Time course of myosin heavy chain transitions in neonatal rats: importance of innervation and thyroid state

G. R. ADAMS, S. A. McCUE, M. ZENG, AND K. M. BALDWIN
Department of Physiology and Biophysics, University of California Irvine, California 92697–4500

Adams, G. R., S. A. McCue, M. Zeng, and K. M. Baldwin. Time course of myosin heavy chain transitions in neonatal rats: importance of innervation and thyroid state. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R954–R961, 1999.—During the postnatal period, rat limb muscles adapt to weight bearing via the replacement of embryonic (Emb) and neonatal (Neo) myosin heavy chains (MHCs) by the adult isoforms. Our aim was to characterize this transition in terms of the six MHC isoforms expressed in skeletal muscle and to determine the importance of innervation and thyroid hormone status on the attainment of the adult MHC phenotype. Neonatal rats were made hypothyroid via propylthiouracil (PTU) injection. In normal and PTU subgroups, leg muscles were unilaterally denervated at 15 days of age. The MHC profiles of plantaris (PLN) and soleus (Sol) muscles were determined at 7, 14, 23, and 30 days postpartum. At day 7, the Sol MHC profile was 55% type I, 30% Emb, and 10% Neo; in the PLN, the pattern was 60% Neo and 25% Emb. By day 30 the Sol and PLN had essentially attained an adult MHC profile in the controls. PTU augmented slow MHC expression in the Sol, whereas in the PLN it markedly repressed IIb MHC by retaining neonatal MHC expression. Denervation blunted the upregulation of IIb in the PLN and of Type I in the Sol and shifted the pattern to greater expression of IIa and IIx MHCs in both muscles. In contrast to previous observations, these findings collectively suggest that both an intact thyroid and innervation state are obligatory for the attainment of the adult MHC phenotype, particularly in fast-twitch muscles.

skeletal muscle; fast twitch; slow twitch; hypothyroidism; denervation

ADULT MAMMALIAN SKELETAL MUSCLE is differentiated into distinct fiber types/motor units, each of which possesses a unique combination of functional, biochemical, and metabolic properties (21). A characteristic feature defining this spectrum of fiber types is the type of myosin heavy chain (MHC) isoform that is expressed in a given fiber type. Available evidence suggests that four adult MHC isoforms have been characterized in rodent skeletal muscle, on the basis of their enzymatic/functional, immunohistochemical, and electrophoretic properties (21, 23). These isoforms have been designated as slow (type I) and fast IIa, fast IIx, and fast IIb (13, 21). Muscles used extensively for antigravity function and postural support chiefly express fibers containing either the type I or IIa MHCs (13, 21), whereas fibers chiefly recruited for high-intensity power output for brief periods of time express primarily the IIx and IIb isoforms (13, 21).

In the spectrum of skeletal muscles that normally support either antigravity or locomotor function in rodents, the MHC phenotype is essentially undifferentiated at birth, and it appears that the adult pattern of MHC expression is not reached until ~4–5 wk of age (4, 5). Available evidence further suggests that the process of attaining an adult phenotype is achieved by replacing embryonic/neonatal forms of MHC with different proportions of the adult MHC isoforms depending on the muscle type (5, 15). Previous findings also have provided evidence that this transition to an adult phenotype is dependent on both neural and hormonal factors, e.g., thyroid hormone (3, 8, 15). In particular, it has been reported that the transition to an adult phenotype in a fast muscle, although dependent on an intact thyroid state, can occur independently of the nerve (8, 15, 20). On the other hand, recent studies on adult animals suggest that both denervation and an altered thyroid state (particularly hypothyroidism) induce skeletal muscle to revert to a relatively undifferentiated state of MHC expression with respect to the adult phenotype (11). One limitation confounding some of the earlier work on myosin expression in developing muscles is that analyses were performed chiefly on native myosins, which do not differentiate the specific fast MHCs involved (8). Also, the inability to both quantify and differentiate expression between the fast IIa and IIx MHCs further limited conclusions regarding the role of the nerve on MHC expression (8, 15, 20).

As such, relatively little information is currently available concerning 1) the time course of these transformations in MHC expression during the critical period of neonatal development, 2) the specific MHC isoforms (i.e., 2 developmental and 4 adult) affected by this transformation process during neonatal development, and 3) the separate and combined influences of thyroid hormone and of an intact nerve in mediating these transformations to the adult MHC phenotype in both slow-twitch and fast-twitch muscles. Therefore, the present study was undertaken to test the hypothesis that both an intact nerve and normal circulating levels of thyroid hormone are essential for both slow and fast muscle types to attain their fully differentiated, i.e., adult MHC, profile. Herein we report that denervation alone prevents both slow and fast muscles from fully attaining their respective slow (type I MHC) and fast (IIb) MHC profiles, while manifesting a large bias to the intermediate fast IIa and IIx isoforms in both muscles. Hypothyroidism, in contrast, results in both a delay and marked attenuation of the adult IIb phenotype, in conjunction with the maintenance of neonatal MHC expression in a fast muscle, while augmenting...
METHODS

Animal Care and Experimental Design

Twelve pregnant rats with similar birth windows (i.e., timed pregnancies) were purchased from Taconic Farms, Germantown, NY, and were housed in individual cages in light- and temperature-controlled quarters. After birth, the pups were randomly redistributed to ensure similar litter sizes of ∼10 pups per dam. Litters were then allocated into four treatment categories: 1) normal control (NC), 2) propylthiouracil injected (PTU), 3) NC plus unilateral denervation, and 4) thyroid-deficient (PTU) plus unilateral denervation. Subgroups (n = 8 each) of the NC animals were selected for study at 7, 14, 23, and 30 days of age. The 30-day end point was chosen on the basis of pilot studies that demonstrated that the cardiac and skeletal muscles of rats at this age had attained the adult MHC phenotype. Pups designated to be hypothyroid began receiving daily injections of PTU (12 mg/kg) at 7 days of age. This dose was selected on the basis that in the adult rat, 12 mg/kg approximates three times the dosage necessary to completely block conversion of L-thyroxine (T4) to 3,5,3′-triiodothyronine (T3) (14). Representative pups (n = 8 each) from this experimental group subsequently were killed at 14, 23, and 30 days of age across the litters.

NC and PTU animal subgroups (n = 8 each) assigned to unilateral denervation were anesthetized with ketamine-acepromazine (80-20 mg/kg) at 15 days of age. After an incision in the popliteal region of the left leg, the sciatic nerve was isolated, ligated in two places, and severed between the ligations so that ∼0.5 cm of the nerve was removed to prevent regeneration. Denervation at this level eliminates innervation of both the anterior and posterior compartments of the lower leg and avoids disrupting the structural integrity of targeted muscles and their blood supply. After suturing, each animal was allowed to recover to full ambulation before being returned to the litter. Unilateral denervation was performed to expose both the denervated and contralateral control muscles to the same hormonal condition. These two denervated groups were studied at 30 days of age along with subgroups of the NC and PTU groups. Fifteen-day-old animals were selected for the denervation protocol because pilot experiments revealed that the muscles were still in an undifferentiated state while the development program leading to an adult phenotype was clearly in progress. Thus any impact of denervation could be clearly delineated relative to the intact normal animals at 30 days of age. The protocols used in the present study were approved by our Institutional Animal Review Committee.

Tissue Preparation and Biochemical Analyses

At the designated time points, the rats were killed by a lethal injection of Nembutal (50 mg/kg). For the aforementioned time points a sample of blood was obtained by cardiac puncture and processed for plasma levels of thyroid hormones and insulin-like growth factor (IGF)-I. Then, the heart, soleus, and plantaris muscles were rapidly removed, trimmed of connective tissue, weighed, and stored in a freezer until analyzed for MHC content. Subsequently, each muscle sample was homogenized in a solution that contained 250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 10 mM Tris base. Total protein concentration of the homogenate was performed using a standard biuret assay (10), and the homogenate protein was diluted to 1 mg/ml in a storage buffer containing 50% glycerol, 100 mM Na2PO4, 5 mM EDTA, and 2 mM 2-mercaptoethanol (pH 8.8) and stored at −20°C until subsequent analyses for MHC protein content.

Skeletal MHCs were separated using an SDS-PAGE technique (22). In our hands, this technique enabled us to separate both neonatal and embryonic MHCs from the four adult MHCs (see Fig. 1, A and D). The separating gel contained 30% glycerol, 8% acrylamide, 1.5 M Tris base (pH 8.8), 1 M glycine, and 10% SDS. The stacking gel contained 4% acrylamide, 30% glycerol, 0.5 M Tris·HCl (pH 6.8), 100 mM EDTA, and 0.4% SDS. Protein samples were denatured by placing 5 µg of sample in 35 µl of sample buffer and heating the solution for 2 min at 100°C. The sample buffer consisted of 5% β-mercaptoethanol, 100 mM Tris base, 5% glycerol, 4% SDS, and bromophenol blue. The gels were run at 275 V for ~22 h at 14–16°C. Afterward, the gels were stained with brilliant blue G 250 (Sigma Chemical), destained, and then scanned and quantified using a Molecular Dynamics densitometer (Sunnyvale, CA). The peaks of interest representing the distinct MHC isoforms were identified in the digitized densitometric data sets. The area of each peak was indicative of the relative MHC isoform that was expressed and was determined by integration. In addition to these analyses, samples of cardiac muscle were prepared and analyzed for native myosin content as a verification of thyroid state using techniques described in detail previously (7).

The neonatal and embryonic MHC bands were identified by Western blot analysis. The MHC gel was run according to the above procedure; at the end of the run, the separated protein bands were electrotransferred to a polyvinylidene fluoride membrane (Immobilon-P, Millipore) using a modified Towbin buffer (25 mM Tris, 193 mM glycine, 10% methanol, pH 8.3). After 2-h transfer at 60 V, the gel was stained with Coomassie blue to localize the different MHC bands, and the membrane was immersed in a blocking solution (5% nonfat milk in T-TBS: 0.05% Tween 20, 20 mM Tris·HCl, pH 7.6, 150 mM NaCl). The membrane was then incubated for 1 h at 22°C with the primary antibody at the appropriate dilutions in the blocking solution. At the end of the incubation the membrane was washed with several changes of washing solution (T-TBS). The electrochemiluminescence detection protocol was used according to the company’s instructions using the supplied anti-mouse horseradish peroxidase antibody (Amersham). The cell lines expressing monoclonal antibodies to embryonic (BF-G6) or embryonic + neonatal (BF-B6) MHC were purchased from ATCC. These antibodies allow for the identification of the neonatal MHC, which has a migration intermediate to that of the adult type IIa and IIb isoforms, and embryonic MHC, which demonstrates the slowest migration, appearing above the IIx band (Fig. 1, B and C).

Plasma IGF-I, T3, and T4 Analyses

Whole blood was centrifuged at 1,000 g for 10 min at 4°C. Plasma was stored at −20°C until analyzed for T3 and T4 content by RIA using a commercially available kit (ICN, Costa Mesa, CA). Plasma IGF-I was measured by RIA using commercially available reagents as described previously (1).

Analyses

All data are reported as means ± SE. Statistical differences between the NC and PTU groups across the time points were tested using a one-way analysis of variance, and when differences were detected, a Newman-Keuls post hoc test was used. All statistical analyses were performed using a com-
puter software package (Statmost). Statistical significance was set at $P < 0.05$.

**RESULTS**

**Body and Muscle Weights**

In euthyroid rats between 7 and 30 days of age there was an approximately fourfold increase in body weight that was paralleled by proportional increases in heart, soleus, and plantaris muscle weights (Figs. 2 and 3). In animals treated with PTU, the gains in both body weight and muscle weight are dramatically reduced throughout this time frame and result in a reduction in the normalized skeletal muscle weights at 30 days of age (Fig. 3). In both NC and PTU rats, denervation during this critical period of development significantly reduced (≤50%) both the soleus and plantaris normalized muscle weights compared with contralateral innervated muscles (Fig. 3). Thus both thyroid hormone and an intact nerve are essential for the normal growth of ankle extensor skeletal muscles used extensively in weight bearing and locomotion. Similar changes in muscle mass were seen in the dorsiflexor muscles of the ankle, but the changes were much less dramatic (unpublished observations).

**Evidence of the Hypothyroid State: Thyroid Hormone and IGF-I Analyses**

Consistent with the reduction in body and muscle weight of the PTU-treated animals, there were dramatic reductions in plasma $T_3$, ranging from $-63\%$ at 14 days to $-43\%$ at 30 days of age (e.g., at 23 days values were $80 \pm 3.6$ vs. $36 \pm 3$ ng/dl in control vs. PTU treated). Further evidence of the attainment of a hypothyroid state in the neonatal rats was seen in the expression of cardiac MHC isoforms, which are highly sensitive to thyroid state (12). The predominant MHC in the adult rat heart is the fast $\alpha$-isoform, which normally comprises $85\%$ of the MHC pool (12). However, at birth this isoform represents $<50\%$ of the total MHC present in the heart, with the $\beta$-isoform making up the difference (Fig. 4). In euthyroid rats at 30 days postpartum, the $\beta$-MHC comprised only $7.5 \pm 4.1\%$ of the total MHC pool, whereas in the PTU rats this isoform represented the predominant isoform (Fig. 3). Thus up to the 30-day time point the normal expression of the adult MHC phenotype was almost completely repressed via PTU treatment.

A portion of the reduction in generalized somatic growth observed in the PTU groups may be a result of...
the interaction between thyroid hormone and the growth hormone (GH)-IGF-I axis. Similar to what has been reported by others (17), we found that PTU rats had significantly lower plasma IGF-I levels than controls at both 23 and 30 days of age (Fig. 5). This is a crucial period when IGF-I levels are normally increasing rapidly to support the developmental growth program of the neonate.

Skeletal MHC Analyses

Developmental patterns in NC animals. At 7 days of age, the MHC pattern of expression in the slow-twitch soleus muscle is biased to a predominance of slow type I (55%), embryonic (30%), and neonatal (10%) MHCs, with only traces of any adult fast isoforms being expressed (Table 1; Fig. 6). In the fast-twitch plantaris muscle, ~85% of the MHC pool is expressed as a combination of the neonatal (60%) and embryonic (25%) isoforms, with relatively small amounts of the adult fast and slow isoforms being detected (Table 1, Fig. 7). During the subsequent 3 wk of development, i.e., until 30 days of age, the embryonic and neonatal isoforms are rapidly repressed in both muscles and essentially replaced by type I and IIa MHCs in the soleus muscle and by the type IIx and IIb MHCs in the plantaris (Figs. 6 and 7). In the soleus the stoichiometry of these transformations suggests that the embryonic form may be the precursor to further increments in expression of the type I MHC. In the plantaris, it appears that the neonatal isoform is likely accounting for the transition to the predominant expression of the IIb MHC isoform,
whereas the embryonic isoform may be involved in the transition to the other fast isoforms such as the IIx. Thus by 30 days of age the adult skeletal phenotype is clearly established in normal, euthyroid animals.

Effects of hypothyroidism. In the soleus muscle, PTU treatment delays the decline in expression of the neonatal isoform that normally occurs, but it does not have any appreciable impact on the downregulation of the embryonic MHC (Fig. 6). In addition, the PTU treatment appears to blunt expression of type IIa MHC and enhance that of type I MHC. Thus the net effects of thyroid deficiency on the development of the MHC phenotype in the soleus are to transform it to an even slower MHC phenotype.

In the plantaris, a hypothyroid state blunts both the normal decline in expression of neonatal MHC as well as the upregulation of the IIb isoform. This response also is coupled to inhibiting normal expression of the fast IIx MHC, whereas expression of the type I MHC is augmented (Table 1, Fig. 7). Thus thyroid deficiency markedly impairs the normal proliferation of the fast IIx and IIb MHCs in a fast muscle.

Effects of denervation. In the soleus muscle, denervation exerted a primary effect by reducing the relative expression of the type I MHC that is normally attained at 30 days of age, and this response was coupled chiefly to the substantial expression of type IIx MHC and the retention of small amounts of the neonatal isoform (Table 1). This alteration provides additional evidence that MHC transformations in normally developing slow skeletal muscle appear to be under the control of neural (trophic) factors apparently linked to an intact innervation (Table 1).

Similarly, in the plantaris muscle, the effects of denervation during development are seen chiefly in the blunting of the normal expression of the IIb MHC (Table 1). However, unlike thyroid deficiency, this response is associated with the downregulation of neonatal MHC, which appears to be coupled with the augmentation of the relative expression of the type IIx MHC. Thus denervation of a fast muscle during a critical stage of development appears to result in a bias toward expression of putatively slower MHCs as well as isoforms normally found in very young neonatal rat muscles.

Table 1. Soleus and plantaris MHC distribution at 7 and 30 days postpartum

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>IIa</th>
<th>IIx</th>
<th>IIb</th>
<th>Emb</th>
<th>Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>55.8±1.1</td>
<td>2.4±0.4</td>
<td>2.8±0.5</td>
<td>29.3±0.8</td>
<td>9.7±0.5</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>79.4±1.6</td>
<td>18.8±1.4</td>
<td>1.3±0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>86.4±0.7a</td>
<td>8.7±0.5a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Den*</td>
<td>47.8±4.2a,6a</td>
<td>25.1±2.0b</td>
<td>21.7±2.6a</td>
<td>4.3±1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Den + PTU†</td>
<td>73.6±2.4b,c</td>
<td>12.4±1.1bc</td>
<td></td>
<td>13.8±1.5bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>5.6±0.3</td>
<td>6.0±0.4</td>
<td>3.6±0.2</td>
<td>24.3±1.2</td>
<td>61.5±0.6</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>4.7±0.3</td>
<td>11.9±0.7</td>
<td>23.6±0.9</td>
<td>61.1±1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>15.3±0.5a</td>
<td>15.0±0.7</td>
<td>8.3±0.7a</td>
<td>8.8±1.0a</td>
<td>52.6±1.4</td>
<td></td>
</tr>
<tr>
<td>30 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Den*</td>
<td>7.7±0.3a</td>
<td>18.5±3.5a</td>
<td>39.6±1.6ab</td>
<td>30.2±3.1ab</td>
<td>4.0±0.7b</td>
<td></td>
</tr>
<tr>
<td>Den + PTU†</td>
<td>8.1±0.9a</td>
<td>12.2±1.4</td>
<td>7.7±0.8ac</td>
<td>15.5±2.5abc</td>
<td>56.8±2.7c</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. MHC, myosin heavy chain; Emb, embryonic; Neo, neonatal; NC, normal control; PTU, thyroid deficient; Den, denervated. Contralateral MHC distribution was similar to that of *NC or †PTU. P < 0.05: a, vs. NC; b, vs. PTU; c, vs Den.
Combined effects of hypothyroidism and denervation. When the intervention of denervation was combined with PTU treatment, their contrasting influences on the slow soleus muscle appear to cancel one another in terms of type I MHC expression, as this isoform appeared to be in a similar range as that seen for the normal euthyroid rat (Table 1). However, the upregulation of the IIa isoform that occurs during normal development in the soleus muscle was markedly reduced, and this response was offset by the maintained expression of neonatal MHC (Table 1). Thus, although the slow MHC phenotype is still manifest in hypothyroid-denervated soleus muscle, the presence of abnormal amounts of the other isoforms clearly suggests that the muscle is in a partially undifferentiated state relative to the adult phenotype.

As presented in Table 1, a similar altered response is apparent for the plantaris muscle in that the combination of hypothyroidism plus denervation severely inhibited the full manifestation of both the fast IIb and the IIx MHC isoforms, and this response was associated with the retention of significant amounts of neonatal MHC in addition to the slight augmentation of the type I MHC. Thus the combination of hypothyroidism and denervation also maintains the developing fast plantaris muscle in a relatively slow and undifferentiated state relative to the normal condition.

DISCUSSION

There were several key findings in the present study: 1) With respect to the adult MHC phenotype, skeletal muscle is undifferentiated shortly after birth (days). In particular, muscle types used more extensively for high power output activities (plantaris) are less differentiated than those types (soleus) used more extensively in antigravity function. 2) The postnatal transformation to the adult MHC phenotype appears to involve stoichiometric changes between the fast IIb/IIx and neonatal isoforms in the development of fast-twitch muscles, whereas the full manifestation of slow type I MHC expression in a slow-twitch muscle appears to coincide with the downregulation of the embryonic isoform. 3) The optimal expression of the fastest isoform (s), e.g., IIb, requires the presence of both intact innervation and a normal thyroid state. 4) Normal body and muscle growth are compromised by thyroid deficiency, and this appears to be associated, in part, with the depression of circulating IGF-I levels, which are a critical factor in the developmental cascade of body and organ growth (e.g., Ref. 9).

Whereas previous findings clearly show that myofiber formation in rodents, particularly the development of the contractile apparatus, largely occurs during the third trimester of fetal development (3, 4), the present findings further suggest that a critical period during the first 4 wk following birth is necessary for attaining the adult MHC phenotype typically expressed in this species. These results, while extending those of others (5, 8, 15, 20), further show that muscle types (e.g., soleus) used more extensively for ground support activities are more mature early on after birth in terms of expressing the adult MHC phenotype relative to their fast-twitch synergetic counterparts that are called on primarily to support high-intensity locomotor activities.

Previous studies involving the development of skeletal muscle during the fetal period also have suggested that there are two critical stages of myofiber expression: 1) an early stage designated as “primary” (slow) fiber formation in which embryonic to slow MHC transitions occur (3) and 2) a later stage designated as “secondary” fiber formation in which neonatal myosin becomes expressed in fibers and serves as a precursor to the eventual expression of type II fibers (3, 4). On the basis of the findings reported herein, it is apparent that neonatal and embryonic isoforms, at least in lower leg extensor muscles, are extensively maintained in expression well into the neonatal stage of development, because at 7 days of age, these two precursor MHC isoforms collectively comprise ~85% of the total MHC pool within the plantaris muscle. In fact, only trace amounts of the adult fast IIb (and IIx) isoforms initially are expressed, i.e., the two chief MHC isoforms normally expressed in adult fast-twitch muscles. These data thus suggest that the transition to fully manifesting the so-called secondary fibers (i.e., chiefly type II fibers) largely occurs during a 3- to 4-wk interval following birth, as illustrated by the pattern of neonatal/IIb MHC changes that are occurring in the fast-twitch plantaris muscle (Fig. 5).

Previous findings have also implicated the important role of thyroid hormone (T3), as well as the involvement of an intact nerve, in mediating the transition from the neonatal condition to the adult phenotype (3, 8, 15, 20). Studies focusing on thyroid hormone suggest that T3 is essential for the normal expression of the adult fast native myosins, (i.e., undenatured myosin protein bands
containing combinations of the light and heavy chain moieties as either hetero- or homodimer complexes (8). However, the analytic approaches used in these studies do not allow one to distinguish which of the adult fast type II MHCs are affected by this transformation. The present study attempts to build on this background by showing that T3 exerts a significant effect on the transition of MHC expression that is both muscle type and MHC isoform specific. For example, by blunting the overall surge in the thyroid axis that normally occurs during development with PTU treatment (6), the time course of downregulating expression of the neonatal MHC isoform is prevented in both the plantaris and soleus muscles, whereas the effects on the embryonic MHC isoform are much less evident, particularly in the soleus muscle (Figs. 6 and 7). These effects due to hypothyroidism on the neonatal MHC isoform transformation in the soleus muscle are seen chiefly in the augmented expression of the type I MHC, while that of the IIa MHC is significantly repressed (Fig. 6, Table 1).

In the plantaris muscle, the effects of PTU treatment on the expression of the neonatal MHC isoform are seen chiefly in blunting the expression of both the IIb and IIx MHC isoforms. Consequently, these data on the PTU-treated animals provide further evidence to suggest that the transition to expressing the type II MHC isoforms in both fast-twitch and slow-twitch muscles is in some way coupled to the downregulation of the neonatal isoform. Clearly these transformations are under the regulation of T3; however, more research is necessary to define the mechanism of this process.

The effects of denervation on MHC expression in the neonatal state are not as clear cut. For example, studies by both Russell et al. (20) and Gamke et al. (8) suggest that denervation, either at birth (20) or during early neonatal development (8), does not impair the normal transition to expressing either the fastest native isoforms (8) or the fast IIb MHC isoform (20). However, an intact nerve appears to be necessary for manifesting a normal neonatal-to-IIa MHC transition (20). However, a confounding problem with these previous studies concerns their inability to differentiate, in a quantitative sense, the response of the various adult fast MHCs, i.e., the relative expression of IIa, IIx, and IIb isoforms in response to these interventions (8, 20). Using the electrophoretic analytic approaches described herein, it is apparent that denervation dramatically impairs the normal developmental program governing expression of 1) slow type I MHC in a slow-twitch muscle and 2) fast IIb MHC in a fast-twitch muscle. Both these events appear to be linked to markedly augmenting the relative expression of the IIa and (especially) the IIx MHCs in both types of muscle (Table 1). Thus these data provide the novel perspective that although so-called primary (slow) fiber development in the embryonic/fetal state does not require the presence of the nerve (4), innervation appears to be obligatory for fiber differentiation that occurs in the neonatal state leading to the optimal expression of the adult phenotypes involving either the slow type I or fast IIb MHCs.

Furthermore, denervation impacts the developing muscle in a different fashion compared with that seen in thyroid deficiency. In the soleus muscle, denervation significantly inhibits the normal expression of type I MHC, whereas it augments expression of the IIa MHC and causes the de novo expression of the IIx isoform, as well as apparently inducing some reexpression and/or maintained expression of neonatal MHC (Table 1). This response is in marked contrast to that seen with PTU treatment as indicated above, i.e., slow MHC is augmented while the IIa MHC is repressed. Interestingly, when PTU treatment and denervation are combined, the two interventions appear to oppose one another in terms of the pattern of MHC expression that is attained in the soleus muscle, i.e., the MHC phenotype more closely resembles that observed in the normal adult state (Table 1).

In the plantaris, the effects of denervation are seen chiefly as a blunting of the expression of the IIb MHC while expression of the IIx, IIa, and type I MHCs are increased, whereas, under thyroid deficiency alone, the IIb MHC is repressed at the expense of retaining expression of the neonatal isoform. Interestingly, when the two treatments are combined, they appear to further repress expression of the IIb MHC while elevating expression of the type I MHC, the latter of which appears to be coupled with retaining expression of the neonatal isoform. Thus these findings clearly suggest that both hormonal (T3) and trophic, i.e., nerve mediated, factors can play both similar and contrasting roles in establishing the adult MHC phenotype, which is both isoform and muscle type specific.

In addition to the muscle-specific (i.e., MHC phenotype) effects of hypothyroidism, the PTU rats experienced a general decline in their rate of somatic growth. As noted, PTU treatment was associated with a marked deficit in circulating levels of IGF-I (Fig. 5), which is not attributable to any known direct effects of PTU on the GH-IGF-I system. This deficit in IGF-I may have contributed to the reduction in generalized somatic growth observed in the PTU groups (Fig. 2). However, others have reported that in hypothyroid rats, administration of GH alone is not sufficient to normalize circulating IGF-I levels (e.g., Ref. 2). This suggests that thyroid hormone may regulate components of the IGF-I system. In support of this concept, Nanto-Salonen and colleagues (17–19) have reported that hypothyroidism in neonatal rats disrupts normal developmental patterns of IGF-I and -II peptide expression and the IGF binding proteins.

There is also evidence that suggests that T3 and IGF-I may converge to impact some of the same processes in skeletal muscle (16). For example, Thelen et al. (24) have found that T3 and IGF-I act synergistically to increase sarcoplasmic reticulum Ca2+-ATPase levels in L6 cells, and Muscat et al. have reported a series of findings (reviewed in Ref. 16) that show that IGF-I, T3, and/or retinoic acid can induce myogenic cell lines to exit the proliferation phase and fuse into myotubes. In addition, thyroid response elements have been identified in genes that are putative targets for IGF-I signal-
ing such as the myoD, myogenin in myogenic cell lines (16). Taken together, these various reports indicate that thyroid state directly affects the IGF-I axis impacting the somatic growth program and that interactions between IGF-I and T₃ signaling pathways may mediate muscle-specific development as well.

In summary, the findings reported herein clearly indicate that rat hindlimb skeletal muscle is in an undifferentiated state following birth, and ~30 days of postnatal development are necessary for the attainment of the adult contractile protein phenotype. Furthermore, both an intact thyroid and innervation states are necessary to mediate this response, although their individual effects appear to be both muscle type and MHC isoform specific. Finally, it appears that there may be synergistic effects between T₃ and IGF-I that play a pivotal role in regulating somatic growth as well as tissue-specific cellular processes impacting phenotypic protein expression.

Perspectives

The collective results of the present study clearly show that the neonatal rat provides a useful model to examine fundamental mechanisms governing muscle plasticity, i.e., the ability of the cell to alter the quantity and quality of the protein milieu it expresses. Furthermore, findings reported herein, as well as unpublished findings, clearly indicate that factors such as thyroid state, innervation state, and the degree of weight-bearing activity chronically imposed on developing muscle are playing a critical role in regulating both muscle growth and contractile protein gene expression. Consequently, future studies using the neonatal animal model in combination with the separate and combined interactions of thyroid hormone, innervation, and load-bearing activity have the potential to provide insight into the subcellular/molecular mechanisms involved in both muscle growth and transcriptional control of MHC genes.

The authors acknowledge the assistance of Sepideh Najaran and Terri Rembuskos.

This study was supported by National Institutes of Health Grant NS-33483 and National Aeronautics and Space Administration (NASA) Grant NAG2–555 (to K. M. Baldwin) and NASA Grant NAGW-4471 (to G. R. Adams).

Address for reprint requests: K. M. Baldwin, Dept. of Physiology and Biophysics, Univ. of California Irvine, 346-D Medical Sciences 1, Irvine, CA 92697–4560 (E-mail: km baldwin@uci.edu).

Received 11 June 1998; accepted in final form 7 December 1998.

REFERENCES


