EFFECT OF THYROXINE ON PHENOTYPE OF INTACT AND DENERVATED GUINEA PIG SKELETAL MUSCLES

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Many aspects of the currently important problem of neurohumoral regulation of skeletal muscles have been examined in the book [2]. Motoneurons have been shown to determine the differentiation of types of skeletal muscle fibers and to affect maintenance of their differential state [1, 2, 4, 6]. It is also known that iodine-containing hormones of the thyroid gland affect the velocity of contraction, content of light myosin chains, and Ca^{++}-ATPase activity of skeletal muscles [7, 9, 10]. The authors cited above have suggested that the influence of thyroxine on muscles is mediated through motoneurons.

In the present investigation, which had the aim of studying the contributions of nervous and hormonal factors in maintenance of the phenotype of skeletal muscles, histochemical and immunologic characteristics of intact and denervated fast and slow muscles were stained in guinea pigs after injection of L-thyroxine (T_4).

EXPERIMENTAL

The plantaris (fast) and soleus (slow) muscles were studied in intact guinea pigs weighing 350-400 g, in animals after division of the nerves supplying the muscles, in animals after injection of T_4 (from Reanal, Hungary), and in guinea pigs which had undergone denervation of the muscles and received T_4 by subcutaneous injection in a dose of 200 μg/kg body weight 24 h after denervation and thereafter on alternate days for 3 weeks. Muscles were studied in all animals 3 weeks after the beginning of the experiment. Each series consisted of six animals. At the end of the experiment the animals were killed under deep ether anesthesia, the muscles isolated, myosin ATPase activity was determined in frozen sections [6], and the number of muscle fibers of the different groups was counted.

Mysoin was isolated from homogenates of the plantaris muscle of adult guinea pigs by the method in [12]; the homogeneity of the preparation was verified by polyacrylamide gel electrophoresis. Antibodies were obtained by immunizing rabbits with purified myosin and the immune serum was exhausted with a homogenate of the soleus muscle, which contains only slow myosin. After exhaustion of the serum, immunoglobulins were removed from it by precipitation with magnesium sulfate by the method in [8]. Myosin was extracted from muscle homogenates of the experimental animals and subjected to the double immunodiffusion test [11]. The reaction was read after the gels had been stained with a solution of Amido black 10B and the gels washed with 7% acetic acid. The appearance of precipitation bands indicated the presence of fast myosin in the muscle. The results of the investigation were subjected to statistical analysis by the t test [3] at a 0.05 level of significance.

EXPERIMENTAL RESULTS

The intact plantaris muscle contains type I (slow) muscle fibers with low myosin ATPase activity and type II (fast) muscle fibers with high activity of that enzyme (Fig. 1a). The number of type I muscle fibers in the denervated plantaris muscle was increased. Injection of T_4 caused no change in the numbers of the various types of muscle fibers in the plantaris muscle. The number of type II muscle fibers was increased after denervation and injection of T_4 (Table 1).

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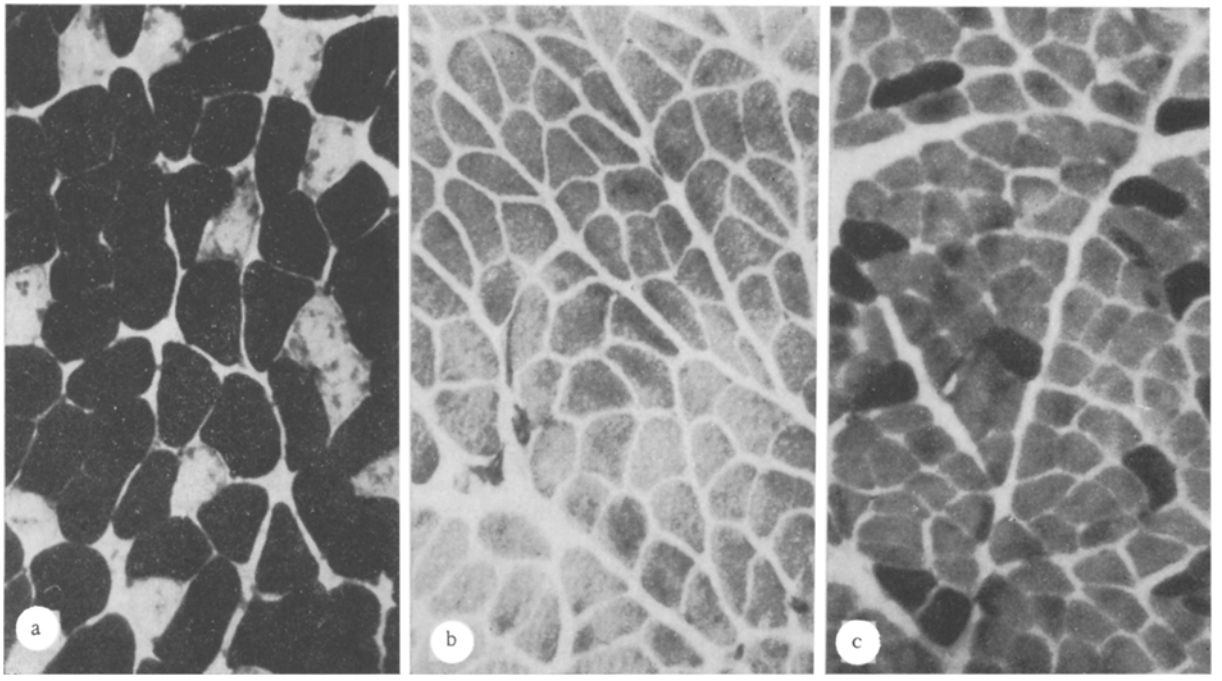

Fig. 1. Myosin ATPase activity in guinea pig muscles. a) Intact plantaris muscle, b) intact soleus muscle, c) soleus muscle after injection of T_4. Pale muscle fibers are type I, dark fibers type II. 63 ×.

TABLE 1. Relative Numbers of Muscle Fibers of Different Types (I, II) in Fast and Slow Guinea Pig Muscles under Different Experimental Conditions ($\bar{x} \pm s_{\bar{x}}$, %)

Experimental conditions	Plantaris muscle		Soleus muscle	
	I	II	I	II
Control	14,1 ± 0,9	85,9 ± 0,9	100	—
Denervation	40,1 ± 1,9*	59,9 ± 7,9*	100	—
Injection of T_4	17,4 ± 1,2	82,6 ± 1,2	91,5 ± 1,3	8,5 ± 1,2
Denervation + injection of T_4	10,6 ± 0,3*	89,5 ± 0,3*	84,9 ± 1,4	15,1 ± 1,4

Legend. *) Differences compared with control are significant.

The intact soleus muscle consists of type I muscle fibers (Fig. 1b). After denervation of the muscle no change was found in its histochemical profile, injection of T_4 led to the appearance of type II muscle fibers (Fig. 1c), and after denervation and injection of T_4 the relative proportion of type II fibers increased even more (Table 1).

By the use of Ouchterlony's double immunodiffusion test a precipitation band was obtained in all series of the experiment. In the soleus muscle of intact and denervated animals the precipitation test was negative, whereas after injection of T_4 and in the series with injection of T_4 after denervation, a precipitation band was present (Fig. 2).

Analysis of the experimental results shows that fast and slow muscles react unequally to injection of T_4. The relative number of muscle fibers in the fast muscle was unchanged, whereas in the slow muscle fast muscle fibers not typical of the intact muscle were observed, i.e., in a proportion of muscle fibers myosin synthesis was reprogrammed. Injection of T_4 after denervation caused an increase in the relative number of fast muscle fibers in both muscles. The most likely explanation of this fact is that thyroxine increases synthesis of fast myosin in skeletal muscles whereas neurotrophic control by motoneurons maintains the level of synthesis of fast or slow myosin that is characteristic of the muscle, and in particular, only slow myosin is synthesized in the slow muscle. Under these circumstances the removal of neurotrophic control by denervation of the muscle, after injection of T_4, ought to

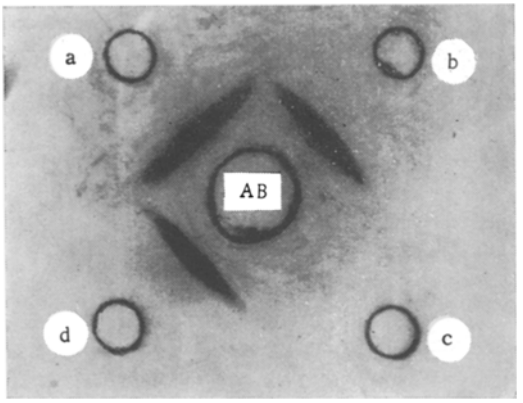

Fig. 2. Immunodiffusion of myosins of plantaris and soleus muscles against antibodies to fast guinea pig myosin. AB) Antibodies against fast myosin, a) myosin from plantaris muscle, b) myosin from degenerated soleus muscle after injection of T_4, c) myosin of soleus muscle, d) myosin of soleus muscle of animals receiving T_4.

lead to an increase in the number of muscle fibers synthesizing fast myosin, and this was indeed observed in the present experiments.

The level of synthesis of fast and slow myosins in muscle fibers is thus determined both by the influence of motoneurons and also by the direct effect of T_4 on the denervated muscle.

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