

Thyroidal and neural control of myosin transitions during development of rat fast and slow muscles

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Experiments with developing euthyroid, hypothyroid and hyperthyroid rats show that the transition from neonatal to adult fast myosin is orchestrated by thyroid hormones acting directly upon fast muscle cells. Denervation studies reveal the switch from neonatal to adult fast myosin synthesis is independent of the motoneuron. However the synthesis of slow myosin during development is critically dependent on innervation.

Myosin Isozyme Transition Control Thyroxine Denervation

1. INTRODUCTION

In [1] a sequence of embryonic and neonatal myosin heavy chains is described which appear prior to the onset of adult fast myosin synthesis in the rat hindlimb. In the present study we have investigated how switching between the production of these distinct myosin isozymes is controlled and how motoneurons and thyroxine modulate the expression of fast and slow forms of myosin in the developing rat soleus, extensor digitorum longus (EDL) and gastrocnemius muscles.

2. MATERIALS AND METHODS

Pyrophosphate gel electrophoresis of myosin was performed on slab gels [2]. For scanning, gels were stained with Coomassie brilliant blue, R-250, individual lanes were digitised in a two-dimensional matrix on a microdensitometer with a step size of 37.5 μ m. Resolution of band peaks was enhanced using a computer program for a con-

strained iterative deconvolution scheme [3]. Peptide maps were prepared by a method modified from [4].

3. RESULTS

3.1. Normal development

We have adopted and modified a nomenclature for myosin isozymes introduced in [5]. Adult fast isozymes are FM1–FM3, and adult slow isozymes are SM1 and SM2, in order of decreasing mobility. Myosins from the embryonic and neonatal stages are f1–f4 and s1 and s2.

At birth, the EDL and gastrocnemius, presumptive fast twitch muscles, contain myosins f1–f4 (fig.1a). Isozymes f3 and f4 are the principal constituents until 5 days postpartum; the proportions of isozyme f4 then decline, and it is a minor band by 10 days. Reciprocally, f1 increases and by 10 days f1, f2 and f3 dominate the gel. Faint traces of a slow component, SM1, are present at all stages.

Between 10 and 15 days the relative proportions of f1–f3, the neonatal components [1], dramatically decline; they are minor constituents at 15 days and are not present by 25 days. They are replaced by the adult fast isozymes FM1–FM3, which

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Results of this report have previously appeared in abstract form [J. Cell Biol. (1982) 95, 366a]

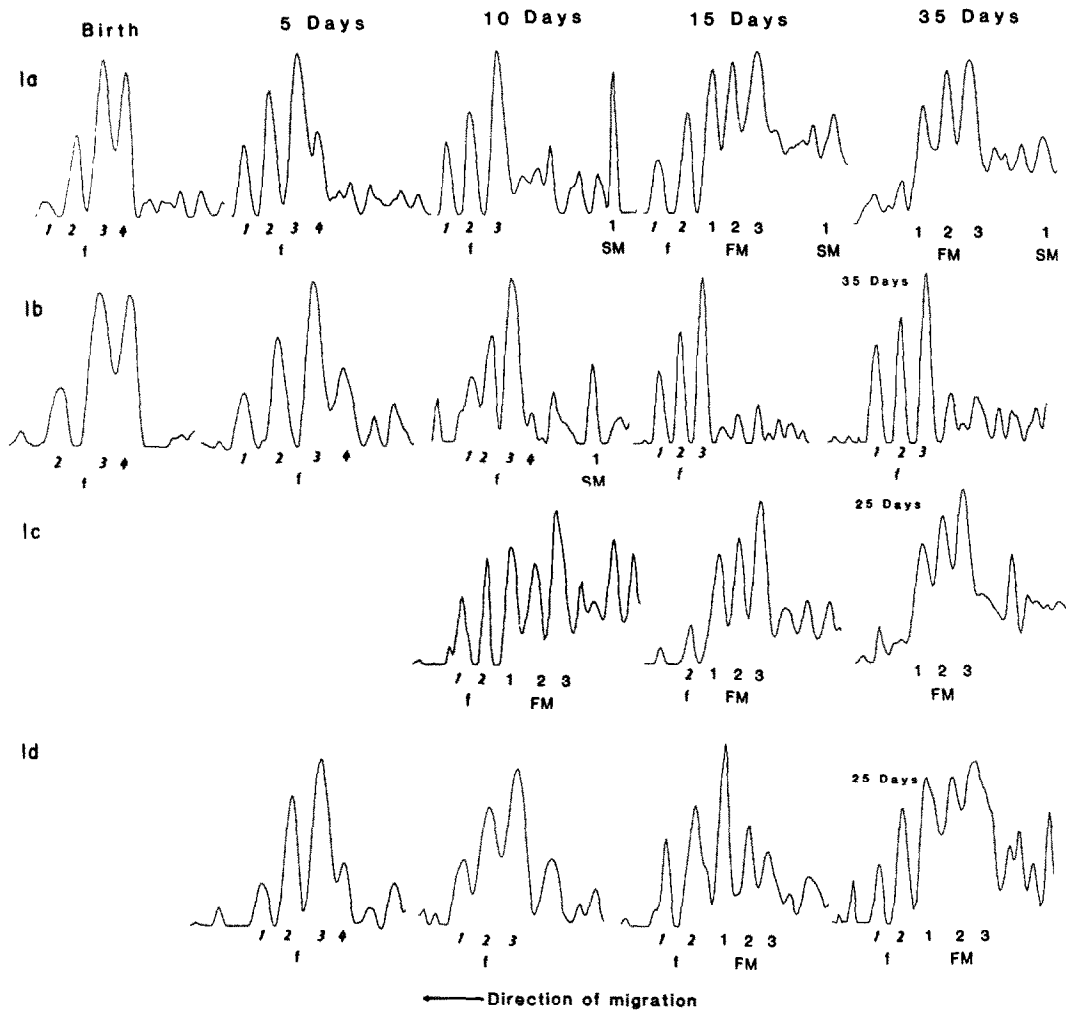


Fig.1. Myosin isozymes of the developing fast EDL. Native myosins were separated on non-denaturing 20 mM pyrophosphate slab gels at pH 8.8 (f1-f4, fetal and neonatal 'fast' isozymes; FM1-FM3, adult fast isozymes). (a) Developing euthyroid animals; FM1-FM3 normally appear at 15 days. (b) Isozymes of rats made hypothyroid from 3 days gestation. FM1-FM3 have not appeared by 35 days. (c) Isozymes of animals made hyperthyroid from 3 days postpartum. FM1-FM3 appear precociously at 10 days. (d) Isozymes of rats denervated at birth. FM1-FM3 appear, but are delayed by 5-10 days.

abruptly appear and dominate the gel at 15 days. These results are similar to, but more complex than, those reported for bulk fast muscle in developing rats [1].

The pattern of differentiation of the slow twitch soleus (fig.2a) has similarities to that of the EDL and gastrocnemius, but at birth and even at 20 days gestation [2] both the upper and lower regions of gels reveal these muscles are already committed to their unique paths of specialization. In contrast to the fast muscles, components f2, f3 and f4 in the

lower part of the gel are most prominent in the newborn soleus. These persist until 15 days and then disappear.

Relative amounts of slow isozymes, s1 and s2, are greater in the fetal soleus than in the fast muscles but cannot be resolved into distinct bands until 5 days. Migration rates of s1 and s2 are distinct from SM1 and SM2 which appear at 10 days. This coincides with the start of antigravity movements by the animal. In the adult, SM2 is the predominant species.

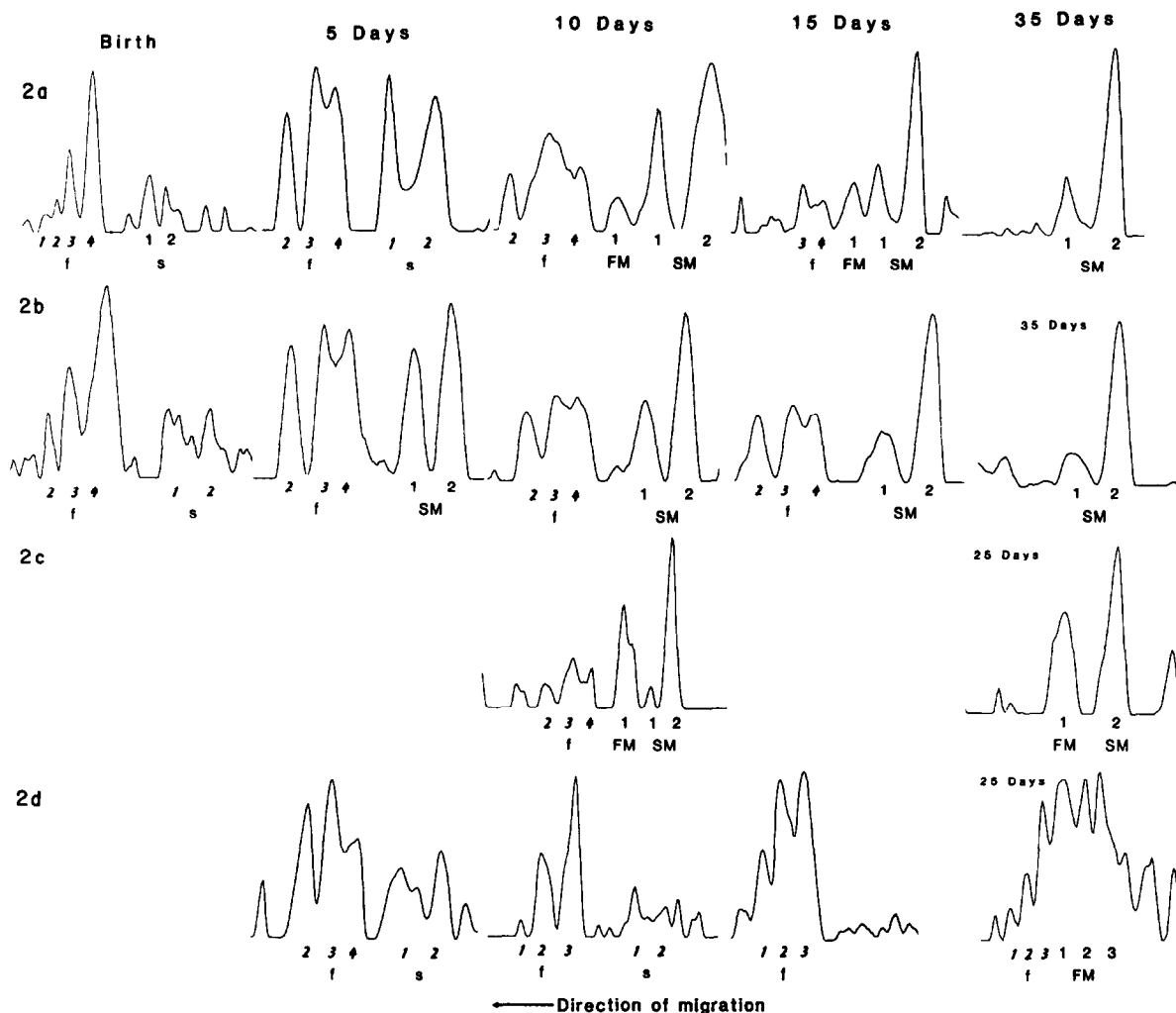


Fig.2. Myosin isozymes of the developing slow soleus (s1-s2, fetal and neonatal 'slow' isozymes; SM1-SM2, adult slow isozymes). (a) Developing euthyroid animals; SM1-SM2 appear at 10 days. (b) Isozymes from hypothyroid animals. SM1-SM2 appear 5 days earlier than in control animals. Neonatal fast isozymes are still present at 35 days. (c) Isozymes from hyperthyroid rats. FM3 appears precociously at 10 days. SM2 does not increase in proportion with development. f1-f4 are no longer present at 15 days. (d) Isozymes from neonatally denervated soleus. SM1-SM2 do not appear. Slow isozymes are imperceptible by 25 days.

3.2. The role of thyroid hormones during development

Serum T4 levels show that the rat is essentially hypothyroid at birth (fig.3). There is a significant rise to peak T4 serum levels at 15 days followed by a slight decline to mature values at 35 days. Thus peak plasma T4 levels coincide with the switch from neonatal to mature fast isozymes.

In view of this, we investigated the effects of hypo- and hyperthyroidism upon the myosin

isozymes in developing muscle. Hypothyroidism was induced with propylthiouracil (PTU) [6] and a low iodine diet (Dyets, Bethlehem PA). Serum T4 levels were undetectable from birth to 15 days and the animals remained severely hypothyroid at 25 days (fig.3). Birth weights of hypothyroid animals were slightly lower than controls; this moderate retardation persisted until 15 days, after which hypothyroid animals stopped growing and clinically became cretinous.

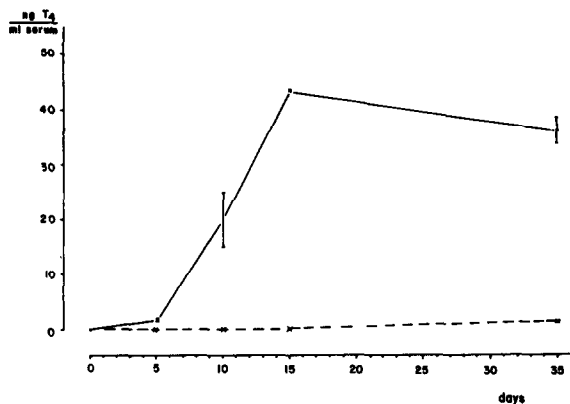


Fig.3. Serum T4 levels of euthyroid and hypothyroid rats during development. Specific RIA measurements of T4 levels at birth, 5, 10, 15 and 35 days. Control rats (—) show a significant rise to peak T4 levels at 15 days. In PTU-treated rats (---), serum T4 levels were undetectable from birth to 15 days. Very low levels of T4 were present at 35 days.

Pyrophosphate gel analyses of the EDL from hypothyroid animals (fig.1b) reveal no distinctions from normal patterns at birth, 5 and 10 days, suggesting the thyroid plays no significant role in modulating the switch from fetal to neonatal rat myosin synthesis. But in hypothyroid animals the adult set of isoforms do not form between 10 and 15 days, and the neonatal isoforms f1–f3, continue to dominate the gel until at least 35 days. At this stage traces of the adult fast isoform FM3 are also present.

In the soleus of hypothyroid animals, neonatal myosin also persisted to 35 days (fig.2b). The slow components of the developing, hypothyroid soleus were indistinguishable from control at birth but achieved the mobility of SM1 and SM2 by 5 instead of 10 days post partum.

To see if addition of exogenous thyroxine at early stages caused precocious maturation, we injected rat pups with 25 μ g T4 on days 3, 5, 7 and 9. At 10 and 15 days serum T4 levels in these animals were above the range of sensitivity of our radioimmunoassay (1 μ g/ml). The switch from neonatal to adult fast myosin was accelerated, for at 10 days FM1–FM3 were the dominant species in the EDL and gastrocnemius and miniscule proportions of neonatal myosin were present by 15 days (fig.1c). In the soleus, neonatal myosin also was virtually eliminated by 15 days (fig.2c).

To see if the thyroidal effect on fast skeletal muscle differentiation is directly imposed on the tissue or indirectly mediated through the nerve, we studied the consequences of neonatal denervation in euthyroid and hyperthyroid animals, by removing a 2 mm segment of the proximal sciatic nerve within 12 h of birth.

Myosin isoforms obtained from denervated muscle are shown in fig.1d and 2d and agree with [7,8]. Despite denervation atrophy, the EDL progresses through its developmental sequence from fetal to neonatal to adult myosin, but the rate of this progression is delayed. This may be due to depression in protein synthesis following denervation. Thus, initiation of mature fast myosin synthesis is not primarily dependent upon the integrity of the motoneuron. Thyroxine acts directly on the muscle cell, for in denervated, hyperthyroid animals the adult isoforms FM1–FM3 appear precociously as in hyperthyroid, innervated fast muscles (fig.1c).

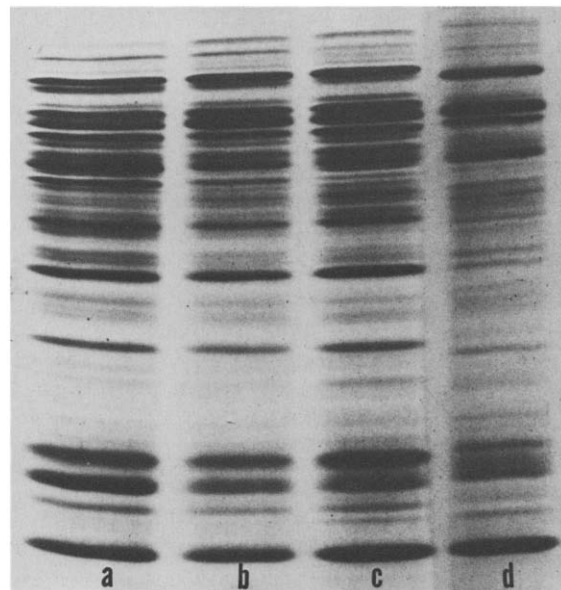


Fig.4. Peptide maps of myosin from euthyroid and hypothyroid rats. Purified myosin from 5 and 25 days euthyroid and hypothyroid gastrocnemius was digested with chymotrypsin (775 μ g/ml) and the peptides separated on SDS–PAGE. Lanes (b) and (d) are from 5 and 25 day euthyroid animals. Lanes (a) and (c) are from 5 and 25 day hypothyroid animals. Lanes (a), (b) and (c) are very similar and distinct from lane (d).

In contrast, slow myosin synthesis in the soleus is sensitive to denervation, for the proportions of slow myosin progressively decline after nerve section, and slow isozymes are no longer detectable by 25 days (fig.2d). At this stage the denervated soleus is composed of myosins which have mobilities similar to those of adult fast isozymes.

Two dimensional gels of fast muscle light chains during development are similar in hypo-, hyper- and euthyroid animals. Elimination of the embryonic light chain is not affected. The target of thyroid control therefore must be the fast myosin heavy chain. Peptide maps of myosins from 5 and 25 day euthyroid fast muscles reveal a clear transition from neonatal to adult fast heavy chains (fig.4b,d). By contrast, maps from 5 and 25 day hypothyroid animals are virtually identical to each other and to the peptide maps from 5 day control animals (fig.4a,c).

4. DISCUSSION

We show there are distinctions in the complement of myosin isozymes between the slow twitch soleus and fast twitch EDL in the rat at birth. This is expressed by preliminary forms of fast and slow myosin. During normal fast muscle development the switch in myosin composition coincides with peak serum levels of T₄, it is inhibited in hypothyroid animals and proceeds precociously in hyperthyroid animals. The findings suggest thyroid hormone orchestrates the transition by activating adult fast myosin synthesis and inhibiting synthesis of neonatal myosin. In developing slow muscle thyroxine probably also turns off synthesis of neonatal myosin for it persists up to 35 days in hypothyroid and is eliminated prematurely in hyperthyroid animals. But our results indicate that thyroxine does not significantly alter the transition from neonatal to adult slow myosin.

Thyroidal regulation of fast muscle maturation appears to be mediated directly upon the muscle cell and is not due to alterations in the physiology of the motoneuron, for despite neonatal denervation the EDL successfully progresses through its maturational sequence and synthesizes the adult set of fast myosin isozymes. Thus, as we have sug-

gested [7], regulation of fast myosin synthesis is not primarily dependent upon integrity of the motoneuron.

By contrast, integrity of the motoneuron is crucial to slow myosin synthesis. After neonatal denervation, proportions of slow myosin in the soleus progressively decline and are no longer detectable by 25 days when the atrophic soleus is composed of myosin isozymes with a mobility very similar to those of adult fast myosin. The present results thus suggest that in the absence of the nerve the prospective slow muscle synthesizes a fast form of myosin which may be under thyroidal control. During normal development the intact slow motoneuron appears to override this propensity and promote synthesis of slow myosin.

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