Organ Specific Expression of Thyroid Hormone Receptor mRNA and Protein in Different Human Tissues

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The major physiologic effect of thyroid hormone is thought to be initiated by the binding of T3 to the DNA binding thyroid hormone receptor (TR). The aim of this study has been to characterize the organ specific expression of thyroid hormone receptor mRNA, as well as its protein distribution and molecular weight in man. Determination of TRα1, α2, β1 and β2 mRNA molecular size was performed using Northern blot analysis in the human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. TRα1, α2 and β1 protein expression was characterized by Western blot analysis of human tissues. TRα1 mRNA of 4.9 kb was detected in all 8 tissues analyzed, with varying abundance in the various tissues. TRα2 mRNA was detected in 2 different sizes, with higher intensity at 5.7 and lower intensity at 3.2 kb. There were, however, multiple TRβ1 mRNA of 8, 2 and 1 kb detected. TRβ1 transcripts of 2 kb and 1 kb showed an organ specific pattern of expression. Multiple TRβ2 mRNA of 6.6, 5.2, 2.5 and 2.4 kb were detected, as well as a unique 1 kb transcript, in the heart. TRβ2 transcripts also displayed tissue specific expression. Western blot analysis displayed a single band of 48 kD for TRα1. The abundance of the TRα1 immunoreactive band was highest in the heart, brain, kidney and skeletal muscle, and lowest in the liver, placenta and lung, while no signals were detected in the spleen. The TRα2 specific antibody detected a band of 58 kD in all the tissues analyzed. The relative intensity of the immunoreactive TRα2 band was highest in the placenta and lung, with a medium concentration range in skeletal muscle, the heart and kidney. The TRα2 protein concentration was lowest in the spleen, liver and brain. Human TRβ1 protein was detected as 55 and 52 kD bands, as well as a unique band of 45 kD in heart. The 52 kD band was detected in all tissues except the kidney and spleen. The 55 kD band was not detected in the brain or liver. Both the 55 and 52 kD TRβ1 immunoreactive bands were detected in the placenta, lung, heart and skeletal muscle with similar intensity.

In conclusion, specific patterns of TR mRNA and protein expression revealed characteristic organ distributions of each subtype. Unique cardiac expression was observed for TRβ1 mRNA and for TRβ2 protein.

Key words TR mRNA; protein; Northern blot; Western blot; molecular weight; human tissue

Thyroid hormones play important roles in various aspects of development and homeostasis by regulating the transcription of specific target genes. These actions are mediated via specific nuclear receptors which are members of the steroid hormone receptor superfamily.1–3 Thyroid hormone receptors (TRs) regulate gene expression through binding to the promoter regions of target genes.4 Various TRs bind as monomers, homodimers or heterodimers depending on the thyroid hormone response element (TRE) and the presence or absence of thyroid hormone.5–7 Heterodimers of TR and retinoid X receptors (RXR) appear to be more stable and bind with greater affinity.6–7 The principal gene, C-erb A, which is part of the genome of the avian erythroblastosis virus, has been shown to be encoded by 2 distinct genes: TRα and β, which are located on chromosome 17 and chromosome 3, respectively.8–11 Alternative mRNA splicing of the TRα gene leads to the generation of functionally different receptors, TRα1 and TRα2. TRα2 has a different C-terminal, which abolishes the hormone binding properties, RXR heterodimerization and transactivation function of the protein.12 Dominant negative regulation by TRα2 is exerted by the suppression of transactivation mediated by products of either the TRα1 or TRβ genes.13–14 The TRβ gene generates 2 different isoforms, TRβ1 and TRβ2, which have different N-terminal variants due to alternative splicing of 5′ exons and differential promoter choice.15,16 The existence of TR has been reported across a variety of species such as human, mouse, Xenopus laevis, rat and chicken.17 The expression of different TR isoforms is regulated transcriptionally and post-transcriptionally, with mRNA encoding each TR isoform displaying characteristic patterns of developmental, tissue-specific, and hormonal regulation.18 It should be noted that mRNA and protein concentrations do not always correlate.19,20 TRα1, α2 and β1 isoforms have been detected in several human tissues.21 The TRβ2 isoform is expressed mainly in the pituitary gland,22 although recent evidence suggests that it is also expressed at very low levels in some other tissues.23–25 However, no study has investigated the distribution and molecular size of all 4 mRNA subtypes, or has detected TRα1, α2 and β1 protein expression in human tissues.

The aim of this study was to evaluate the organ specific expression of thyroid hormone receptor mRNA and protein distribution in different human tissues, especially in the heart.

MATERIALS AND METHODS

Northern Blot Analysis Human TRα1 and TRβ2 specific DNA was synthesized using human heart cDNA amplified with specific primers which were derived based on published sequences: 1) hTRα1 (human thyroid hormone receptor α1, Gen EMBL X55005), 5′ primer: bases 1573–1599, 3′ primer bases 1825–1800, expected PCR fragment: 252 bp; 2) hTRβ2 (human thyroid hormone receptor β2, Gen EMBL X74497), 5′ primer: bases 120–143, 3′ primer bases 380–355, expected PCR fragment: 260 bp. The PCR reactions were run according to Shahrara et al.22 The size of the

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PCR product was compared with a 1 kb marker (Gibco, Sweden) on a 4% NuSieve agarose gel. The PCR products were cloned in a pGEM-T vector (Promega, U.S.A.) according to the manufacturer's specification. The ligations were confirmed by sequencing. The inclined PCR products were cleaved with Sac II and Spe I and were purified on a 1.5% low melting gel for labeling. The TRa2 was a kind gift from Karolinska Institute, Sweden, and it was cleaved with Ava II to a 206 bp region from n.t. 1295—1500. The TRβ1 was a gift from Birgitta Moller and it was cut with Bam H I and Sp I at n.t. 263—485, generating a fragment of 222 bp. 100 ng of a 2.0 kb human β-actin cDNA control probe was included in the Multiple Tissue Northern blot (MTN, Clontech, U.S.A.). DNA probes were labeled by random priming. For this purpose, 100 ng of the DNA fragment and 1 μg/μl random primer were mixed and diluted to 10 μl. The mixture was heated for 5 min at 95—100°C and placed on ice. Two micro-liter of 10× klenow buffer, 6 μl of 32P-dCTP, 2 μl of 2.5 μM dNTP (dCTP) and 1 μl 2 U/μl klenow enzyme was added to the mixture, which was then incubated for 1 h at room temperature. The solution was diluted to 80 μl with 10 mM Tris—1 mM EDTA, (pH 8). The probe was purified with fine G-50 (Pharmacia, Sweden). Typically, 100 ng of fragment yielded 8—16×10^6 precipitable counts, which were used for individual hybridization. Human Multiple Tissue Northern blot was purchased from Ambion (U.S.A.) and contained poly A+ RNA from multiple tissues or cell lines of a single species. The human tissues analyzed were from the heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. TR subtype and β-actin probes were hybridized twice to the commercial membrane at the optimized temperature in order to check the reproducibility. The prehybridization/hybridization solution contained 5×SSPE (3 μM NaCl, 0.2 mM NaH2PO4, H2O and 0.02 M Na2EDTA), 10×Denhardt's solution, 100 μg/ml freshly denatured and sheared salmon sperm DNA and 0.5% SDS. The MTN membranes were prehybridized for 3 h. The prehybridization/hybridization temperature for different CDNA were: TR α1, α2 and β1, 60°C; TR β2, 55°C; and β-actin, 65°C. The prehybridization solution was replaced with fresh hybridization solution containing a radiolabeled probe for 18 h. The probe was denatured at 95°C for 4 min before being added to the hybridization solution. The blot was rinsed in 2×SSC and 1% SDS twice for 20 min at the same temperature as hybridization. Thereafter, it was washed in 0.1×SSC and 0.5% SDS twice for 20 min at 5°C lower than the hybridization temperature. The membrane was then exposed to Kodak X-Omat film. The film exposure time was different in each TR mRNA subtype, depending on the intensity of the signals in each case. The densitometric scanning of Northern blots was done using an Advanced Image Data Analyzer Aida Version 2.0. Since the relative amount of β-actin was different in various tissues, the concentration of TR mRNA was normalized by the concentration for β-actin for each tissue.

Western Blot Analysis Human tissue-specific protein extracts were purchased from Clontech (U.S.A.). The protein extracts were isolated from whole tissue homogenates under conditions to minimize proteolysis and to ensure maximal representation of tissue specific proteins. After finding the optimal primary and secondary antibody dilution, Western blot analysis of TR subtypes was performed at least twice in order to check the reproducibility. The human tissues analyzed were from the heart, brain, placenta, lung, liver, skeletal muscle, kidney and spleen. Equal amounts of total protein extract (70 μg per lane from each tissue) were loaded on 9% SDS-polyacrylamide gel electrophoresis according to Laemmli. Transfer of the protein to a nitrocellulose filter was performed as described by Towbin et al. The filter was blocked in TBS [0.15 M NaCl and 10 mM Tris–HCl, pH 7.5], containing 0.05% Tween 20 and 10% non-fat milk for 2 h. The filter was later incubated with anti-human TRα1, TRα2 and TRβ1 rabbit polyclonal antibodies (Affinity BioReagents, U.S.A.) at 1:1600 dilution in 1×TBS-T containing 10% non-fat milk at 4°C overnight. Amino acid sequences recognized by these antibodies in human TRα1, TRα2 and TRβ1 are a.a. 403—410, a.a. 431—451 and a.a. 62—82, respectively, according to Falcone et al. The human TRα1 antibody recognizes the extreme C-terminal region of TRα1 downstream from the point of sequence divergence from α2, whereas the TRα2 antibody detects within the 80 a.a. sequence unique for TRα2. The human TRβ1 antibody is designed to recognize the N-terminal specific for the TRβ1 subtype. Bound IgG molecules were detected with alkaline phosphatase conjugated goat anti-rabbit IgG (Fc) secondary antibodies (Promega, U.S.A.) at a 1:7500 dilution. The protein/antibody complex was visualized with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium tablets (BCIP/NBT alkaline phosphatase substrate) (Sigma, Germany). The filters were densitometrically scanned using the Advanced Image Data Analyzer Aida Version 2.0. The number of TR protein bands is expressed relative to the value for each tissue in percent. The amount of actin was the same in all tissues analyzed, since 70 μg of each protein extract was loaded per lane.

RESULTS

As determined in 8 human tissues, specific patterns of TR mRNA and protein expression were present for each subtype. A single human TRα1 transcript was detected in all 8 tissues analyzed. The molecular weight of the TRα1 mRNA is 4.9 kb, with varying abundance in various tissues. The intensity of the hTRα1 transcript was highest in skeletal muscle, the heart and pancreas. hTRα1 was detected with similar intensity in the brain, placenta and lung (Fig. 1a, Table 1).

Human TRα2 was detected in 2 different sizes. The intensity of the 5.7 kb transcripts was highest in skeletal muscle, the heart and pancreas. The liver and kidney showed similar abundance, whereas the density of the detected 5.2 kb mRNA was lower and similar in the placenta and lung. The 3.2 kb transcript was detected in the heart, skeletal muscle and pancreas only (Fig. 2a, Table 1).

Some of the tissues analyzed expressed multiple sizes of hTRβ1. A hTRβ1 transcript of 8 kb was present in all tissues analyzed, with the highest abundance in the brain, skeletal muscle and kidney. A shorter transcript of 2 kb was present in the placenta, lung, liver and skeletal muscle. A 1 kb hTRβ1 transcript was detected only in the placenta and skeletal muscle (Fig. 2b, Table 2).

Multiple expression of hTRβ2 was detected in several tissues. A 5.2 kb transcript was present in all tissues. A longer hTRβ2 mRNA of 6.6 kb was present in the heart, brain and
Fig. 1a. Human Multiple Tissue Northern Blot No. 5 Hybridized with Human TRα1 cDNA
The total radioactivity of the probe was 9 × 10^7 cpm. The time of exposure was overnight.

Fig. 1b. Human Multiple Tissue Northern Blot No. 5 Hybridized with Human TRβ1 cDNA
The total radioactivity of the probe was 11 × 10^7 cpm. The time of exposure was 48 h.

Fig. 1c. Human Multiple Tissue Northern Blot No. 5 Hybridized with Human β-Actin cDNA
The total radioactivity of the probe was 16 × 10^7 cpm. The time of exposure was overnight.

Table 1. The Concentration of Human TRα1 mRNA 4.9 kb, TRα2 mRNA 5.7 and 3.2 kb in Human Tissues Was Normalized by the Concentration of β-Actin (TR/Actin)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TRα1 mRNA 4.9 kb (%)</th>
<th>TRα2 mRNA 5.7 kb (%)</th>
<th>TRα2 mRNA 3.2 kb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>81</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>Brain</td>
<td>20</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>25</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>52</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>41</td>
<td>39</td>
<td>91</td>
</tr>
<tr>
<td>Pancreas</td>
<td>80</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

The relative amounts of mRNA were estimated by densitometric scanning of Northern blot, and are expressed relative to the value for skeletal muscle in percent. The amount of actin was 100% in skeletal muscle, 95% in the heart, brain, placenta, lung, kidney and pancreas and 52% in the liver.

Table 2. Relative Abundance of Human TRβ1 mRNA 8 and 2 kb in Human Tissues Was Normalized by the Concentration of β-Actin (TR/Actin)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TRβ1 mRNA 8 kb (%)</th>
<th>TRβ1 mRNA 2 kb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>68</td>
<td>50</td>
</tr>
<tr>
<td>Lung</td>
<td>65</td>
<td>30</td>
</tr>
<tr>
<td>Liver</td>
<td>72</td>
<td>43</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>

The relative amounts of mRNA were estimated by densitometric scanning of Northern blot. The abundance of the TRβ1 transcript of 8 kb is shown in comparison to the value for brain in %, whereas in TRβ1 mRNA of 2 kb, the relative abundance is calculated in comparison to the intensity of the TRβ1 mRNA detected in skeletal muscle in %. The amount of actin was 100% in skeletal muscle, 95% in the heart, brain, placenta, lung, kidney, pancreas and 52% in the liver.

Table 3. Relative Abundance of Human TRβ2 mRNA 6.6 and 5.2 kb in Human Tissues Was Normalized by the Concentration of β-Actin (TR/Actin)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TRβ2 mRNA 6.6 kb (%)</th>
<th>TRβ2 mRNA 5.2 kb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td>Brain</td>
<td>75</td>
<td>79</td>
</tr>
<tr>
<td>Placenta</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>

The relative amounts of mRNA were estimated by densitometric scanning of Northern blot and are expressed relative to the value for skeletal muscle in %. The amount of actin was 100% in skeletal muscle, 95% in the heart, brain, placenta, lung, kidney, pancreas and 52% in the liver.

skeletal muscle. The abundance of the 5.2 kb transcript was highest in skeletal muscle, the brain and pancreas. Transcripts of 2.5 and 2.4 kb were detected in the placenta and lung, respectively. Unexpectedly, a TRβ2 1 kb transcript was detected only in the heart (Fig. 1b, Table 3).

The β-actin control probe has generated a signal in each lane, with similar intensities and an approximate length of 2 kb. However, in both the heart and skeletal muscle, there
Fig. 2a. Human Multiple Tissue Northern Blot No. 3 Hybridized with Human TRα2 cDNA
The total radioactivity of the probe was 8×10^7 cpm. The time of exposure was overnight.

Fig. 2b. Human Multiple Tissue Northern Blot No. 3 Hybridized with Human TRβ1 cDNA
The total radioactivity of the probe was 13×10^7 cpm. The time of exposure was 48 h.

Fig. 2c. Human Multiple Tissue Northern Blot No. 3 Hybridized with Human β-Actin cDNA
The total radioactivity of the probe was 14×10^7 cpm. The time of exposure was overnight.

Fig. 3a. Western Blot Analysis of Human Tissues Hybridized with Human TRα1 Rabbit Polyclonal Antibody
Protein extracts (70 µg) were from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and spleen. The primary anti-human TRα1 was diluted 1:1600, and the dilution of the secondary alkaline phosphatase conjugated goat anti-rabbit IgG was 1:7500.

Fig. 3b. Western Blot Analysis of Human Tissues Hybridized with Human TRα2 Rabbit Polyclonal Antibody
Protein extracts (70 µg) were from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and spleen. The primary anti-human TRα2 was diluted 1:1600, and the dilution of the secondary alkaline phosphatase conjugated goat anti-rabbit IgG was 1:7500.

Fig. 3c. Western Blot Analysis of Human Tissues Hybridized with Human TRβ1 Rabbit Polyclonal Antibody
Protein extracts (70 µg) were from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and spleen. The primary anti-human TRβ1 was diluted 1:1600, and the dilution of the secondary alkaline phosphatase conjugated goat anti-rabbit IgG was 1:7500.

Table 4. Relative Abundance of the Human TRα1 Band of 48 kD, TRα2 Band of 58 kD and TRβ1 Band of 52 kD in Human Tissues Was Estimated by Densitometric Scanning of Western Blot, and Is Expressed Relative to the Value for Each Tissue in percent.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TRα1 protein 48 kD (%)</th>
<th>TRα2 protein 58 kD (%)</th>
<th>TRβ1 protein 52 kD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>100</td>
<td>75</td>
<td>93</td>
</tr>
<tr>
<td>Brain</td>
<td>100</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Placenta</td>
<td>18</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lung</td>
<td>9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>32</td>
<td>49</td>
<td>36</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>89</td>
<td>81</td>
<td>91</td>
</tr>
<tr>
<td>Kidney</td>
<td>100</td>
<td>69</td>
<td>90</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

The amount of actin was the same in all tissues analyzed, since 70 µg of each protein extract was loaded per lane.

There are 2 forms of β-actin mRNA, a 2 kb and a 1.6—1.8 kb form. This difference in size is not due to mRNA degradation but to probe hybridization to either the α or γ form of actin. Other isoforms of actin may also be detected in some tissues.
Western blot analysis displayed a single band of 48 kD for TRα1. The abundance of the TRα1 immunoreactive band was highest in the heart, brain, kidney and skeletal muscle and lowest in the liver, placenta and lung. No signal was detected in the spleen (Fig. 3a, Table 4).

The TRα2 specific antibody was detected by a band of 58 kD in all the tissues analyzed. The relative intensity of the immunoreactive TRα2 band detected was highest in the placenta and lung. The density of the 58 kD band was at the same concentration range in the skeletal muscle, heart and kidney. The TRα2 protein concentration was lowest in the spleen, liver and brain (Fig. 3b, Table 4).

Human TRβ1 protein was detected at sizes of 55 and 52 kD, as well as a unique band of 45 kD in the heart. The TRβ1 52 kD band was detected in all tissues except the kidney and spleen. The 55 and 52 kD immunoreactive bands were detected in the placenta, lung, heart and skeletal muscle with almost the same intensity. The 55 kD immunoreactive band was not detected in the brain and liver (Fig. 3c, Table 4).

**DISCUSSION**

In the present study we have determined the molecular weight and distribution of different subtypes of human TR expression at mRNA and protein levels in various tissues.

In accordance with earlier studies, we have detected human TRα1 of 4.9 kD. However, our data is not consistent with results from Sakurai et al. who detected multiple human TRα1 transcripts of 6 and 3.2 kD. Laude et al. showed that human TRα1 of 5.8 kD is encoded by a large messenger RNA which contains a long 3′ untranslated region. This feature is common among nuclear receptor genes. The human glucocorticoid receptor contains a 3′ untranslated region of 2.3 kD.

Human TRα2 was detected in 2 different sizes with a higher intensity at 5.7 kD and lower intensity at 3.2 kD. Laude et al. and Miyajima et al. determined TRα2 mRNA of 2.7 kD, and Nakai et al. showed a human TRα2 transcript of 5 and 2.5 kD, whereas Sakurai et al. reported a single band of 3.2 kD. Our results correlate partly with the findings of Sakurai et al.

We have detected multiple TRβ1 mRNA of 8, 2 and 1 kD in human tissues. Angel et al. determined a single band of 6 kD TRβ mRNA in human liver and placenta. Sakurai et al. have reported that the human TRβ mRNA is expressed at 10, 5, 3 and 2 kD. Interestingly, Weinberger et al. also detected 5, 3, 2.5 and 2 kD TRβ transcripts in the human placenta. Sakurai et al. argue that the 5 and 3 kD TRβ mRNA might be smaller forms or degradation products of 10 kD mRNA or alternative splicing products of TRβ because of their nonreproducibility when using poly A+ RNA from the same tissues. Furthermore, Sakurai et al. suggested that the short human TRβ transcript (2 kD transcript) has a short 3′ untranslated region, whereas the long TRβ mRNA has a long 3′ untranslated region. This might suggest that long and short mRNA forms have different regulatory mechanisms and different physiological roles. The TRβ transcript that has commonly been detected in our lab and by the other 2 groups is the 2 kD size, which is in the same size range as the predicted molecular mass of human TRβ1 mRNA. Sakurai et al. have used the whole human TRβ1 gene as a probe. However, Weinberger et al. and Angel et al. have used a 650 bp Bam HI-Pst I fragment. The human TRβ1 subtype (Gen EMBL X04707) has the least homology to human TRβ2 subtype between bases 1—470. Thus, in these studies, the whole human TRβ1 and the 650 bp Bam HI-Pst I fragment can bind both to human TRβ1 and β2. Angel et al. suggested that the 2 different rat transcripts which hybridize to the TRβ probe could arise either from two separate genes or from differential processing of the transcript of a single gene. Hodin et al. confirmed later that TRβ1 and β2 represent alternative splice products of a single gene, but they have different amino-terminals which could not be distinguished by a common TRβ probe. We believe that by using subtype specific probes, we were able to distinguish between the human TRβ1 and β2 subtypes. By doing repeated measurements and having intact poly A+ RNA, we provide more reliable molecular sizes for the different TR mRNA subtypes.

Using Northern blot analysis, Hodin et al. also reported that the mouse 6.4 kD TRβ2 mRNA was limited to thyrotrope cells. Multiple human TRβ2 mRNA of 6.6, 5.2, 2.5, 2.4 kD were detected, as well as a 1 kD transcript, in the heart. We were the first group that has been able to detect specific, multiple TRβ1 and TRβ2 mRNA in different human tissues using Northern blot analysis.

Although earlier studies also used Northern blot analysis, there are discrepancies in our results. This may be due to the mRNA preparation method, probe specificity, or hybridizing conditions. We have designed TR subtype specific cDNA probes to identify different isoforms.

The predicted molecular mass of human TR cDNA α1, α2, β1 and β2 are 1.2, 1.6, 1.4 and 1.3 kD, respectively, mRNA in the size range of the predicted sizes were not detected in the human tissues. This is probably due to polyadenylation at different sites, or less likely, to differences in the 5′ untranslated region. Sakurai et al. suggested that the multiple mRNAs are derived from differences in the 5′ portion of the mRNA and poly A site selection. Yaiota et al. reported that the 5′ untranslated region can comprise a short region of TRβ mRNA in Xenopus laevis, whereas the remaining 8 kD of these mRNA is located in the 3′ untranslated region. Generation of transcripts with different 3′ untranslated regions could influence message stability, but would probably not affect the translation products. Another explanation for the different sized TR messages is that the translated proteins are of different sizes.

Chamba et al. reported a protein band of 48 kDa as human TRα1, a band of 58 kDa as human TRα2 and bands of 52, 55 and 65 kDa as human TRβ1. On the other hand, Yen et al. determined a single band of 62 kDa as human TRβ2 translation product.

The subtype specific probes had different GC content. TRβ1 had the highest GC content (70% GC), whereas TRα1 and α2 had almost the same GC content (60%, respectively 61%). Furthermore, TRβ2 had the lowest GC content (44%). That is probably why the prehybridization/hybridization temperature for TRα1, α2 and β1 cDNA (60°C) was higher compared to TRβ2 (55°C). The commercially available Northern blot membrane from Clontech contained pools of RNA preparation from eight different tissues from individu-
als of varying age and gender. The samples were taken at different time points. This was done in order to have a representative group from the population.

Variation in transcripts among tissues may indicate that their expression of TR is regulated in different ways, and may imply that they have different functions or are targeted for different intracellular sites for regulating the crucial effects of T3. It is possible that the expression of TR mRNA is increased to compensate for low serum thyroid hormone concentrations. Unoccupied T3 receptors repress some genes that are usually stimulated by T3.2) The multiple expression of the different TR isoforms and the presence of organ specificity suggests complex mechanisms of control of thyroid hormone responses.

We have used the polyclonal antibodies characterized by Falcone et al.26) which are able to specifically recognize each TR isoform. Bands of 48 and 58 kD were identified as TRα1 and TRα2, respectively. Human TRβ1 protein was detected at 55 and 52 kD, as well as a unique band at 45 kD in the heart. These results correlate with the findings of Falcone et al., Chambra et al. and Macchia et al.26,34,36) The TRβ1 band of 45 kD in the heart has not been reported previously. The unique band detected in the heart might be translated from a different translation start site, or the proteins detected with higher molecular weight might represent a further modified form of the receptor.

Schwartz et al.37) have compared the concentration ratios for protein to mRNA for TRα1 and TRβ1 isoforms in the liver, brain, kidney and heart. They demonstrated higher protein/mRNA levels in rat heart, brain and kidney for both TRα1 and β1 subtypes. In accordance with their findings, we have also detected higher TRα1 protein distribution in the same tissues. In each TR subtype, the pattern of protein and mRNA distribution is different in some tissues and similar in others. Strait et al.16,17) and others18–20) have shown that there can be a wide discordance in TR protein/mRNA levels in different tissues.

One of the possible reasons for the lack of correlation may be due to translational or posttranslational modification of the expressed TR.

Tagami et al.38) suggested that the modification of receptor protein by acetylation or phosphorylation may affect TR protein metabolism, causing a dissociation between TR protein and its mRNA level. TR proteins are known to interact with TR proteins themselves (homodimerization) or with other nuclear receptors (heterodimerization). It is conceivable that such a protein–protein interaction could influence the stabilization of TR proteins. Another important factor is the stability of TR mRNA. It has been reported that some mRNAs are protected39) whereas others are degraded40) during translation to another.

In accordance with previous findings12,13) the size of the TR mRNA and protein detected in this study does not correlate. Laudet et al.12) and Sakuragi et al.31) demonstrated that both human TRα and β have a long 3’ untranslated region downstream from the poly A sequence. They further reported that using a primer extention method, using a primer 3’ to the termination codon, they were able to convert a large mRNA to a cDNA of the right size.

In conclusion, this is the first study to show the distribution and molecular sizes of different TR subtypes at both mRNA and protein levels in various human tissues. Unique cardiac expression was observed for TRβ2 mRNA and for TRβ1 protein.

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