Distinct Tissue-Specific Roles for Thyroid Hormone Receptors $\beta$ and $\alpha_1$ in Regulation of Type 1 Deiodinase Expression

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Type 1 deiodinase (D1) metabolizes different forms of thyroid hormones to control levels of T$_3$, the active ligand for thyroid hormone receptors (TR). The D1 gene is itself T$_3$-inducible and here, the regulation of D1 expression by TR$\alpha_1$ and TR$\beta$, which act as T$_3$-dependent transcription factors, was investigated in receptor-deficient mice. Liver and kidney D1 mRNA and activity levels were reduced in TR$\beta^{-/-}$ but not TR$\alpha_1^{-/-}$ mice. Liver D1 remained weakly T$_3$ inducible in TR$\beta^{-/-}$ mice whereas induction was abolished in double mutant TR$\alpha_1^{-/-}$TR$\beta^{-/-}$ mice. This indicates that TR$\beta$ is primarily responsible for regulating D1 expression whereas TR$\alpha_1$ has only a minor role. In kidney, despite the expression of both TR$\alpha_1$ and TR$\beta$, regulation relied solely on TR$\beta$, thus revealing a marked tissue restriction in TR isotype utilization. Although TR$\beta$ and TR$\alpha_1$ mediate similar functions in vitro, these results demonstrate differential roles in regulating D1 expression in vivo and suggest that tissue-specific factors and structural distinctions between TR isotypes contribute to functional specificity. Remarkably, there was an obligatory requirement for a TR, whether TR$\beta$ or TR$\alpha_1$, for any detectable D1 expression in liver. This suggests a novel paradigm of gene regulation in which the TR sets both basal expression and the spectrum of induced states. Physiologically, these findings suggest a critical role for TR$\beta$ in regulating the thyroid hormone status through D1-mediated metabolism. (Molecular Endocrinology 15: 467–475, 2001)

INTRODUCTION

Type 1 iodothyronine deiodinase (D1) in peripheral tissues converts the main product of the thyroid gland, T$_4$ into T$_3$, the ligand of the thyroid hormone receptor (TR) (1–3). Although the thyroid gland secretes T$_3$, up to 70% of circulating T$_3$ may be derived from $5'$-deiodination of T$_4$ by D1 (4–7). D1 also has an inactivating $5'$-deiodination role that converts T$_4$ to rT$_3$ and T$_3$ to T$_2$ (3,3$'$-diiodothyronine), products that do not activate the TR (8). D1 is abundant in liver and kidney, where it is itself induced by T$_3$ and suppressed by hypothyroidism in a form of autoregulation that adapts to changes in the thyroid hormone status (2, 9, 10). T$_3$ induces D1 expression at the transcriptional level, and T$_3$ response elements (T$_3$REs) have been identified upstream of the human D1 gene (11–13). Despite the physiological importance of D1, the TR pathways that control D1 expression are unknown.

TRs are T$_3$-dependent transcription factors and belong to the family of nuclear receptors (14, 15). Distinct genes encode the TR$\alpha_1$ and TR$\beta$ receptors, which are closely related in their central DNA binding and C-terminal T$_3$-binding domains but which diverge in their N termini. In vitro, TRs can mediate both T$_3$-dependent and T$_3$-independent transcriptional control (16–18). TR$\alpha_1$ and TR$\beta$ can transactivate through similar T$_3$REs, although cotransfection assays also suggest that they differ in their regulation of certain genes including the TRH and pcp-2 genes (19, 20). Such distinctions may reflect TR structural differences that confer preferences in DNA binding or transactivation in T$_3$RE-specific fashion (21, 22).

TR$\alpha_1$ and TR$\beta$ have both specific and common roles in vivo, as revealed in TR-deficient mouse strains. TR$\alpha_1^{-/-}$ mice have a reduced heart rate (23) whereas TR$\beta^{-/-}$ mice exhibit deafness and a hyperactive pituitary-thyroid axis (24). TR$\beta^{-/-}$ mice provide a recessive...
model of human resistance to thyroid hormone (RTH), which is associated with TRβ mutations (25). TRα1+/– TRβ−/− double mutant mice are runted and have an array of exacerbated phenotypes, indicating the existence of common pathways in which TRα1 and TRβ cooperate with or can substitute for each other (26–28).

Here, the receptor mechanisms that regulate D1 expression in vivo were investigated using TR-deficient mice. The results show that TRβ has the major role in regulating D1 expression in liver and kidney. Remarkably, the deletion of all known TRs not only abolished T3-inducibility but also abrogated basal expression of D1 in liver. Thus, the D1 gene illustrates a novel paradigm of regulation where, rather than modulating expression around a basal level determined by other factors, TRs set basal as well as T3-inducible expression.

The findings suggest that D1 deficiency may contribute to the hormonal imbalances caused by TRβ mutations in mice or in human RTH syndrome.

RESULTS

D1 Abnormalities in TR-Deficient Mice

In TRβ−/− mice, liver D1 activity and mRNA levels were reduced to ≤30% of wild type (wt) levels (P < 0.001), demonstrating that TRβ was required for the maintenance of basal D1 expression (Fig. 1A). The D1 deficiency occurred despite the approximately 3-fold elevated thyroid hormone levels (total T4, TT4, and total T3, TT3), reported previously (24) (Table 1). This emphasized the need for TRβ for sustaining D1 expression as T3 increases would normally induce D1. A similar trend occurred in kidney, where D1 mRNA and activity levels were reduced approximately 50% (P < 0.05) below normal.

In contrast, in TRα1+/– mice, liver D1 expression was not reduced but was 2-fold elevated (P < 0.01, Fig. 1B). In accord with the above indicated role for TRβ, this probably represents a TRβ-mediated response to the slightly elevated TT3 levels present in TRα1+/– mice (see below) (Table 1). Kidney D1 activity was slightly (1.49-fold) elevated in TRα1+/– mice (P < 0.01) although this was not accompanied by corresponding increases in mRNA levels (see Fig. 3, C and D).

In TRα1+/– TRβ−/− mice, a distinct phenotype arose that represented an extreme form of the defect in TRβ−/− mice (Fig. 1C). Liver D1 activity fell to the detection limit and was ≤0.03% of normal, while mRNA levels were undetectable despite very high TT3 levels that would normally induce D1 (Table 1) (27). The sensitivity of the Northern assay was high as poly(A)-selected mRNA was analyzed, and prolonged exposures failed to detect signals (see Materials and Methods), suggesting that any putative, residual D1 mRNA expression was at very low levels. The results indicate a requirement for either TRα1 or TRβ to sustain any detectable D1 expression in liver. In contrast, kidney D1 expression was only 50–60% reduced, resembling the kidney D1 deficiency in TRβ−/− mice (see Fig. 3). Thus, unlike in liver, the additional loss of TRα1 did not significantly worsen the phenotype in TRα1+/– TRβ−/− mice, indicating a unique dependence on TRβ with no detectable role for TRα1 in kidney.

D1 expression levels varied according to the genetic background of the strains that carried the TR mutations, which differed due to the gene targeting approaches used (see Materials and Methods). Liver D1 activity was 3- to 4-fold greater in wild-type (wt) mice a
on the 129/Sv × C57BL/6J (TRβ+/−) than on the 129/OlaHsd × BALB/c (TRα1+/−) mixed backgrounds (Fig. 1, A and B, compare left hand columns in activity graphs). In kidney, a similar but less pronounced trend occurred. These data are consistent with reports that D1 activity is relatively high in the C57BL/6J strain, intermediate in a 129 substrain (129/J), and low in the BALB/c strain (29).

Liver D1 Regulation by T3 in TR-Deficient Mice

To investigate the TR specificity in the adaptive response of D1 expression to changes in T3 levels, TR-deficient mice were studied under normal, hypo-, and hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methylazolate and a low iodine diet (LID), or were made hyperthyroid using methimazole and a high iodine diet (HID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions. Groups received a normal diet (ND) or were made hypothyroid using methimazole and a low iodine diet (LID), or were made hyperthyroid conditions.

Table 1. Thyroid Hormone Levels in TR-Deficient Mice under Different Treatments

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hormone</th>
<th>Normal Diet (ND)</th>
<th>Hypothyroid Diet (LID)</th>
<th>Hyperthyroid Diet (LID + 0.5 μg/ml T3)</th>
<th>Hyperthyroid Diet (LID + 5.0 μg/ml T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>T4 (μg/dl)</td>
<td>4.7 ± 0.2</td>
<td>0.17 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>TRβ−/−</td>
<td>T4 (μg/dl)</td>
<td>16.4 ± 1.4</td>
<td>0.02 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>wt</td>
<td>T3 (ng/dl)</td>
<td>49.6 ± 3.0</td>
<td>7.5 ± 0.8</td>
<td>580.4 ± 102.4</td>
<td>2,581.5 ± 293.0</td>
</tr>
<tr>
<td>TRβ−/−</td>
<td>T3 (ng/dl)</td>
<td>144.1 ± 11.1</td>
<td>8.3 ± 2.3</td>
<td>434.9 ± 150.5</td>
<td>4,162.9 ± 402.9</td>
</tr>
<tr>
<td>wt</td>
<td>T4 (μg/dl)</td>
<td>5.6 ± 0.3</td>
<td>0.27 ± 0.03</td>
<td>0.08 ± 0.03</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>TRα1−/−</td>
<td>T4 (μg/dl)</td>
<td>5.3 ± 0.8</td>
<td>0.34 ± 0.06</td>
<td>0.07 ± 0.02</td>
<td>0.13 ± 0.11</td>
</tr>
<tr>
<td>wt</td>
<td>T3 (ng/dl)</td>
<td>50.2 ± 3.1</td>
<td>13.5 ± 1.4</td>
<td>519.4 ± 214.7</td>
<td>4,787.5 ± 1,108.4</td>
</tr>
<tr>
<td>TRα1−/−</td>
<td>T3 (ng/dl)</td>
<td>70.1 ± 8.0</td>
<td>12.1 ± 2.3</td>
<td>362.0 ± 75.9</td>
<td>4,785.4 ± 1,003.7</td>
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<tr>
<td>wt</td>
<td>T4 (μg/dl)</td>
<td>7.2 ± 0.5</td>
<td>0.15 ± 0.05</td>
<td>0.61 ± 0.10</td>
<td></td>
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<tr>
<td>TRα1−/−β−/−</td>
<td>T4 (μg/dl)</td>
<td>51.6 ± 6.6</td>
<td>0.31 ± 0.07</td>
<td>1.7 ± 0.13</td>
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<tr>
<td>wt</td>
<td>T3 (ng/dl)</td>
<td>80.8 ± 12.5</td>
<td>6.7 ± 1.3</td>
<td>9,440.0 ± 2,110.9</td>
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<tr>
<td>TRα1−/−β−/−</td>
<td>T3 (ng/dl)</td>
<td>2,722.5 ± 494.3</td>
<td>6.5 ± 0.7</td>
<td>14,937.5 ± 1,347.7</td>
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Total serum T4 and T3 levels in TRα1−/−, TRβ−/−, and TRα1−/−TRβ−/− mice under normal diet (ND), hypo- (LID), or hyperthyroid (LID + 0.5 and 5.0 μg/ml T3) conditions. T4 and T3 levels were significantly higher in TRβ−/− and TRα1−/−TRβ−/− mice than in wt mice under ND (P < 0.001). In all mice LID resulted in suppression of both T4 and T3. Administration of T3 increased serum T3, but not T4. Groups contained 8–10 mice, except TRα1−/−TRβ−/− and wt controls which consisted of three–five mice.

Table 2. TR-Specific Regulation of D1 Expression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hormone</th>
<th>Normal Diet (ND)</th>
<th>Hypothyroid Diet (LID)</th>
<th>Hyperthyroid Diet (LID + 0.5 μg/ml T3)</th>
<th>Hyperthyroid Diet (LID + 5.0 μg/ml T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>D1 activity</td>
<td>4.2-fold</td>
<td>4.2-fold</td>
<td>4.2-fold</td>
<td>4.2-fold</td>
</tr>
<tr>
<td>TRβ−/−</td>
<td>D1 activity</td>
<td>4.2-fold</td>
<td>4.2-fold</td>
<td>4.2-fold</td>
<td>4.2-fold</td>
</tr>
<tr>
<td>wt</td>
<td>mRNA</td>
<td>6.5-fold</td>
<td>6.5-fold</td>
<td>6.5-fold</td>
<td>6.5-fold</td>
</tr>
<tr>
<td>TRβ−/−</td>
<td>mRNA</td>
<td>6.5-fold</td>
<td>6.5-fold</td>
<td>6.5-fold</td>
<td>6.5-fold</td>
</tr>
</tbody>
</table>

TRSpecificity 7-fold above the very low basal levels in TRβ−/− mice (P < 0.001). This corresponded to an absolute activity level that was only approximately 1.5-fold above normal levels in wt mice. Thus, liver D1 induction was severely blunted but not abolished in TRβ−/− mice, suggesting that TRβ has a major role while TRα has a limited role or can partially substitute for TRβ.

In both wt and TRα1−/− mice, T3 stimulated normal increases in liver D1 expression, indicating that TRα was nonessential for D1 induction, in contrast to the requirement for TRβ (Fig. 2, C and D). Although the degree of increased expression was greater in TRα1−/− than in TRβ−/− mice, the absolute levels attained were comparable, suggesting that the greater degree of increase in TRα1−/− mice was due to the lower initial D1 levels on this genetic background.

To ascertain whether the residual T3 inducibility of D1 in TRβ−/− mice was mediated by TRα1, responses to changes in T3 levels were determined in TRα1−/−TRβ−/− mice (Fig. 2, E and F). Liver D1 mRNA was undetectable and D1 activity fell to the detection limit in TRα1−/−TRβ−/− mice under ND, a phenotype that was similar to the suppression of D1 caused by hypothyroidism in wt mice. Even high T3 doses failed to induce any detectable D1 mRNA. This indicated that TRα1 accounts for the residual basal and T3-inducible expression of liver D1 in TRβ−/− mice.

A marginal increase in D1 activity (P < 0.05) above the almost undetectable levels in TRα1−/−TRβ−/− mice (Fig. 4, A and B) was observed in the presence of very high (>180-fold above normal wt levels) T3 levels. Although non-TR-mediated responses to T3 have been suggested in other systems (30), this is not likely to explain the present data given the failure of such high T3 levels to induce a more significant D1 increase. The diminutive increase may reflect variability in the D1 assay at the detection limit.
Hypothyroidism suppressed kidney D1 expression in wt and \( \text{TR}^{\beta -/-} \) mice (Fig. 3, A and B). Unlike in liver, however, significant activity and mRNA levels were still detectable, indicating that D1 was less sensitive to \( \text{T}_3 \)-dependent regulation in kidney than in liver. In wt mice, \( \text{T}_3 \) induced dose-dependent increases in D1 expression whereas in \( \text{TR}^{\beta -/-} \) mice, no significant increase occurred, even with high \( \text{T}_3 \) doses. This total block of induction indicated an absolute requirement for \( \text{TR}^{\beta} \) for induction of kidney D1 expression. Kidney D1 expression responded normally in wt and \( \text{TR}^{\alpha 1-/-} \text{TR}^{\beta -/-} \) mice to changes in \( \text{T}_3 \) levels (Fig. 3, C and D). Thus, \( \text{TR}^{\beta} \) but not \( \text{TR}^{\alpha 1} \) was essential for regulation of D1 expression in kidney.
Kidney D1 expression in TRα1−/−TRβ−/− mice under ND was reduced by approximately 50% (P < 0.001, Fig. 3, E and F), which was markedly less severe than in liver where D1 was almost abolished (Fig. 2, E and F). Thus, in the absence of TRs, basal expression of D1 is set at a higher level in kidney than in liver. In wt mice under hypothyroid conditions, the suppressed D1 activity was not significantly different from the deficient values in TRα1−/−TRβ−/− mice (P > 0.78). The reduced D1 activity in TRα1−/−TRβ−/− mice did not vary significantly under any condition (P > 0.20). In TRα1−/−TRβ−/− mice, as in TRβ−/− mice, kidney D1 expression was noninducible by T3 (Fig. 3, E and F). The absence of TRα1 functions in D1 induction indicated that TRβ was solely responsible for the T3-dependent expression of D1 in kidney.

It is noteworthy that all of the T3-dependent regulation of D1 mRNA expression could be attributed to TRβ and TRα1 in liver and to TRβ in kidney. This supported the conclusion that TRβ and TRα1 represent the entire complement of nuclear TRs and argued against the existence of other hypothetical TRs in these tissues (27, 28).

TR Gene Expression in TRα1−/− and TRβ−/− Mice

To support a direct role for TRs in D1 gene regulation, the presence of TRα1 and TRβ proteins in liver and kidney was demonstrated by electrophoretic mobility shift assay (EMSA) (27) (Fig. 4A). Using a DR4 T3RE as a probe, two specific shifted bands were detected in wt nuclear extracts. Antibodies against TR or retinoid X receptors (RXR) abolished or supershifted these bands, indicating that they represented TR-RXR heterodimeric complexes bound to the DNA. The lower TR-specific band was absent in TRα1−/− extracts, the upper band in TRβ−/− extracts, and both bands in TRα1−/−TRβ−/− extracts, consistent with the bands representing TRα1 and TRβ, respectively. Although not quantitative, the EMSA suggested that in liver the presumptive TRβ band was more abundant than that of TRα1, whereas in kidney, TRα1 was somewhat more abundant.

To determine whether changes in TRα1 expression in TRβ−/− mice could explain the ability of TRα1 to substitute for TRβ in liver but not in kidney, TRα1 mRNA levels were investigated (Fig. 4B). Over- or underexpression of TRα1 was excluded as TRα1 mRNA levels were similar in both wt and TRβ−/− mice. T3 administration led to a slight decrease in TRα1 mRNA in liver in both wt and TRβ−/− mice, agreeing with previous reports for rat liver (31). Conversely, TRβ mRNA was not up-regulated in the absence of TRα1 (Fig. 4C), suggesting that the normal D1 regulation in TRα1−/− mice was achieved through normal levels of TRβ. The lack of major changes in TRα1 expression in TRβ−/− mice suggested that tissue-specific differences other than changes in TR expression levels account for the restriction of TRα1 function to liver.

![Fig. 4. TR Expression in TR-Deficient Mouse Strains](https://academic.oup.com/mend/article-abstract/15/3/467/2747948/Distinct-Tissue-Specific-Roles-for-Thyroid-Hormone/317x217-528x647)
Weight Changes in Hypo- and Hyperthyroidism

To rule out major differences in the general condition of TRβ−/− and TRα1−/− mice as an influence over the D1 phenotypes, weight gain was assessed under the hypo- and hyperthyroid treatments (Fig. 5). For wt mice, hypothyroidism produced a decline in body weight, which was reversed with moderate T3 doses (compare Fig. 5A to 5B and 5C). Very high T3 doses, however, did not rescue weight gain but typically caused further loss of weight, presumably through hyperstimulated metabolism (Fig. 5, D, E, and F). Both TRβ−/− and TRα1−/− mouse strains showed a similar response as wt mice, indicating that their overall responses were not grossly impaired. The sole exception was that very high T3 doses in TRβ−/− mice resulted in a weight gain rather than loss (Fig. 5E). This suggested that TRβ mediated the weight loss caused by T3 excesses in wt mice. In TRα1−/−TRβ−/− mice, neither hypo- nor hyperthyroidism produced significant changes in weight (Fig. 5D), consistent with these mice lacking all nuclear TRs.

DISCUSSION

This study reveals the critical role of TRs in the control of expression of the D1 gene in its natural context in vivo. The most fundamental role was the obligatory requirement for a TR, whether TRα1 or TRβ, for any detectable D1 expression in liver. Thus, the D1 gene in liver illustrates a novel paradigm of regulation in which TRs set the basal expression as well as the spectrum of T3-induced states. This contrasts with the scenario suggested by many cotransfection and in vivo studies in which the TR modulates expression around a basal level set by other factors. There is less dependence on TRs in kidney where there is substantial basal expression of D1 in the absence of TRs. Thus, other transcription factors play a more prominent role in kidney than in liver. This reveals differential utilization of TR signaling in the regulation of the same gene in different tissues.

On certain T3REs in vitro the TR exhibits a bimodal function, as it not only mediates T3-dependent activation but in the absence of T3, actively represses expression below basal levels (16–18). These T3-independent actions of the TR provide a possible mechanism for repression of basal transcription in hypothyroidism. For D1 expression, however, the deletion of all TRs caused an equally strong suppression as did hypothyroidism, indicating that TRs and any T3-independent function of TRs are unnecessary for suppression. Thus, models of exchange between repression and activation by the TR (32, 33) may have limited applicability in vivo and would vary depending on the target gene. Rather, the combined requirement for both the TR and T3 for any level of liver D1 expression is consistent with expression in this case being set by a continuous gradient of T3-dependent, positive activation states of the TR.

The utilization of such a positive mode of regulation, even in the sub-basal range of expression in liver, could account for the greater sensitivity of D1 induction in liver than in kidney (Figs. 2 and 3). This may also confer upon the liver D1 enzyme a greater importance of T3-induced states. This contrasts with the scenario suggested by many cotransfection and in vivo studies in which the TR modulates expression around a basal level set by other factors. There is less dependence on TRs in kidney where there is substantial basal expression of D1 in the absence of TRs. Thus, other transcription factors play a more prominent role in kidney than in liver. This reveals differential utilization of TR signaling in the regulation of the same gene in different tissues.

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in response to modest fluctuations in thyroid hormone levels. In its highly tuned sensitivity and activation-based mode of regulation, this role of TRs is reminiscent of that of the positively inducible signaling pathways that activate cellular immediate early genes (34). As these mutant mice lack TRs throughout life, it is not excluded that uncharacterized developmental defects or other indirect changes, involving for example, other transcription factors, contribute to the deficiency in D1 expression.

The results demonstrate a clear TR isotype specificity in the T3-inducible expression of D1, which is mediated predominantly by TRB in liver and exclusively by TRB in kidney. This cannot be explained solely by differential expression of TR isotypes since both TRB and TRA1 are expressed at levels that may be expected to allow regulation of D1. Kidney contains relatively abundant levels of TRB and TRA1 mRNA (48), but in vitro (31, 35–37) and T3 binding proteins, as suggested by immunoprecipitation with antibodies against TRs (38), D1 expression is, however, enriched in the kidney proximal tubules (39, 40). Thus, segregation of TR isotypes in different cell types might in theory contribute to differential regulation by TRB and TRA1, although this seems unlikely given the widespread distribution of TRA1 in many tissues. Alternatively, differences in the structure or conformation of TRA1 and TRB could result in differential recognition of the target T3RE, exposure of activation domains, or interaction with co-factors (33, 41). Indeed, TRA1 tends to form monomeric and TRB homodimeric DNA binding complexes (21, 22). Two TRB REs have been identified upstream of the human D1 gene (11, 12), but these do not occur in the mouse, despite D1 being T3 inducible in both species (42). Clariﬁcation of the physiologically relevant T3REs may allow investigation of the TR speciﬁcity in control of D1 expression.

The permissiveness of liver for some TRA1 function occurs despite TRA1 being expressed at relatively low levels in this tissue where it is approximately 4-fold less abundant than TRB (35, 38, 43). Several corepressors and co-activators have been shown to modify TR activity in vitro (32, 33, 41, 44, 45) and conceivably, tissue- or TR isotype-specific cofactors in liver could facilitate, or in kidney preclude, a role for TRA1. A precedent may be the differential interaction of the SMRT corepressor with retinoic acid receptors α, β, or γ (46). Thus, intrinsic differences between TR isotypes as well as tissue-specific factors are likely to extend the speciﬁcity of the functions of TRB and TRA1 in different physiological situations.

Interestingly, TRB has been suggested to have the primary role in other liver functions as well as in the control of D1 expression. Thus, TRB−/− mice show defective T3-dependent regulation of cholesterol metabolism (47) and impaired T3-inducibility of malic enzyme and spot 14 mRNAs (48). TRB is known to have a critical role in the feedback control of the pituitary-thyroid axis and the thyroid secretion of thyroid hormones, as TRB−/− mice or human RTH patients with TRB mutations have goiter and overproduce thyroid hormones (24, 25, 49). The present study extends the functions of TRB to regulating the thyroid hormone status at the level of the peripheral metabolism of thyroid hormones. This raises the possibility that the thyroid hormone excesses caused by TRB mutations are due, in part, to D1 deficiency. RTH typically results from heterozygous TRB mutations that generate dominant inhibitory proteins (25), and virally mediated expression of such proteins in mice impairs the T3-induction of liver D1 mRNA (50). Our results predict that in RTH, any D1 deficiency would be the result of inhibition of a TRB rather than TRA1 pathway.

The consequences of D1 deficiency may be complex given that D1 has alternative activities that either convert T4 into T3 or that inactivate T3 (8). Assuming that the induction of D1 by T3 serves to protect against excesses of active hormone through the inactivating role of D1, then D1 deficiency may reduce hormone clearance rates. This could cause the accumulation of serum T4 and T3. Partial D1 deficiency, attributed to changes in the 5′-region of the D1 gene, occurs in the C3H/HeJ mouse strain (29, 51), and these mice have also been suggested to have reduced T3 clearance rates (42). It has not been ruled out that changes in D1 activity in the pituitary and thyroid glands (10, 52) or changes in type 2 or type 3 deiodinases (2, 3) also contribute to the net hormone changes in TR-deficient mice.

MATERIALS AND METHODS

Mouse Strains

The TRB mutation, deleting both TRB1 and TRB2 products, was maintained on a mixed background of 129/Sv × C57BL/6J strains (24); the TRA1 mutation was on a 129/OlaHsd × BALB/c background (23). The strains were crossed to derive TRA1+/−/TRA1−/− double mutant mice that were devoid of detectable nuclear T3 binding capacity in brain and liver (27). wt mice were derived with the same genetic backgrounds as each mutant strain. Adult males, ranging in age at the start of experiments from 6–11 weeks (TRA1+/−/TRA1−/−) and 6–8 weeks (TRA1−/−, TRA1+/−) were studied. Mice were housed with 12-h light/12-h dark cycles. All animal experiments followed approved institutional protocols.

Diet Treatments

ND-fed mice received defined pellets containing iodine at 5 mg/kg (ICN Biochemicals, Inc., Cleveland OH) and distilled drinking water for 4 weeks. Other groups were fed the same pellets with reduced iodine (0.05 mg/kg) and were provided with water containing 0.05% MMI and 1% potassium perchlorate (KClO4) (LID) to induce hypothyroidism, for 4 weeks. Subgroups under LID were made hyperthyroid by the addition of T3 at 0.5 μg/ml or 5.0 μg/ml in the drinking water containing MMI/KClO4 (LID + T3) for an additional 8–14 days. Given the limited numbers of TRA1+/−/TRA1−/− double mutant mice available (due to fertility problems and the complex breeding program) (27), these groups received ND, LID, or LID with only a single T3 dose (5.0 μg/ml).
Analysis

Poly (A)-selected mRNA was prepared from pooled liver or kidney samples (TRα1+/– and TRβ2/– strains, n = 5; TRα1+/–TRβ2/– strain, n = 3–5). Northern blots were prepared as described (36) using a D1 probe, a 357-bp fragment from the mouse D1 that was cloned by RT-PCR, using primers homologous to rat D1 sequence (5′-primer, 5′-GGAGCTTGAGATGGGTTCAAGCTGATG-3′; 3′-primer, 5′-CAGGA-GGGGTCCTGCCTTTGAATGAAATTCCCCAGGTTTGA-CTT-3′) (9). Sequence analysis verified the identity of the resulting clone. Northern lanes contained 5 μg of poly(A)-selected mRNA for all samples except TRα1+/–TRβ2/– and their wt control samples which contained 7.5 μg. A mouse glyceraldehyde-3-phosphatedehydrogenase (G3PDH) probe was used to ascertain integrity and quantity of RNA samples. Signals were recorded by autoradiography (1- to 2-day exposure) and were quantified using a Phosphorimager (Molecular Dynamics, Inc., Sunnyvale, CA). In D1-deficient samples (from liver in TRα1+/–TRβ2/– mice and LID-treated groups) prolonged exposures (1–2 weeks) were used but failed to detect additional signals. Moreover, spacer lanes were used to avoid possible spillover between D1-expressing and D1-deficient sample lanes. Spacer lanes were cut out from final figures.

D1 Enzyme Assays

Liver and kidney (n = 4–9) samples were homogenized individually on ice in 5–6 vol of 25% (wt/vol) sucrose in 10 mM HEPES (pH 7.0) containing 10 mM dithiothreitol (DTT) and frozen immediately. 5′-D1 activity was determined in diluted aliquots of the homogenate by the release of radioiodide from [125I]rT3 (NEN Life Science Products-Dupont, Boston, MA) in the presence of 5 mM DTT, as described previously (54). Aliquots of the homogenate by the release of radioiodide from [125I]rT3 (NEN Life Science Products-Dupont) tracers, as described previously. RIAs were performed for total T3 and T4 using antiserum bodies (Sigma, St. Louis, MO) and [125I]T3 (NEN Life Science Products-Dupont) tracers, as described previously (54).

EMSAs

The DR4 DNA probe sequence was: 5′-GGAGGCCTGGAGTCACCTCAGGTTCAAGCTGATG-3′ and the β-fibrinogen probe was described as described (27). Nuclear protein extracts were prepared and EMSAs prepared as described previously, using the F2 TαRE (27). Supershift assays were performed with antibodies against mouse RXR (4RX-1D12), which detect all three RXRs (a kind gift of Dr. P. Chamoun), and against full-length TRβ, which detects both TRα and TRβ (27).

Hormone RIAs

Blood was collected when mice were killed, and serum was separated by centrifugation at 2000 × g and immediately frozen. RIAs were performed as described (28) using antibodies (Sigma, St. Louis, MO) and [5,125I]T3 and [3,125I]T3 (NEN Life Science Products-Dupont) tracers, as described previously (54).

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