletal Muscle*

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Summary. Both hind limbs of male rats were immobilized in casts. After 4 weeks, serial sections of hind limb muscles were stained for myosin ATPase and NADH-diaphorase. The soleus from immobilized limbs had significantly fewer muscle fibers than the control soleus. Moreover, the soleus from immobilized limbs had a significantly lower percentage and lower number of fibers with low myosin ATPase activity than the soleus from control rats. Immobilization also resulted in the speed of contraction for the soleus being significantly faster than the soleus from control rats. There were no significant differences in the contractile properties or in the percentages of fibers with low myosin ATPase between rectus femoris muscles from immobilized and control limbs. The deep portion of the rectus femoris from immobilized limbs had a significantly smaller percentage of muscle fibers with high NADH-diaphorase activity than did the rectus femoris from control rats.

Key words: Muscle Atrophy — Contraction Times — Muscle Fiber Number — Muscle Fiber Type — Histochemistry.

Three types of muscle fibers have been identified in rodent skeletal muscle [11,12]. These are (a) fibers with low myosin ATPase activity, moderately high oxidative enzyme activity and low glycolytic enzyme activity (SO); (b) fibers with high myosin ATPase, high oxidative and high glycolytic activities (FO); and (c) fibers with high myosin ATPase, low oxidative and high glycolytic activities (F). The soleus muscle of the rat is predominantely composed of SO fibers with a small percentage of FO fibers [12] and has a slow speed of contraction [8].

An increase in the speed of contraction of the soleus has been noted after chronic immobilization [15,18]. One explanation for this finding

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might be an increase in myosin ATPase activity in fibers that initially had a low myosin ATPase activity. In favor of this explanation is the finding that when the soleus muscle is denervated and then reinnervated with the nerve from a fast-contracting muscle, myosin ATPase activity per gram and the speed of contraction are increased [3]. A second possible explanation for the speeding of the contraction of the soleus after immobilization could be a preferential loss of SO fibers, resulting in an increase in the percentage of FO fibers.

One purpose of this study was to evaluate the latter possibility. The hind limbs of the rats were immobilized with plaster casts for 4 weeks. The total number of SO fibers and of FO fibers were then compared in soleus muscles from rats which had their hind limbs immobilized and from normal pair-fed controls.

The oxidative capacity of FO and F fibered muscle increases in response to endurance exercise training [2]. In addition, the percentage of FO fibers increases in skeletal muscle consisting of FO and F fibers as a result of endurance training [2,4,11]. Another purpose of this study was to determine if immobilization results in a decrease in the percentage of FO fibers in the rectus femoris, a muscle consisting of FO and F fibers [1].

Materials and Methods

Male, Sprague-Dawley rats, 70—80 days old, were randomly divided into groups of control and casted animals. Plaster of paris was applied to both hind limbs of rats so that ankle, knee and hip joints were fixed approximately at their resting angles. Details concerning methods of casting and the resulting muscular atrophy are described elsewhere [6]. Control rats were pair fed with the casted animals. About 2 weeks after casting, the initial cast was replaced with a second one around the now smaller limb. After a total of 4 weeks in the casts, rectus femoris or soleus muscles from control and casted rats were studied. Contractile properties were studied in one set of rats and histochemical properties in a second set of rats.

Contractile Properties. Rats were anesthetized with sodium pentobarbital (50 mg/kg). Either the soleus or rectus femoris were dissected free of surrounding tissue, so that only blood vessels, nerves, and the proximal tendon of the muscle remained intact. The distal tendons of the muscles were attached to transducers for the measurement of contractile properties. The femur or the tibia were clamped to prevent their movement during contraction of either rectus femoris or soleus muscles, respectively. The muscle was indirectly stimulated via its nerve.

Krebs-Ringer-bicarbonate solution was continuously dripped onto the *in situ* muscle preparation, and the external muscle temperature was maintained at 35° C. All recordings were made with the muscle length for optimal twitch (rectus femoris) and for optimal tetanic tensions (soleus). Contraction times, half relaxation times, and tetanus fusion frequencies were measured according to Close and Hoh [9].

Histochemistry. Serial sections, 6 μ thick, from the bellies of the soleus and rectus femoris muscles from control and casted rats were stained concurrently for myosin ATPase [21] and for NADH-diaphorase [20]. Fiber types were classified as follows [21]: Fibers with high enzyme activities for both myosin ATPase and NADH-diaphorase were called FO (fast-twitch and high oxidative fibers); fibers with high activity of myosin ATPase and low activity of NADH-diaphorase were termed F (fast-twitch and low oxidative fibers); and fibers with low activity of myosin ATPase and moderate activity of NADH-diaphorase were called SO (slow-twitch and moderate oxidative fibers) [2,11,12].

In the soleus, all fibers were identified as to type and were counted. Photographs (100×) were taken of the serial cross-sections stained for myosin ATPase and NADH-diaphorase. The photographs were fitted together so that a magnified picture of the soleus cross-section was constructed. The same fiber was found in these magnifications of the myosin ATPase and NADH-diaphorase cross-sections, was identified as to fiber type and was numbered.

The rectus femoris is not a homogeneous mixture of the three muscle-fiber types. The superficial portion of the rectus femoris consists almost totally of F fibers, while the deep portion of the muscle has approximately equal proportions of FO and F fibers, with a smaller percentage of SO fibers. A vertical connective tissue layer, which runs superficial to deep within the muscle, was used as a landmark for three counting areas. Consistency in sampling areas in muscle from different rats was obtained by using the deep and superficial borders of the connective tissue layer within the rectus femoris as centers for square counting areas of approximately 250 fibers each. The middle counting area was centered on the connective tissue layer approximately halfway between the deep and superficial borders. Fiber types for the rectus femoris were identified as described for the soleus.

Results

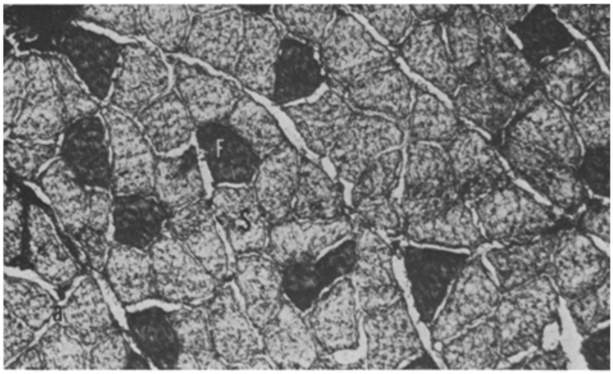
In the soleus from the immobilized limb a significant decrease occurred in both the total number and percentage of SO fibers (Table 1 and Fig. 1). There was a significant increase in the percentage of FO fibers (Table 1 and Fig. 1). The contraction time of the soleus from the immobilized limb was significantly shorter than that of the soleus from control rats (Table 2). Although the half-relaxation time of the soleus from immobilized limbs tended to be shorter than those of controls, the difference was not statistically significant. The tetanus fusion frequency for the soleus

Table 1. The number and percentages of muscle fiber types in control and casted soleus muscles

	Control		Casted	
	Total number (5)[a]	Percentage (7)	Total number (6)[a]	Percentage (7)
Fast-twitch oxidative (FO)	361 ± 62	13 ± 2	516 ± 93	31 ± 7*
Fast-twitch low-oxidative (F)	0	0	0	0
Slow-twitch oxidative (SO)	2463 ± 42	87 ± 2	1629 ± 154*	69 ± 7*
Total number of fibers/muscle	2824 ± 59	100	2145 ± 162*	100

Means ± SE. * $P < 0.05$ from control. (n) is number rats.

[a] Two control and one casted were not used in total fiber number due to parts of these muscles being torn. Fiber numbers reported were made from direct fiber counts on photomicrographs of complete cross sectional views of soleus.



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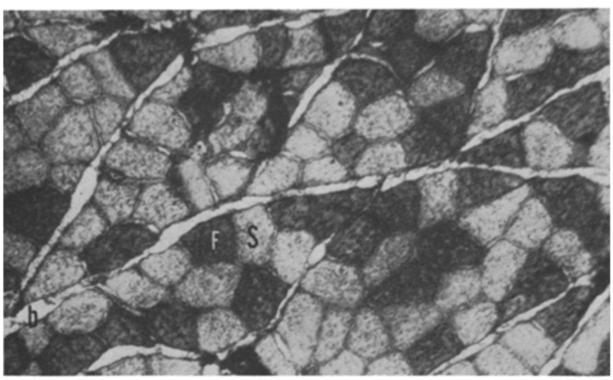

b

Fig. 1 a and b. Cross sections of soleus muscles of rats incubated for myosin ATPase activities. Fibers are identified as follows: *F*, fast-twitch; and *S*, slow-twitch. a Control. b Limb immobilized in cast for 4 weeks. (×150)

Table 2. Tetanus fusion frequency, contraction time, and half-relaxation times for control and casted rectus femoris and soleus muscles

Muscle	Group	Tetanus fusion frequency (Hertz)	Contraction time (msec)	Half-relaxation time (msec)
Rectus femoris	Control (6)	95.8 ± 2.4	14.0 ± 0.4	19.1 ± 0.2
	Casted (6)	91.7 ± 4.4	13.5 ± 0.8	16.3 ± 5.3
Soleus	Control (6)	15.0 ± 2.2	49.0 ± 12.5	157.0 ± 34.3
	Casted (6)	31.0 ± 7.3*	19.0 ± 2.4*	104.0 ± 49.1

Means ± SE. * $P < 0.05$ from control. (n) is number rats.

Table 3. Percentages of muscle fiber types for control and casted rectus femors muscles in deep, middle and superficial areas

	Fast-twitch Oxidative (FO)		Fast-twitch low oxidative (F)		Slow-twitch Oxidative (SO)	
	Control (4)	Casted (4)	Control (4)	Casted (4)	Control (4)	Casted (4)
Deep	52 ± 2	45 ± 2*	41 ± 3	52 ± 2*	7 ± 2	4 ± 1
Middle	37 ± 3	32 ± 2	62 ± 3	68 ± 3	1 ± 1	1 ± 1
Superficial	8 ± 3	6 ± 3	92 ± 3	94 ± 3	0	0

Means $\pm$ SE. * $P < 0.05$ from control. (n) is number rats.

from immobilized limbs was significantly higher than for the soleus from control rats (Table 2).

No significant changes in the percentages of fiber types were observed in the superficial and the middle areas of the rectus femoris from immobilized limbs (Table 3). However, the deep area of the rectus femoris from immobilized limbs showed a significant decrease in the percentage of FO fibers, while the percentage of F fibers was significantly higher (Table 3). No significant differences in contractile times, half-relaxation times or tetanus fusion frequencies were noted in rectus femoris muscles from control and immobilized limbs (Table 2).

Discussion

Previously reported values for the total number of muscle fibers [17] and for the percentages of SO and FO fibers [1] in the rat soleus are similar to the values found in the soleus from control rats in this study. Our findings are in accordance with previous reports that the loss of muscle fibers can occur. Anatomical evidence of muscle-cell disintegration in the soleus of the cat as a result of limb immobilization has been reported [10]. Aging is associated with the loss of muscle fibers in rat soleus [17] and in guinea pig plantaris [13, 14]. In contrast to the increased rate of muscle fiber loss as a result of immobilization, increased levels of muscular activity appear to protect against the decrease in muscle fiber number in guinea pig plantaris with aging [13, 14].

The significant loss in the number of SO fibers in the soleus as a result of immobilization accounted both for the decrease in the total number of soleus muscle fibers and for the increased percentage of FO fibers in soleus after immobilization. In agreement with the preferential loss of SO fibers in soleus muscles from immobilized limbs is the recent observation on electron micrographs that SO fibers in the soleus muscle undergo a more rapid and marked degeneration as a result of tenotomy than do either FO and F fibers in the vastus lateralis muscle after tenotomy [22]. These observations help to explain the faster contraction times noted in the soleus muscle as a result of muscular inactivity in the following reports: immobilization of ankle, knee and hip joints in the present study; immobilization of ankle and knee joints [15]; and tenotomy of the rabbit soleus [7,23].

Decreased concentrations of myosin ATPase and faster contraction speeds have been reported in the soleus approximately 1 year after cross-innervation of soleus and extensor digitorum longus muscles [3]. In view of this evidence that myosin ATPase activity in muscle can change, it seems possible that an alteration in myosin ATPase concentration in SO fibers, as a result of immobilization could have contributed to changes in the contraction times of the soleus. However, this possibility was not tested in the present study.

The failure in some previous studies to observe changes in the contractile properties [19,24] or in the histochemical characteristics [19] of the soleus after immobilization of only the ankle joint is probably related to the failure of ankle-joint immobilization to significantly reduce muscular activity [16,19]. Decreased electromyographic activity from the soleus during immobilization of ankle and knee joints [15,16] and after tenotomy of the soleus [23] was associated with an increase in contraction speed of the soleus.

The deep portion of the rectus femoris from immobilized hind limbs had a decreased percentage of FO fibers and an increased percentage of F fibers as compared to control values. Percentages of fiber types noted in rectus femoris from control rats were similar to previous measurements of fiber percentages in this muscle [1]. The decreased percentage of FO fibers in the deep rectus femoris is in agreement with our finding (unpublished data) that cytochrome oxidase activity, a marker for oxidative capacity, decreased in the deep rectus femoris in response to immobilization. The adaptive response to increased physical activity is the reverse of that seen with immobilization. There is an increase in the percentage of FO fibers [2,4,11], and an increase in the level of activity of cytochrome oxidase [2] in muscle of rats subjected to endurance exercise training. Apparently an inverse relationship between the level of muscular activity and the level of oxidative enzymes in a muscle composed both of FO and F fibers exists. The observation that the contraction times of the rectus femoris did not change significantly in response to immobilization is in agreement with previous studies in which little effect of muscular inactivity on the contraction times of muscle composed of FO and F fibers was reported [7].

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