Effects of hypothyroidism on myosin isozyme transitions in developing rat muscle

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Hypothyroidism was induced in young rats by methylthiouracil treatment of pregnant mothers from 18 days of gestation to 4 weeks after birth. Electrophoretic analysis of native myosin isozymes revealed a persistence of neonatal and embryonic myosin in developing fast and slow muscles up to at least 28 days after birth. The appearance of adult fast myosin was inhibited in 28-day old animals, however adult slow myosin was found in the soleus muscle. Immunocytochemical results on the soleus demonstrate a cellular heterogeneity in the response to hypothyroidism. About half fibers have a normal complement of slow myosin and do not contain neonatal myosin. Only the remaining fibers contain the large amounts of neonatal myosin demonstrated by electrophoresis.

Immunocytochemistry *Native myosin isozyme* *Methylthiouracil* *Soleus* *Gastrocnemius*

1. INTRODUCTION

During rat skeletal muscle development, several myosin isozyme transitions take place [1–4]. In muscle fibers destined to contain adult fast-type myosin, embryonic and neonatal myosin isozymes are found before the adult form becomes the predominant isozyme [1–3]. In muscles such as the soleus, which is primarily composed of slow-type fibers in the adult rat, the situation is more complex. Immunocytochemistry using antibodies to different myosin isozymes has shown that two major types of fibers are present in the developing soleus muscle in the early post-natal period [4,5]. One type contains adult slow myosin by one week after birth [4]. The other type undergoes isozyme transitions similar to developing fast fibers, and then these fibers are gradually converted to slow fibers over a period of several months [4,6]. Continued innervation seems to be required for the maintenance or the induction of slow myosin during development [7–10] but the appearance of adult fast myosin does not require innervation [3,10]. Thyroid hormone levels also exert a major influence on myosin isozyme transitions in both developing and adult animals [10–13]. We have here used biochemical and immunocytochemical approaches to evaluate the effect of hypothyroidism on myosin isozyme transitions occurring in developing rat fast and slow muscle fibers.

2. MATERIALS AND METHODS

Young rats were rendered hypothyroid by oral administration of methylthiouracil to pregnant mothers from day 18 of gestation until 4 weeks after birth. The gastrocnemius and soleus muscles were removed from 28-day old rats and mounted for histochemical and immunocytochemical analysis. Immunocytochemistry was performed as in [3] using the myosin antibodies described in [4]. After cytochemical analysis the muscles were thawed, extracted and processed for native myosin electrophoresis as in [4].

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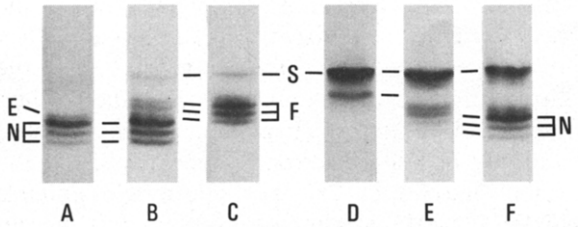

Fig.1. Electrophoretic analysis of native myosin isozymes. The samples analyzed were gastrocnemius muscles from (A) a normal 7-day old rat, (B) a 28-day old hypothyroid rat, (C) a normal 28-day old rat, and soleus muscles from (D) a normal 28-day old rat, (E) a 28-day old hypothyroid rat. The sample in (F) is a mixture 28-day hypothyroid soleus and 7-day normal gastrocnemius. The bands corresponding to the different myosins are indicated, and the abbreviations are E (embryonic myosin), N (neonatal myosin), F (fast myosin), and S (slow myosin).

3. RESULTS

Electrophoresis of native myosin permits an evaluation of the different myosin isozymes present in small amounts of unpurified muscle extracts [14,15]. This technique is particularly useful in conjunction with immunocytochemical analysis since it can provide confirmatory biochemical evidence for the presence of myosins detected by the antibodies. The electrophoretic analysis of gastrocnemius muscles is shown in fig.1A–C. At 7 days after birth in normal animals (fig.1A), 3 bands are present representing neonatal myosin [2]; a fourth band which migrated more slowly than the neonatal bands is also seen and is due to embryonic myosin still present at this time [2]. At 28 days after birth (fig.1C), 3 bands of adult fast

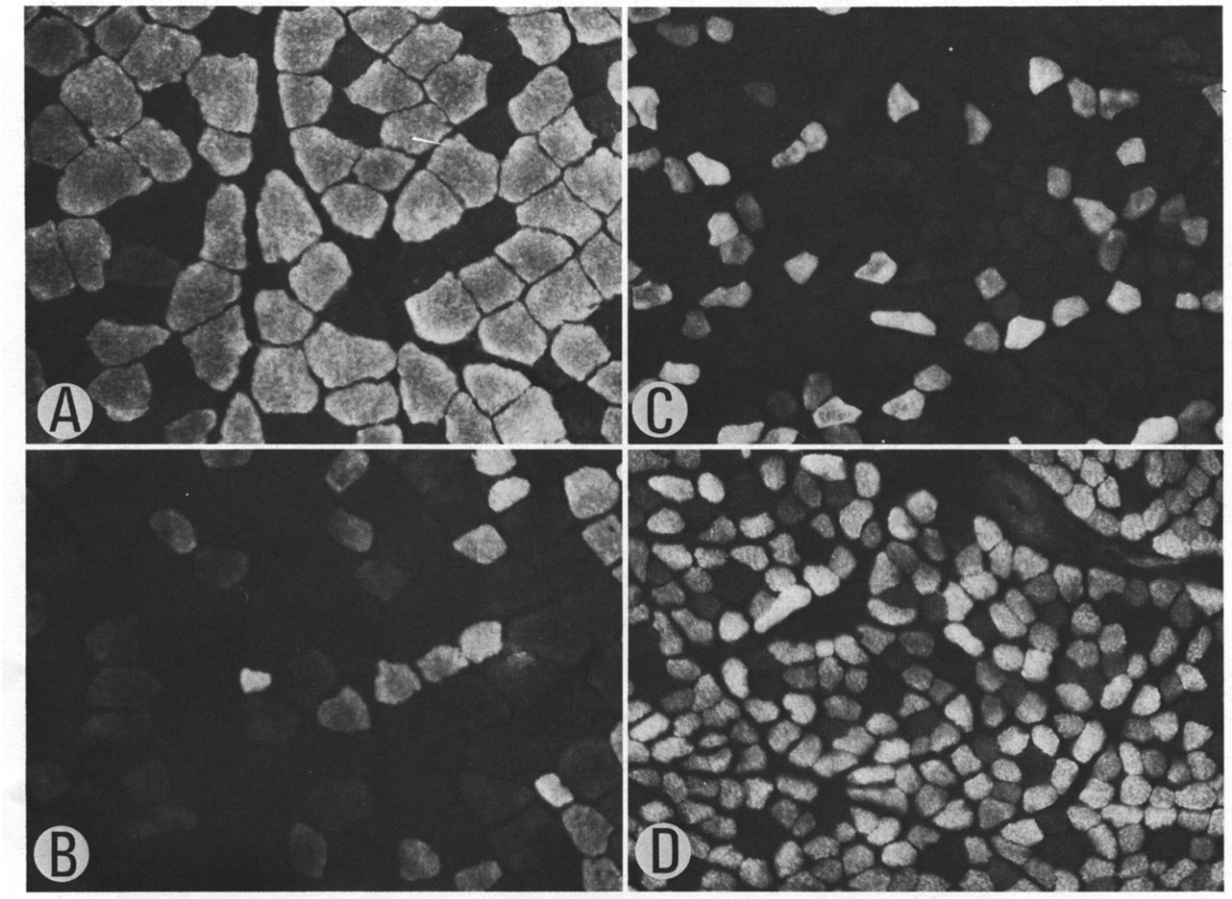

Fig.2. Immunocytochemistry on gastrocnemius muscles. Indirect immunofluorescence was carried out on muscles from normal (A,B) and hypothyroid (C,D) 28-day old rats. The antibodies used were against fast (A,C) and neonatal (B,D) myosins. A few fibers are unreactive with either antibody; they were however stained with slow myosin antibody (not shown).

myosin are present in control animals along with a minor band of slow myosin corresponding to the small number of slow fibers in the gastrocnemius muscle. In contrast, the gastrocnemius muscles of animals rendered hypothyroid during the first month of life still contain large amounts of neonatal myosin (fig.1B). In the hypothyroid muscles, minor bands can be seen in the region corresponding to adult fast myosin, and a minor band of slow myosin is also apparent.

The electrophoretic analysis of soleus muscles is shown in fig.1D–F. At 28 days after birth (fig.1D), the soleus contains one major band corresponding to adult-type slow myosin and a second faster-migrating band corresponding to the myosin found in the fast fibers that comprise about 30–40% of all fibers in the soleus at this age [6]. This soleus fast myosin does not co-migrate with the gastrocnemius fast isozymes [4] and may reflect differences between the myosins of the histochemical type IIA and type IIB fibers found in the rat soleus and gastrocnemius muscles, respectively [16]. Electrophoretic analysis of the soleus muscles of 28 day hypothyroid animals (fig.1E) reveals slow myosin, but the fast myosin band is absent. In addition, bands corresponding to embryonic and neonatal myosin are clearly present. This was determined by analyzing a mixture of the 28-day hypothyroid extract with an extract from a 7-day old gastrocnemius muscle: the embryonic and neonatal bands in the latter (see fig.1A) clearly co-migrate with the bands in the hypothyroid soleus sample (fig.1E). The myosin types seen in this 28-day old hypothyroid soleus

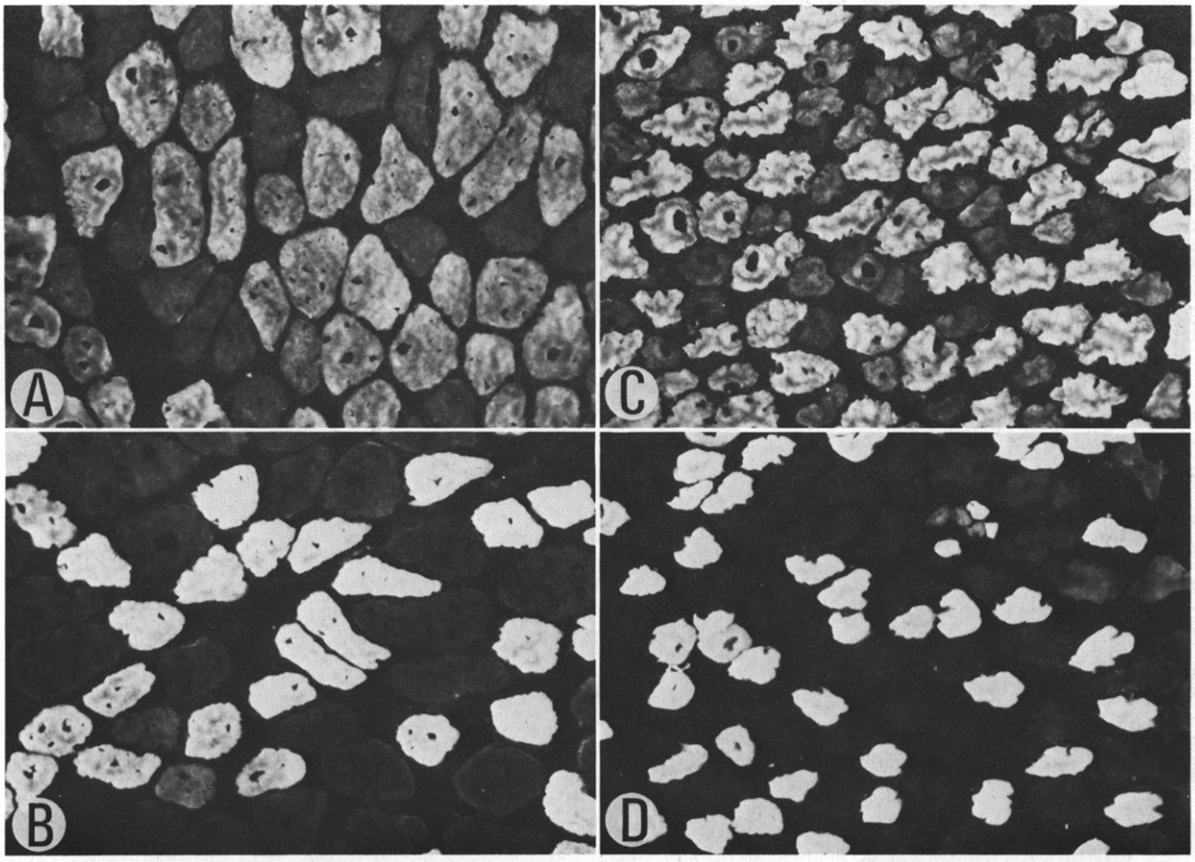

Fig.3. Immunocytochemistry on soleus muscles. Indirect immunofluorescence was carried out on muscles from normal (A,B) and hypothyroid (C,D) 28-day old rats. The antibodies used were against slow (A,C) and neonatal (B,D) myosins. The dark, unstained areas within many of the fibers are due to holes in the sections arising from freezing artifacts during storage and transport.

muscle are thus similar to those found in a normal 7-day old soleus muscle, as shown in [4].

Fig.2 shows the results of immunocytochemical analysis of the 28-day old gastrocnemius muscles. Most of the fibers in the normal muscle are stained with fast myosin antibody (fig.2A) while a smaller proportion are stained by anti-neonatal myosin (fig.2B). A few fibers react with both antibodies. In contrast, almost all the fibers of the hypothyroid gastrocnemius are stained with anti-neonatal (fig.2D). The immunocytochemical analysis also shows that the small amount of fast myosin observed in the electrophoretic analysis (fig.1B) is concentrated in a small proportion of the fibers (fig.2C) rather than being uniformly distributed in all fibers. Those fibers which contain fast myosin also contain neonatal myosin (cf. fig.2C,D).

Fig.3 shows the immunocytochemical results for the 28-day old soleus muscles. More than half the fibers of the normal soleus stain with antibody to slow myosin (fig.3A), while the remaining fibers are stained with anti-neonatal (fig.3B). Neonatal myosin can still be detected by immunocytochemistry at this stage of normal development (fig.3B and [4]), although electrophoresis shows that it must be a minor component in normal 28-day soleus muscles (fig.1D and [4]). In the soleus of 28-day old hypothyroid animals, two populations of fibers can also be clearly seen, one staining with anti-slow and the other staining with anti-neonatal myosin. Thus, the persistence of neonatal myosin in hypothyroid soleus muscles seen biochemically (fig.1E) does not affect all fibers uniformly but rather is localized in a discrete population of soleus muscle fibers.

It is also clear from the immunocytochemical results shown in fig.2,3 that the muscle fibers of the 28-day hypothyroid gastrocnemius and soleus muscles are of smaller diameter than those of normal 4-week old rats. A more detailed analysis of this phenomenon is given in [17].

4. DISCUSSION

We demonstrate here that hypothyroidism in young rats, induced by the anti-thyroid drug methylthiouracil, results in a persistence of neonatal myosin and an inhibition of the appearance of adult-type fast myosin in developing fast and slow muscle fibers until at least 28 days after birth. Thus, in the rat gastrocnemius muscle, neither the accumulation of adult fast myosin nor the disappearance of neonatal myosin requires continued innervation [3], however both processes are severely affected by hypothyroidism. The small amount of fast myosin in the gastrocnemius muscle of hypothyroid animals is localized to a discrete population of fibers. This may indicate that some fibers are partially resistant to the effects of drug-induced hypothyroidism or that they are recovering from the effects of treatment more rapidly than the majority of fibers.

The biochemical and immunocytochemical results concerning the presence of slow myosin in the hypothyroid soleus can be interpreted within the context of our study of myosin types in developing soleus muscles [4]. We have suggested that all muscle fibers can undergo the embryonic $\longrightarrow$ neonatal $\longrightarrow$ adult fast transitions, but that at any time the accumulation of slow myosin can be induced. About half of all soleus fibers contain slow myosin as early as 1 week after birth as determined by immunocytochemistry [4]. A second population of fibers acquires slow myosin only at later times, starting at 3–4 weeks after birth [4] and continuing until at least 6 months of age [6]. Thus hypothyroidism may be affecting only this latter population of fibers since neonatal myosin is found in about half the fibers in the 28-day hypothyroid soleus. The presence of slow myosin in the remaining fibers may be actively promoted by the effects of hypothyroidism, as suggested by results on adult rat muscles [12,13]. Alternatively it may be independent of the thyroid status in developing animals. Neuroendocrinological control of thyroid hormone levels occurs after birth in rats [18] and it is thus possible that slow myosin accumulates in some of the normal soleus fibers before this time.

Finally, it is not certain that hypothyroidism directly affects the developing muscle fibers. It is known that postnatal maturation of the central nervous system is critically dependent on thyroid hormone levels [19] and this might influence the neuromuscular system. In addition, both nerve cells and muscle fibers could be subject to the secondary influences of lowered thyroid hormone levels. The postnatal increase in growth hormone is regulated in part by thyroid hormone status [20]

and the effects of growth hormone are thought to be largely indirect, acting via the production of somatomedins [21]. Thus the endocrinological control of myosin transitions during muscle development may be under the influence of factors other than thyroid hormone per se.

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