This study examined nuclear thyroid receptor (TR) maximum binding capacity ($B_{max}$), dissociation constant ($K_d$), and TR isoform ($\alpha_1$, $\alpha_2$, $\beta_1$) mRNA expression in rodent cardiac, "fast-twitch white," "fast-twitch red," and "slow-twitch red" muscle types as a function of thyroid state. These analyses were performed in the context of slow-twitch type I myosin heavy-chain (MHC) expression, a 3,5,3'-triiodothyronine ($T_3$)-regulated gene that displays varying responsiveness to $T_3$ in the above tissues. Nuclear $T_3$ binding analyses show that the skeletal muscle types express more TRs per unit DNA than cardiac muscle, whereas the latter has a lower $K_d$ than the former. Altered thyroid state had little effect on either cardiac $B_{max}$ or $K_d$, whereas hypothyroidism increased $B_{max}$ in the skeletal muscle types without affecting its $K_d$. Cardiac muscle demonstrated the greatest mRNA signal of TR-$\beta_1$ compared with the other muscle types, whereas the TR-$\alpha_1$ mRNA signals were more abundant in the skeletal muscle types, especially fast-twitch red. Hyperthyroidism increased the ratio of $\beta_1$ to $\alpha_1$ and decreased the ratio of $\alpha_2$ to $\alpha_1 + \beta_1$ mRNA signal across the muscle types, whereas hypothyroidism caused the opposite effects. The nuclear $T_3$ affinity correlated significantly with the TR-$\beta_1$ mRNA expression but not with TR-$\alpha_1$ mRNA expression. Collectively, these findings suggest that, despite a divergent pattern of TR mRNA expression in the different muscle types, these patterns follow similar qualitative changes under altered thyroid state. Furthermore, TR expression pattern cannot account for the quantitative and qualitative changes in type I MHC expression that occur in the different muscle types.

Heart; slow-twitch muscle; fast-twitch muscle; maximum binding capacity; hyperthyroidism; hypothyroidism; thyroid receptor mRNA

Thyroid hormones exert profound effects on striated muscle, and they produce alterations in gene and isoform expression that result in modified contractile/mechanical and metabolic characteristics (2, 3, 8, 22, 24, 27). The biological action of thyroid hormones is thought to be mediated in part via interaction of 3,5,3'-triiodothyronine ($T_3$) with high-affinity receptors [thyroid receptors (TRs)] located in the nucleus (7, 8, 10, 11, 23, 35). The TRs are ligand-dependent transcription factors that regulate gene transcription via interaction with specific DNA sequences known as thyroid-responsive elements (TREs) located in the promoter of $T_3$-responsive genes (3, 11). Nuclear TRs are products of the cellular erythroblastosis A (c-erbA) protooncogene and are members of a superfamily of nuclear receptors that includes the steroid hormones, vitamin D, and retinoic acids (3, 7, 10, 11, 23, 35). These receptors collectively regulate the transcription of complex gene networks and subsequently control diverse aspects of growth, development, and differentiation (3, 7, 10, 11, 17, 23, 35). To date, two distinct TR genes have been characterized, namely the $\alpha$- and $\beta$-genes (3, 7, 10, 11, 23, 28). The TR-$\alpha$ gene produces two alternatively spliced mRNA isoforms, $\alpha_1$ and $\alpha_2$, which are translated into a functional receptor, TR-$\alpha_1$, and a non-ligand-binding protein, erbA-$\alpha_2$, respectively (7, 10, 11, 17, 19). The TR-$\beta$ gene also produces two alternatively spliced mRNA isoforms, $\beta_1$ and $\beta_2$, both of which are translated into functional TRs, but TR-$\beta_2$ is expressed primarily in the anterior pituitary gland (10, 11, 17, 23, 28). TR-$\alpha_3$, TR-$\beta_1$, and erbA-$\alpha_2$ mRNA are widely distributed among tissues, including striated muscle, and their expression is developmentally regulated in a tissue-specific manner (11, 14, 15, 23).

Thyroid hormones are among the most potent regulators of muscle gene expression, and their effects appear to dominate other regulatory factors such as mechanical activity and metabolic state (3). TREs have been identified on several muscle genes including the glucose transporter (GLUT-4) gene, the sarcoplasmic reticulum calcium-ATPase (SERCA) genes, the $\alpha$-actin gene, and the $\alpha$- and $\beta$- (type I) myosin heavy-chain (MHC) genes (see Ref. 3 for review). For example, the type I MHC gene is the most widely expressed MHC isoform in striated muscle, and its expression in the heart and skeletal muscle appears to be tightly regulated by thyroid hormone at the pretranslational level (2, 12, 16) as well as at the transcriptional level (9, 29, 31). Specifically, type I MHC expression is downregulated by $T_3$, and this repression has been linked to a putative negative TRE located in proximity to the TATA box of the gene (9).

The rodent heart is most unique in its properties in the euthyroid state. It is characterized by a high intrinsic contractility state, high myosin ATPase activity, and high oxidative capacity that can only be mimicked in larger animals under a hyperthyroid state (27).

Hypothyroidism severely alters these properties and transforms the rodent heart into a hypokinetic state (8). These changes involve a large number of genes expressed in the heart, including the myofibrillar proteins, metabolic enzymes, transporters, receptors, channels, and other regulatory genes (see Ref. 3 for review; Ref. 8). In contrast, in response to hyperthyroidism, the hyperkinetic functional state of the rodent heart is upregulated even further, but the magnitude of change is much smaller than that seen in the thyroid hormone-induced transformations. Thus these observations suggest that the rodent heart is more sensitive to a hypothyroid state than to a hyperthyroid state. In contrast, studies examining the effects of thyroid hormone on gene expression in skeletal muscles suggest...
that T<sub>3</sub> exerts a differential effect on gene expression in different types of muscle fibers (3, 12, 16). Other studies suggest that, among skeletal muscle types, slow-twitch muscle is more sensitive than fast-twitch muscle to the effects of thyroid hormone (22, 24). The molecular basis of these differences is not clear, but they suggest differences in the thyroid hormone receptors expressed in these muscles.

Presently, relatively little is known concerning 1) the relative level of expression of TRs and their isoform species and 2) the plasticity of TR levels in response to altered thyroid states in different types of striated muscle. Therefore, in view of the key role that TRs play as a transcriptional factor in muscle gene regulation, the present study was undertaken to examine nuclear T<sub>3</sub> binding capacity and the pattern of TR mRNA expression in the rodent heart and skeletal muscles of varying fiber type as a function of thyroid state. Also, we investigated the possible existence of differential regulation of TR mRNA expression by T<sub>3</sub> in the different types of striated muscle. These determinations were performed in the context of analyses of type I MHC expression, which served as a marker gene that is highly sensitive to thyroid state in different types of skeletal muscle.

METHODS

Animal care and experimental groups. Seventy-two adult female Sprague-Dawley rats (240–250 g; Taconic Farms, Germantown, NY) were randomly separated into three groups (n = 24 each) designated as normal control (NC), hypothyroid (PTU), and hyperthyroid (T<sub>3</sub>). The animals were housed in groups of four in light- and temperature-controlled quarters and were fed food and water ad libitum. Hypothyroidism was induced with propylthiouracil (PTU; 12 mg/kg ip daily), whereas hyperthyroidism was induced with T<sub>3</sub> (150 µg/kg ip). The isola-

Isolation of cardiac and skeletal muscle nuclei. For each individual sample, the reaction was run in duplicate for each of the tested [125I]T<sub>3</sub> concentrations. B<sub>max</sub> was expressed in both femtomoles per milligram nuclear DNA and femtomoles per gram tissue. For the latter, tissue total DNA concentration was used in the conversion.

RNA and protein extraction. Total cellular RNA and total muscle proteins were simultaneously coextracted from frozen muscle samples by using the TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the company’s protocol, which is based on the method described by Chomczynski (6). Total proteins were separated in the organic phase and subsequently precipitated with isopropanol, washed with guanidine hydrochloride and ethanol, and suspended in 1% SDS. Protein concentration was adjusted to 1 mg/ml with 1% SDS, and samples were subsequently analyzed for MHC isoform composition as determined by SDS-PAGE (38). Extracted RNA was precipitated from the aqueous phase with isopropanol, washed and suspended in a small volume of 0.1 M Tris, pH 8.0, and 1 mM EDTA. The RNA concentration was determined by optical density at 260 nm (using an OD<sub>260</sub> unit equivalent to 40 μg/ml). The RNA samples were stored frozen at −80°C until subsequent processing for Northern hybridization.

Northern analysis for mRNA. The Northern procedure used to identify MHC and TR mRNA isoforms was essentially as reported previously (13). For the MHC mRNA analyses, 32P-labeled 5′-end-labeled oligonucleotides, highly specific for
each MHC isoform, were used in the hybridization (12). For TR mRNA analyses, 32P-labeled random-primed cDNA probes were used. Plasmids for the α1-, α2-, and β1-TR mRNA isoforms were kind gifts from Dr. Ronald Evans (The Salk Institute, San Diego, CA), Dr. Mitchell Lazar (Harvard Medical School, Boston, MA), and Dr. Howard Towle (University of Minnesota, Minneapolis, MN), respectively. After hybridization, band intensities on the autoradiogram were quantified using a laser-scanning densitometer (Molecular Dynamics, Sunnyvale, CA), and each specific mRNA signal (MHC or TR) was normalized to its corresponding 18S rRNA signal.

Statistical analyses. All data are reported as means ± SE. All statistical analyses were performed using a computer software package (Prism, GraphPad Software). For each variable analyzed, differences among groups were determined using one-way ANOVA and Newman-Keuls post hoc tests. Statistical significance was set at the 0.05 probability level.

RESULTS

Confirmation of altered thyroid state. The PTU group exhibited a marked, significant reduction in circulating T3 and T4 levels relative to the euthyroid group (NC), whereas the T3 group responded with elevated levels of plasma T3 obtained 24 h after the last injection (Table 1). The low T4 levels in this group were attributed to a T3-induced inhibition in T4 production due to the high weight data paralleled the T3 data (Table 1), which exhibited a marked, significant reduction in circulating T3 and T4 levels relative to the euthyroid group (NC). The absolute and relative heart protein data do not fully reflect the impact of altered hormone on cardiac MHC expression, whereas cardiac nuclei had a greater T3 affinity for T3 as an index of TR number and was expressed on the basis of nuclear DNA as well as per unit muscle mass. These data (Table 2) revealed collectively that the three types of skeletal muscle have a greater Bmax than cardiac muscle as expressed per milligram of nuclear DNA, whereas cardiac nuclei had a greater Kd than skeletal muscle nuclei. However, when Bmax is expressed on the basis of muscle mass, the data show that slow-twitch skeletal muscle contains a greater density of TRs than the fast-twitch types (Table 2). This difference likely has to do with slow-twitch skeletal muscles having more nuclei per cell volume compared with their fast-twitch counterparts (1).

Type I MHC mRNA and protein expression. Type I MHC (β-MHC) is negatively regulated by T3 at the transcriptional level (3, 9, 31), and the results presented in the current study agree with this notion in that hyperthyroidism upregulated type I MHC (β-MHC) mRNA expression, whereas hyperthyroidism resulted in the opposite response (Fig. 1). This regulation was observed in both heart and skeletal muscle, with the former showing much greater sensitivity, as the type I mRNA signal in the heart varied 10-fold because of extremes in thyroid state (Fig. 1). The type I MHC protein data do not fully reflect the impact of altered thyroid state on this phenotype, especially in the soleus and VI muscles (Fig. 1). This discrepancy likely has to do with the duration of the experimental manipulation. With consideration that the MHC protein half-life is ~7–10 days, a steady state at the protein level cannot be reached in 3 wk. However, it has been previously shown that a longer duration of hypothyroidism compared with that used herein transformed the soleus MHC protein profile to 100% type I (3), whereas hyperthyroidism decreased type I MHC expression by ~60% (24).

Collectively, these findings on type I MHC expression in the different striated muscle types suggest both a diversity and a different sensitivity/responsiveness to T3. Thus it was of interest to determine whether these diverse levels of expression and the different T3 sensitivity of type I MHC in different striated muscles can be explained on the basis of either quantitative or qualitative differences in the TR receptor expression.

TR protein expression. Striated muscle TR expression was examined at both the protein and mRNA levels (Tables 2 and 3). Muscle nuclei T3 Bmax was used as an index of TR number and was expressed on the basis of nuclear DNA as well as per unit muscle mass. These data (Table 2) revealed collectively that the three types of skeletal muscle have a greater Bmax than cardiac muscle as expressed per milligram of nuclear DNA, whereas cardiac nuclei had a greater T3 affinity (lower Kd) than skeletal muscle nuclei. However, when Bmax is expressed on the basis of muscle mass, the data show that slow-twitch skeletal muscle contains a greater density of TRs than the fast-twitch types (Table 2). This difference likely has to do with slow-twitch skeletal muscles having more nuclei per cell volume compared with their fast-twitch counterparts (1).

The response to altered thyroid state differed somewhat between cardiac and skeletal muscle. In the heart, altered thyroid state had little effect on Bmax or Kd (Table 2). In contrast, hyperthyroidism induced an upregulation of TR expression in all three types of skeletal muscle without appreciably affecting the Kd (Table 2). Moreover, hyperthyroidism tended to reduce TR expression in skeletal muscle, and the effect was significant in the slow-twitch and red MG (RMG) muscles when both extremes were compared, i.e., PTU group vs. T3 group. Collectively, these data suggest that TRs are differentially expressed and regulated by T3 in a muscle type-specific fashion. These quantitative and qualitative differences in nuclear T3 binding properties might be partially responsible for differences in muscle-specific responsiveness to T3.

TR mRNA expression. Although it is known that TRs exist as different isoforms, our binding assays did not involve a technique that differentiates between the various TR isoforms at the protein level. Thus the reported Bmax consists of both TR-α1 and TR-β1 binding activities, not considering the expression of the α2- isoform, which does not bind T3 (3, 23). Also it is not quite clear how the Kd would be affected by the different isoform composition within a given muscle type. Therefore, in an attempt to gain some insight on this issue in the context of the present study, analyses concerning TR isoforms were performed using Northern analyses.
with cDNA probes specific for the TR-α₁, TR-β₁, and erbA-α₂ isoforms. Figure 2 depicts TR isoform mRNA signals for the different muscle types under the euthyroid state. In the performance of these analyses, the soleus and VI muscles were analyzed individually as separate representatives of the slow-twitch type. The mRNA data analyses show that the expression pattern of the three TR mRNA isoforms in the soleus and VI was not different. Furthermore, TR-β mRNA is most abundant in the heart, i.e., the signal was two- to threefold greater compared with that of the skeletal muscles (Fig. 2B). In contrast, TR-α₁ is most abundant in the RMG fast-twitch skeletal muscle type and least abundant in the heart. Also, these results clearly show that the ratio of α₂ to α₁+β-mRNA is relatively constant across the striated muscle types, whereas the ratio of the β- to α₁-signal was significantly higher in the heart compared with skeletal muscle (Fig. 2C).

Slow muscles (soleus, VI) also exhibited a larger ratio of TR-β to TR-α₁ mRNA than fast-twitch muscles (RMG, white MG).

TR isoform mRNA expression appeared to be affected differently by altered thyroid state across the different muscle types. For example, the TR-α₁ mRNA expression was decreased by the hyperthyroid state in the fast-twitch skeletal muscle, whereas it was increased by the hypothyroid state in all skeletal muscle types (Table 3). The pattern of change of the erbA-α₂ mRNA in response to altered T₃ state was the same as TR-α₁, i.e., it increased in the PTU group and decreased in the T₃ group (Table 3). These changes were more significant in the VI (a predominantly slow-twitch muscle with slow-twitch MHC type I consisting of >60% of MHC pool) and fast-twitch skeletal muscle. The pattern of change of the TR-β₁ mRNA in response to altered T₃ state was completely different from that of the

![Fig. 1. Type I myosin heavy chain (MHC) protein and mRNA expression in striated muscles under altered thyroid state. Proteins are expressed as percentage of total MHC pool. mRNA signals are expressed relative to 18S rRNA. VI, vastus intermedius; RMG, red medial gastrocnemius. No type I MHC was detected in any of the white muscles of any of the groups. Data are means ± SE. *P < 0.05 vs. normal control (NC). #P > 0.05, not significant.](http://ajpendo.physiology.org/)

*Fig. 1.*
Table 2. Nuclear thyroid receptor K_d and number (B_max) in heart and in different skeletal muscle types across experimental groups

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>PTU</th>
<th>T3</th>
</tr>
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<tr>
<td><strong>Heart</strong></td>
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<td></td>
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<tr>
<td>K_a, PM</td>
<td>256 ± 77</td>
<td>379 ± 86</td>
<td>409 ± 30</td>
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<td>B_max, fmol/mg DNA</td>
<td>228 ± 20</td>
<td>237 ± 34</td>
<td>188 ± 40</td>
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<td>B_max, fmol/g muscle wt</td>
<td>610 ± 52</td>
<td>735 ± 106</td>
<td>412 ± 58</td>
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<td><strong>Slow-twitch muscle (n = 4)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>K_a, PM</td>
<td>779 ± 260</td>
<td>720 ± 198</td>
<td>584 ± 214</td>
</tr>
<tr>
<td>B_max, fmol/mg DNA</td>
<td>581 ± 102</td>
<td>972 ± 47</td>
<td>472 ± 77</td>
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<td>B_max, fmol/g muscle wt</td>
<td>1,244 ± 18</td>
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<tr>
<td><strong>RMG (n = 4)</strong></td>
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<tr>
<td>K_a, PM</td>
<td>569 ± 47</td>
<td>488 ± 39</td>
<td>571 ± 183</td>
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<tr>
<td>B_max, fmol/mg DNA</td>
<td>594 ± 61</td>
<td>853 ± 20</td>
<td>517 ± 106</td>
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<tr>
<td>B_max, fmol/g muscle wt</td>
<td>802 ± 71</td>
<td>1,109 ± 22</td>
<td>714 ± 127</td>
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<td><strong>WMG (n = 4)</strong></td>
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<tr>
<td>K_a, PM</td>
<td>626 ± 49</td>
<td>463 ± 76</td>
<td>684 ± 86</td>
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<td>632 ± 25</td>
<td>928 ± 61</td>
<td>642 ± 127</td>
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<tr>
<td>B_max, fmol/g muscle wt</td>
<td>733 ± 34</td>
<td>1,058 ± 69</td>
<td>732 ± 145</td>
</tr>
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</table>

Data are means ± SE; n = no. of pooled samples. Slow-twitch muscle represents a pool of soleus and vastus intermedius (VI) muscles in a proportion of 1 soleus to 1 VI. Maximum binding capacity (B_max) is expressed as fmol/mg DNA and as fmol/g muscle wt; the latter was deduced on the basis of whole muscle DNA concentration. RMG and WMG, red and white medial gastrocnemius, respectively; K_a, dissociation constant. *P < 0.05 vs. NC; †P < 0.05, T3 vs. PTU. ‡P > 0.05, not significant. §P > 0.05 by ANOVA, and P = 0.01 vs. NC by t-test. When data were analyzed across different muscle types: 1P < 0.05 vs. all; 1P < 0.05, heart vs. slow-twitch muscle within a thyroid state.

α isoforms. The TR-β1 signal was increased in all muscle types in response to hyperthyroidism, whereas it tended to decrease in response to hypothyroidism. Analysis of the ratios of TR mRNA species in muscles under altered thyroid state demonstrates that regardless of the muscle type, the ratio of β- to α1-species tended to decrease in the PTU group, whereas it increased in the T3 group (Fig. 3). In contrast, the ratio of α2- to α1+β1-mRNA species appears to increase in the PTU group and decrease in the T3 group; this pattern was observed in all types of muscles (Fig. 3). Collectively, these findings show that although the pattern of TR mRNA isoform expression is muscle type specific (Fig. 2), its response to altered thyroid state is qualitatively similar across the muscle types (Fig. 3).

**DISCUSSION**

Thyroid hormone exerts its biological effects largely by influencing gene expression via interactions with the high-affinity TRs located in the nucleus. Both the concentration of the nuclear receptors and T3 affinity play a major role in determining T3 responsiveness of a given tissue. However, other factors are also involved. For example, T3 transport from the bloodstream to the nucleus could play a critical role in determining the sensitivity to T3. A cytosolic protein has been shown to bind T3 and thus modulate its availability to the nucleus (5). This cytosolic T3 binding protein has been shown to be expressed and regulated by T3 in a tissue-specific fashion (5). Also, TR activation or repression of transcription is not a simple process. Rather, it involves complex multilevel interactions with other nuclear factors that could also be tissue specific and may affect the T3 response (26). Despite this complexity and as a first approach to studying this problem, it seemed logical to examine TR expression in striated muscle types and how this expression is altered by extremes in thyroid state.

Although previous reports have established that heart and skeletal muscle express the TR-α1, TR-β1, and erbA-α2 forms (2, 3, 11, 15), relatively little is known about the pattern of TR expression in specific skeletal muscle types and whether TR isoform expression is sensitive to altered thyroid state in a muscle type-specific fashion. Thus one of the goals of the present study was to examine the expression of TR isoforms both in different muscle types and as a function of thyroid state. We examined the TR protein expression using T3 binding assays, which give the nuclear T3 B_max and affinity. Surprisingly, these assays demonstrated that the rodent heart expresses the fewest nuclear receptors based on its lower B_max when expressed per milligram DNA, but it exhibited a higher affinity to T3 than any of the skeletal muscle types. Both slow-twitch and fast-twitch skeletal muscles have similar T3 affinity and similar B_max as expressed per milligram DNA. However, slow-twitch skeletal muscle had a higher B_max when expressed per unit muscle mass. This difference likely has to do with slow-twitch muscle having more DNA, i.e., more nuclei per muscle mass (1), and is consistent with slow-twitch muscles being more responsive to altered thyroid state than fast-twitch muscles (3, 22, 24). Under an altered thyroid state, the heart B_max did not change, whereas skeletal muscle B_max increased in the hypothyroid state and tended to decrease somewhat in the slow-twitch (19%) and RMG (13%) muscles in the hyperthyroid
Although these binding assays do not differentiate among the different receptor isoforms that are expressed or account for $\alpha_2$-isoform expression, they suggest both a qualitative and quantitative difference between $T_3$ binding properties of cardiac vs. skeletal muscle nuclei. Also, they show a differential response to altered thyroid state, with the heart being the least sensitive in terms of altering its binding capacity. In agreement with previous reports, we show that altered thyroid state does not alter the binding affinity in striated muscles (21).

The reported TR mRNA data demonstrated that cardiac muscle expresses the greatest proportions of TR-$\beta$ mRNA, whereas the RMG, a fast-twitch skeletal
vice versa. On the other hand, the ratio of state, i.e., increased in response to hypothyroidism and species appears to be reciprocally regulated by thyroid.

Interestingly, it would appear that this inhibition would be expected to be diminished under the state of hyperthyroidism (Fig. 3), and hence the influence of T3 would be augmented in this state on thyroid-sensitive genes. Conversely, during hypothyroidism, the increased relative expression of the c-erbA-α2 would likely potentiate the effects of any reduction in the availability of T3. Clearly, more studies are needed to address these issues.

With regard to the relationship between TR mRNA pattern, TR isoform protein composition, and T3 binding properties, previous studies suggest that the TR-α1 has a lower Ke than TR-α2 when analyzed in purified forms (33). However, other studies suggest that TR isoform composition cannot predict the Ke when TRs are analyzed as a component of the nuclear extract (34). Also, it has been proposed that tissue thyroid responsiveness (rather than Bmax) correlates better with TR mRNA expression in a broad spectrum of mammalian tissue, including liver, kidney, brain, and heart (36). However, in this study (36), the Ke of the binding in the different tissue was not reported. In the present study, our findings agree with the notion that there is no definitive relationship between Bmax and TR mRNA expression; however, a somewhat different perspective can be drawn from the relationship between Ke and TR-β mRNA expression. For example, in the euthyroid animals, the mean Ke values negatively correlated with the mean TR-β mRNA signals, with a correlation coefficient of -0.79. However, because of the low number of rats included for the analyses, the statistical significance was low (P = 0.10). When the different Ke values were correlated with TR mRNA expression across all of the muscle types and under altered T3 state, a significant negative correlation (P = 0.03) was found between Ke and TR-β mRNA, with a correlation coefficient (r) of -0.56. However, when TR-α1 mRNA was used instead of TR-β, this correlation was very poor (r = -0.024, P = 0.47). These findings suggest that the higher affinity for T3 in cardiac nuclei could be due in part to its disproportionately greater expression of the TR-β isoform (especially in the euthyroid state), assuming that cardiac vs. skeletal muscle TR proteins are expressed to the same relative proportions as their respective mRNA levels.

It is clear from this study that the diversity in type I MHC expression in striated muscle and its regulation under altered thyroid state (Fig. 1) do not correlate well with either the T3 binding capacity of the tissue or TR mRNA isoform expression in the spectrum of muscles examined in the present study. For example, in the heart, type I MHC expression varies from 0 to 100%
depending on the T₃ state, whereas Bₓmax does not change significantly. These observations suggest that the level of circulating T₃ and hence its availability to the cardiac myocyte nuclei are critically important in cardiac myosin isoform regulation. In skeletal muscle, type I MHC expression also does not correlate well with the T₃ Bₓmax or with TR mRNA expression. For example, in the comparison of the fast-twitch white muscle with the slow-twitch red muscle, type I MHC expression goes from being undetectable in the white muscle to accounting for >80% of the MHC pool in the slow-twitch muscle (Fig. 1). However, Bₓmax is approximately equal in these two muscle types as expressed per milligram DNA. Also, although white muscle is not responsive to T₃ state in terms of type I MHC expression, it was highly plastic in terms of TR expression. In fact, white muscle showed more plasticity than slow-twitch muscles in TR mRNA expression under altered T₃ state (Fig. 3). It should be emphasized that although TR expression did not account for the changes in MHC expression, this may not be generalized to all T₃-regulated genes, i.e., MHC regulation may not be the best marker in this case. These observations suggest that although the TR is a transcription factor involved directly with transcriptional regulation of MHC expression in striated muscle, it would appear that other muscle-specific factors are also important in this regulation. The TRs appear to be widely expressed transcription factors affecting the expression of different genes in a different fashion. Of further interest, TRs may affect the same gene differently depending on the muscle type (3, 12, 16). Therefore, because of their diverse action, TRs must work in concert with other factors or cofactors, which in turn must be more muscle type specific. This latter concept can be illustrated by the fact that the fast-twitch IIb MHC gene is sensitive to thyroid state even though no TRE has been reported on its promoter (3).

In summary, the present study has provided information to suggest that 1) TRs are differentially expressed, both quantitatively and qualitatively, in skeletal vs. cardiac muscle types; 2) this pattern of expression does not necessarily correspond to the differences in responsiveness between cardiac and skeletal muscle to altered thyroid state, in which the former is markedly greater than the latter; 3) there is a differential quantitative response between cardiac vs. skeletal muscle concerning the pattern of TR adaptation to altered thyroid state; and 3) all striated muscle types share a similar pattern of adaptation concerning TR isoform plasticity as examined at the mRNA level (Fig. 3). Despite the new findings reported herein, more research is necessary to establish the physiological context of these adaptations in terms of thyroid-responsive gene regulation.

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REFERENCES


