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Effects of hypophysectomy on soleus muscle fibers and spinal motoneurons in rats

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Abstract The fiber type distribution of the soleus muscle in male and female rats was investigated 4 weeks after hypophysectomy. Oxidative enzyme activity of the soleus motoneurons in the spinal cord was also examined by enzyme histochemical assay. In male rats, the total number of fibers in the soleus muscle was not changed after hypophysectomy, but the percentage of intermediate (INT) fibers (with intermediate adenosine triphosphatase activity following alkaline preincubation, and high succinate dehydrogenase and α-glycerophosphate dehydrogenase activities) was increased. All types of fibers in the soleus muscle of hypophysectomized rats showed high adenosine triphosphatase activity following acid preincubation. Oxidative enzyme activity of the motoneurons innervating the soleus muscle was not changed after hypophysectomy. Similar results were obtained in female rats. It is suggested that the increased percentage of INT fibers in the rat soleus muscle after hypophysectomy is due to a lack or reduced levels of growth hormones, and that the metabolic capacities of the muscle fibers and of the innervating motoneurons are affected independently by hypophysectomy.

Key words Muscle fiber type distribution · Oxidative enzyme activity · Soleus muscle · Spinal motoneuron Hypophysectomy

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Introduction

Mammalian skeletal muscle fibers are classified according to their enzyme histochemical profiles. Adenosine triphosphatase, succinate dehydrogenase, and α-glycerophosphate dehydrogenase activities are used to classify muscle fibers as fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), or slow-twitch oxidative (SO) types [17].

Chronic administration of hormones can cause the type shift of muscle fibers. The anabolic hormones nandrolone phenylpropionate [5] and nandrolone cypionate [4] increase the percentage of FOG fibers, while decreasing that of FG and/or SO fibers in the extensor digitorum longus, medial gastrocnemius, tibialis anterior, and soleus muscles in female rats. In male rats, increases in the percentage of FOG fibers at the expense of SO fibers in the soleus muscle [14, 15], and at the expense of FG and SO fibers in the extensor digitorum longus muscle [14] have been observed after administration of the thyroid hormone L-triiodothyronine. Furthermore, the thyroid hormones L-thyloxine and L-triiodothyronine increase the percentage of FOG fibers in the soleus muscle in both male [8] and female [6] rats.

On the other hand, few studies have dealt with the effects of hormone deficiency, especially the influence of hypophysectomy, on the fiber type distribution in muscles. Therefore, we investigated the fiber type distribution in the soleus muscle in both male and female hypophysectomized rats. We also examined oxidative enzyme activity of spinal motoneurons innervating the soleus muscle after hypophysectomy.

Materials and methods

Animals and treatment

Ten 4-week-old specific-pathogen-free male Wistar strain rats were housed, one per cage, in a controlled environment with 12-h daylight and 12-h darkness. Food and water were provided ad libitum and the room temperature was maintained at 22 ± 2° C. Five animals were assigned to the control group (CONT) and the other five to the hypophysectomized group (HYPO). Eight age-matched female rats were examined according to the same protocol.

Hypophysectomy

At the age of 7 weeks the animals were quickly hypophysectomized under anesthesia induced by intraperitoneal (i.p.) administration of sodium pentobarbital (35 mg/kg) under sterile conditions [20]. Briefly, a syringe was used to remove the pituitary body. The syringe needle, the size of which was adjusted according to the body weight of individual animals, was inserted into the auricular canal and the tympanic membrane was broken. After the bone wall was broken with the needle, the hypophysis was then drawn into the syringe barrel.

Tissue preparation and histochemical procedures

Four weeks after hypophysectomy, the animals were anesthetized with sodium pentobarbital (35 mg/kg, i.p.), and 10 μl of a 2% solution of nuclear yellow dissolved in distilled water was injected into the left soleus muscle. Care was taken to inject the dye slowly and to check for leakage, and any dye that leaked back out of the muscle was washed away with saline. One day after injection, the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The lumbosacral enlargement of the spinal cord and the soleus muscles of both sides were rapidly removed.

The lumbosacral enlargement of the spinal cord was frozen in isopentane cooled in a dry ice and acetone mixture, and longitudinal sections, 20 μm thick, were cut on a cryostat at –20° C. Soleus motoneurons were identified by a fluorescence microscopy at a wavelength of 360 nm by their golden-yellow fluorescent nuclei on untreated fresh frozen sections. After the motoneurons were identified, the same sections were stained for succinate dehydrogenase (SDH) activity. SDH activities of the identified motoneurons were determined by a microspectrophotometer at 470 nm. The measuring field had a spot diameter of 5–9 μm, and the average value for 2 or 3 spots within the cytoplasm of a given neuron was calculated and expressed as absorbance in arbitrary units. During photometric analyses, all lighting conditions, magnifications, and reference points were kept constant.

The right soleus muscle was weighed while moist and then frozen in isopentane cooled in a dry ice and acetone mixture, and serial transverse sections, 10 μm thick, of the widest point of the muscle belly were cut on a cryostat at –20° C and incubated in a battery of histochemical media. Serial sections were preincubated at an alkaline (pH 10.3) or an acid (pH 4.3) prior to determination of adenosine triphosphatase (ATPase) activity [3, 16]. SDH and α-glycerophosphate dehydrogenase (α-GPD) activities were also determined in serial sections [13, 22]. The soleus muscle fibers were classified as FOG (high ATPase activity following alkaline preincubation, and high SDH and α-GPD activities), intermediate (INT; intermediate ATPase activity following alkaline preincubation, and high SDH and α-GPD activities), or SO (low ATPase activity following alkaline preincubation, high SDH activity, and low α-GPD activity), [7, 9]. The muscle fiber type distribution was calculated by counting the total number of muscle fibers of each type seen on the whole muscle transverse section.

The left soleus muscle was frozen in isopentane cooled in a dry ice and acetone mixture, and transverse sections, 20 μm thick, were cut on a cryostat at –20° C. These sections were used to confirm that nuclear yellow was injected into the entire muscle using a fluorescence microscope at 360 nm.

Statistics

Means and standard deviations (SD) were calculated from individual values using standard procedures. Student's *t*-test was used to determine the significant difference between the CONT and HYPO groups.

Results

Body weight and muscle weight

Body weights in the male and female HYPO groups were lower than those in the male and female CONT groups, respectively. Soleus muscle weights in the male and female HYPO groups were lower than those in the male and female CONT groups, respectively, while the muscle weight per body weight in the male HYPO group was higher than that in the male CONT group (Table 1).

Muscle fiber type distribution

There were no differences in the total number of fibers in the soleus muscle between the male CONT and HYPO groups or between the female CONT and HYPO groups (Table 2). Both HYPO groups had a higher percentage of INT fibers in the muscle than the respective CONT groups (Table 2). ATPase activities following acid preincubation were higher in all types of muscle fibers in both HYPO groups compared with the CONT groups (Figs. 1, 2).

Table 1 Body weight and soleus muscle weight in control and hypophysectomized rats. Values are means ± SD (*n* number of animals analyzed, *MW* muscle weight, *RMW* muscle weight/100 g body weight, *CONT* control group, *HYPO* hypophysectomized group)

		n	Body weight (g)	Muscle weight (mg)	
				MW	RMW
Male	CONT	5	325 ± 19	132 ± 10	41 ± 2
	HYPO	5	162 ± 5**	73 ± 2**	45 ± 2*
Female	CONT	5	203 ± 5	86 ± 7	42 ± 3
	HYPO	3	134 ± 5**	58 ± 2**	43 ± 1

* $P < 0.01$, ** $P < 0.001$ compared with CONT

Table 2 Fiber type distribution and total number of fibers in the soleus muscle in control and hypophysectomized rats. Values are means ± SD (*n* number of animals analyzed, *FOG* fast-twitch oxidative glycolytic, *SO* slow-twitch oxidative, *INT* intermediate, *CONT* control group, *HYPO* hypophysectomized group)

	n	Muscle fiber type (%)			Total
		FOG	SO	INT	
Male					
CONT	5	13.2 ± 5.1	83.2 ± 6.1	3.6 ± 1.4	2541 ± 275
HYPO	5	14.0 ± 2.2	77.5 ± 3.9	8.5 ± 2.8*	2505 ± 255
Female					
CONT	5	14.2 ± 5.6	81.9 ± 4.7	3.9 ± 1.3	2545 ± 173
HYPO	3	13.1 ± 2.5	77.6 ± 2.8	9.3 ± 1.5*	2440 ± 117

* $P < 0.01$ compared with CONT

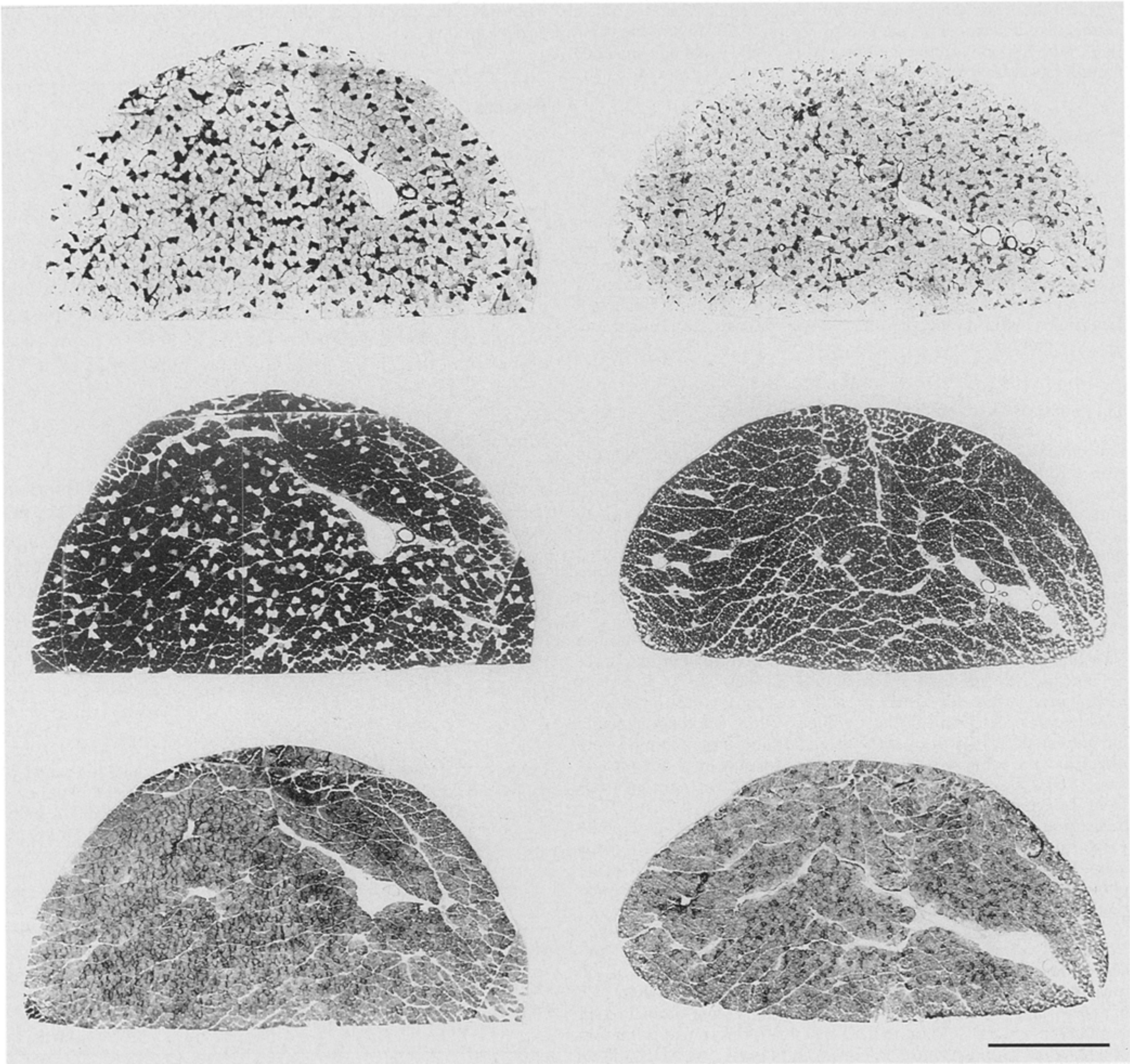

Fig. 1 Transverse sections of the soleus muscle stained for adenosine triphosphatase activity following alkaline (*top*) and acid (*middle*) preincubation, and for succinate dehydrogenase activity (*bottom*) in male control (*left*) and hypophysectomized (*right*) rats. *Bar* indicates 1 mm

Oxidative enzyme activity of motoneurons

There were no differences in the mean oxidative enzyme activities of motoneurons innervating the soleus muscle between the male CONT and HYPO groups (Table 3). The same result was obtained in comparison between the female CONT and HYPO groups (Table 3).

Discussion

Vaughan et al. [21] found that castrated male mice had a lower percentage of FOG fibers in the soleus muscle than control males, and concluded that the androgen deficit influences ATPase activity in the muscle fibers. Similarly, Matoba et al. [12] and Johnson et al. [10] observed that hypothyroidism decreased the percentage of fast-twitch (FG or FOG) fibers and increased that of SO fibers in the male rat extensor digitorum longus, soleus, and diaphragm muscles. Therefore, hormone deficiency might affect ATPase levels in the muscle fibers and inhibit the fast-type ATPase activity in specific fiber types.

In the present study, the percentage of INT fibers in the soleus muscle was increased 4 weeks after hypophysectomy. We interpret this as indicating a type shift of muscle

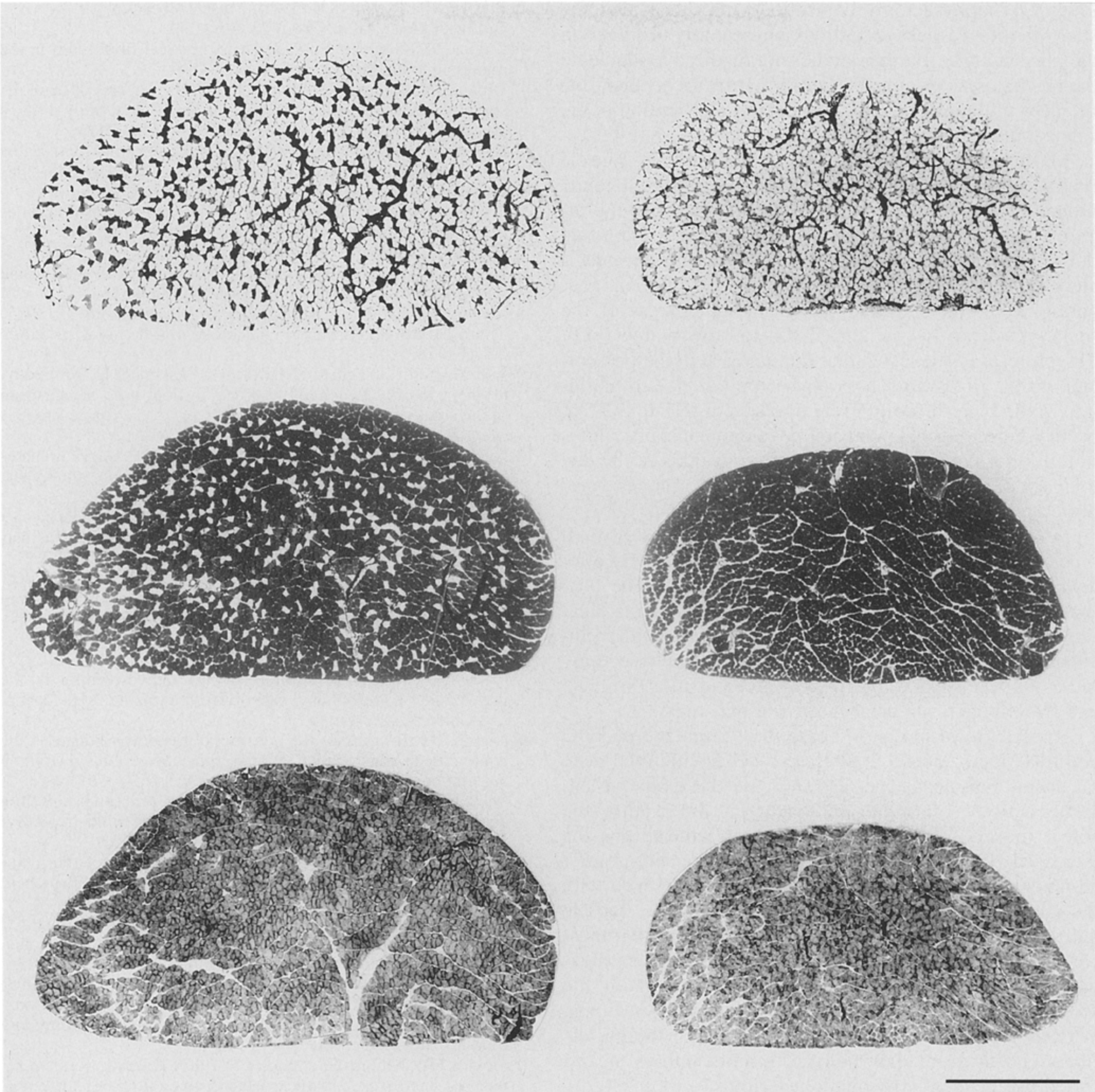

Fig. 2 Transverse sections of the soleus muscle stained for adenosine triphosphatase activity following alkaline (*top*) and acid (*middle*) preincubation, and for succinate dehydrogenase activity (*bottom*) in female control (*left*) and hypophysectomized (*right*) rats. *Bar* indicates 1 mm

fibers toward INT, because the total number of muscle fibers was not changed. Identification of this shift in muscle fiber type was based on ATPase staining intensity and was interpreted as indicating a change in myosin synthesis in the muscle fibers. On the other hand, a previous study [18] in male rats showed that hypophysectomy over periods of 500 days increased the percentage of SO fibers in the soleus muscle. These results over a long period fol-

Table 3 Oxidative enzyme activity of motoneurons innervating the soleus muscle in control and hypophysectomized rats. Values are means ± SD (*n* number of animals analyzed, A_{470} absorbance at 470 nm, *CONT* control group, *HYPO* hypophysectomized group)

		n	SDH activity (A_{470})
Male	CONT	5	0.689 ± 0.029
	HYPO	5	0.729 ± 0.028
Female	CONT	4	0.688 ± 0.029
	HYPO	3	0.739 ± 0.026

lowing hypophysectomy contrasted with those over the short period (4 weeks) after hypophysectomy observed in the present study. Further studies are required to elucidate the mechanisms responsible for these time-dependent differences in the effects on the fiber type distribution in the soleus muscle following hypophysectomy.

Maltin et al. [11] found that a type shift of muscle fibers from FOG (high ATPase activity following alkaline preincubation) to SO (low ATPase activity following alkaline preincubation) occurred in the rat soleus muscle during postnatal development. INT fibers with intermediate ATPase staining intensity following alkaline preincubation were found during the changing process of the fiber type from fast-twitch (FOG) to slow-twitch (SO). Therefore, it is suggested that the increase in the percentage of INT fibers after hypophysectomy is due to inhibition of the type shift of muscle fibers from INT to SO that occurs during normal postnatal development. This inhibition in type shift of muscle fibers is considered to be due to a lack or a reduction in level of growth hormones as reported previously in the study in male rats [1].

In hypophysectomized rats in the present study, all types of muscle fibers showed high ATPase activity after acid preincubation, as shown in Figs. 1 and 2. The fast-twitch fibers showed high ATPase activity following alkaline preincubation, but low activity following acid preincubation [2]. The mechanisms by which hypophysectomy increases ATPase activity (at acid preincubation) in FOG and INT fibers is not clear in the present study.

Hyperthyroidism can increase the percentage of FOG and INT fibers and decrease that of SO fibers in the male rat soleus muscle; it can also increase the oxidative enzyme activity of spinal motoneurons innervating the soleus muscle [19]. These observations indicate that the increased oxidative capacities in the motoneurons after administration of thyroid hormones correspond well with the increased oxidative enzyme activities in the muscle and with the increased percentage of high oxidative muscle fibers. However, in the present study, the mean oxidative enzyme activity of the motoneurons innervating the soleus muscle was not changed after hypophysectomy. We conclude that the metabolic properties of the muscle fibers and those of the innervating motoneurons are affected independently by hypophysectomy.

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