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Differential expression of α and β thyroid hormone receptor genes in rat brain and pituitary

(*erbA/in situ* hybridization histochemistry)

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ABSTRACT **Multiple thyroid hormone receptor cDNAs have previously been identified in rat and are classified into α and β subtypes. Alternative splicing of the α gene gives rise to the functional receptor, rTRα1, and the non-thyroid hormone-binding isotype, rTRα2. Recent evidence suggests the β gene encodes two functional receptors, rTRβ1, and the pituitary-specific receptor, rTRβ2. By using synthetic DNA probes common to rTRβ transcripts and specific for rTRα1 and rTRα2 mRNAs, we mapped the expression of these transcripts in adult rat brain and pituitary by hybridization histochemistry. We also localized mRNAs encoding the putative nuclear receptor REV-ErbAα, a portion of which is derived from the opposite strand of the rTRα gene. rTRα1 and rTRα2 transcripts were widely distributed in a similar, if not identical, pattern. Highest levels of rTRα1 and rTRα2 transcripts were found in the olfactory bulb, hippocampus, and granular layer of the cerebellar cortex. REV-ErbAα and rTRβ mRNAs were found in more restricted patterns of expression distinct from those of rTRα1 and rTRα2. REV-ErbAα mRNA was highest in the neocortex. High levels of rTRβ transcripts in the anterior pituitary and the parvocellular part of the paraventricular hypothalamic nucleus suggest rTRβ gene products may mediate thyroid hormone feedback regulation of thyroid-stimulating hormone and thyrotropin-releasing hormone. Our results identify nuclei and structures in the mammalian central nervous system in which regulation of gene expression by specific thyroid hormone receptor subtypes may occur.**

The cellular actions of thyroid hormone [3,5,3′-triiodothyronine (T_3)] are mediated by nuclear receptors (1). T_3–receptor complexes bind to thyroid hormone-responsive elements (TREs) associated with target genes and stimulate or inhibit expression of these genes (2). Although the brain is considered unresponsive to T_3 as measured by traditional biochemical criteria, such as oxygen consumption (3), the presence of high-affinity T_3 nuclear binding sites in rat brain has been demonstrated by several investigators (4–7). T_3 is essential for normal brain development (8), and numerous abnormalities, including delayed myelin deposition and mental retardation, are associated with its deficiency (9). In adult humans, alterations in T_3 levels have been associated with neurologic and behavioral disturbances (10).

T_3 receptors are encoded by the cellular homologue of the viral *erbA* oncogene (c-*erbA*) (2). Significant homology between the v-*erbA* oncogene product and steroid hormone receptors led to the recognition that the c-*erbA* gene product binds T_3 and its analogs with affinities and specificities characteristic of biochemically analyzed T_3 receptors (11, 12). In addition, c-*erbA* polypeptides induce T_3-dependent regulation of gene expression (13–16). Taken together, these findings suggest that c-*erbA* encodes the physiologic T_3 receptor.

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Multiple c-*erbA* cDNAs have been identified and are classified into α and β subtypes based on sequence homology and mapping to human chromosomes 17 and 3, respectively (11, 12, 14, 16–24). In the rat, alternative splicing of the α thyroid hormone receptor gene gives rise to a functional receptor, rTRα1, and a non-T_3-binding isotype, rTRα2 (15, 21, 24) (Fig. 1). rTRα1 and rTRα2 are identical in their first 370 amino acids, at which point their sequences abruptly diverge (21, 24). Both rTRα1 and rTRα2 bind to TREs (15, 24). Unlike rTRα1, however, rTRα2 fails to regulate gene expression in a T_3-dependent manner (15). A putative nuclear receptor, REV-ErbAα, is encoded by the DNA strand opposite to that encoding rTRα1 and rTRα2, and a portion of REV-ErbAα mRNA is complementary to rTRα2 transcripts (25). REV-ErbAα shares homology with members of the steroid and thyroid hormone receptor superfamily. Like rTRα2, REV-ErbAα fails to bind T_3 or other tested ligands, and its function is unknown (25).

Recently, a second functional rat β thyroid hormone receptor, rTRβ2, was described by Lazar and coworkers (16), who have suggested that rTRβ1 and rTRβ2 arise from the same gene. The two β receptors share the same putative DNA- and hormone-binding domains but differ in their amino termini. Unlike rTRα1, rTRα2, REV-ErbAα, and rTRβ1 mRNAs which have been found in both rat brain and pituitary by Northern analysis (14, 18, 21, 23–25), rTRβ2 transcripts have been detected only in the anterior pituitary (16).

Regional localization of nuclear T_3 receptors in rat brain has previously been determined by ^{125}I-labeled T_3 binding to brain homogenates (5–7). To identify neural target tissues specific for α and β thyroid hormone receptor subtypes and to gain insight into what roles multiple c-*erbA* and related gene products might play in regulation of neural gene expression, we used specific DNA probes to map rTRα, rTRβ, and REV-ErbAα transcripts in rat brain and pituitary. Our results show that rTRα1 and rTRα2 mRNAs have apparently identical distributions in these tissues, and that genes encoding rTRα1, rTRβ, and REV-ErbAα transcripts have partially overlapping but distinct patterns of expression in the central nervous system. Brain structures with rTRα1 and rTRβ transcripts may be targets for T_3-dependent regulation of gene expression.

MATERIALS AND METHODS

Animals. Adult male Sprague–Dawley rats (300–350 g, Taconic Farms) were housed at 25°C on a 12-hr on, 12-hr off

Abbreviations: T_3, 3,5,3′-triiodothyronine; TRE, thyroid hormone-responsive element; rTRα, rat α thyroid hormone receptor; rTRβ, rat β thyroid hormone receptor; REV-ErbAα, a putative nuclear receptor; GR, glucocorticoid receptor; PVN, paraventricular hypothalamic nucleus.

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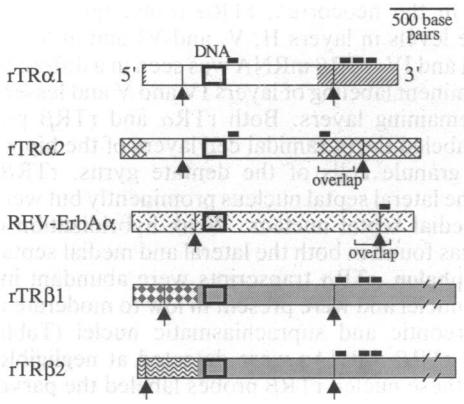

FIG. 1. Probes complementary to rTRα1, rTRα2, rTRβ1, rTRβ2, and REV-ErbAα cDNAs. Oligonucleotide probes used for hybridization histochemistry and Northern analysis are represented by bars. Start and stop codons (indicated by arrows) and putative DNA-binding domains (indicated by heavy borders) are shown as described (14, 16, 18, 24, 25). Unique sequences are hatched or shaded. The region of opposite strand overlap between rTRα2 and REV-ErbAα is shown. A single probe common to both rTRα1 and rTRα2 cDNAs was complementary to published sequences 682–729 (18) and 881–928 (24). rTRα1 mRNA was mapped by using three specific probes in combination. These probes were complementary to rTRα1 cDNA sequences 1611–1658, 1721–1768, and 1801–1848 (18). Similarly, probes common to rTRβ1 cDNA sequences 1412–1459, 1601–1648, and 1651–1698 (14) and to rTRβ2 cDNA sequences 1627–1674, 1816–1863, and 1866–1913 (16) were used together to map rTRβ transcripts. Single specific probes used to map rTRα2 and REV-ErbAα mRNAs were complementary to sequences 1637–1684 (24) and 2035–2082 (25), respectively. A sense, control probe (not shown) coding for the last 16 amino acids of the rat vasopressin glycopeptide was also used (26).

lighting schedule and given food and water ad libitum. The animals were sacrificed by decapitation, and their brains and pituitaries were immediately frozen in −20°C isopentane and stored at −80°C prior to use.

Probes. Synthetic 48-base oligodeoxyribonucleotide probes common to rTRβ1 and rTRβ2 cDNAs and specific for rTRα1, rTRα2, and REV-ErbAα cDNAs (Fig. 1) were prepared on an Applied Biosystems DNA synthesizer and purified on an 8 M urea/8 M polyacrylamide gel. A control, sense probe (27) encoding the last 16 amino acids of rat vasopressin (26) was similarly synthesized and purified. Probes were 3′ end labeled by using terminal deoxynucleotidyl transferase and either dATP[α-^{35}S] (NEN, >1000 Ci/mmol; 1 Ci = 37 GBq) for hybridization histochemistry or [α-^{32}P]dATP (NEN, 3000 Ci/mmol) for RNA analysis.

RNA Analysis. Total RNA was isolated by using guanidine isothiocyanate and enriched for poly(A)$^+$ RNA by oligodeoxyribothymidylate cellulose chromatography as described (28). RNA was analyzed according to Koller *et al.* (29) using 2×10^6 cpm of ^{32}P-labeled probe per ml of hybridization medium.

Hybridization Histochemistry. Hybridization histochemistry was performed according to Young *et al.* (30) with few modifications. Briefly, two 16-μm-thick coronal sections were mounted per slide and processed as described (30). To reduce nonspecific binding of probes, sections were prehybridized for 2 hr at 37°C in a buffer containing 50 μM [α-thio]dATP (NEN). A total of 2×10^6 cpm of probe per 30 μl of the same medium used for prehybridization was then applied to each slide. The sections were then incubated in a humid chamber for 20–24 hr at 37°C. Following hybridization, sections were briefly washed three times in 1× standard sodium citrate (SSC, pH = 7.2) at room temperature and for 1 hr in four changes of 2× SSC/50% formamide at 42°C. Sections were then soaked for 1 hr in two changes of 1× SSC at room temperature and dipped into water and 70% ethanol prior to air drying. Northern analysis and hybridization histochemistry were done under equivalent hybridization and wash stringencies. Wash temperatures were ≈19°C below the calculated probe-mRNA melting temperature (31, 32).

Autoradiography. Sections were apposed to film (Kodak X-Omat AR and Amersham Hyperfilm-Bmax) for 1–8 weeks. Selected sections were coated with 50% Kodak NTB-3 emulsion and exposed for 10 weeks prior to development and staining with toluidine blue.

RESULTS

Hybridization Specificity. Several steps were taken to ensure specificity of probe hybridization. (*i*) Probes had low sequence complementarity to non-target c-*erbA* cDNAs. (*ii*) Probes derived from adjacent regions of the same cDNA produced identical patterns by hybridization histochemistry (data not shown). (*iii*) A messenger sense vasopressin probe (26) was used in parallel with antisense probes as a measure of nonspecific hybridization (27). (*iv*) Under equivalent hybridization and wash stringencies used for hybridization histochemistry, probes were found to hybridize to rat brain RNAs of previously reported sizes on Northern blots (14, 16, 18, 21, 23–25). Probes specific for rTRα1 and rTRβ transcripts hybridized only faintly to total RNA on Northern blots. Therefore, poly(A)$^+$-selected rat brain RNA was used to confirm the hybridization of probes to mRNAs of expected sizes (Fig. 2). A probe common to rTRα1 and rTRα2 cDNAs detected RNAs of ≈2.6, ≈5, and ≈6 kb (Fig. 2*A*, lane 1). Probes specific for rTRα1 hybridized to 5- and 6-kb transcripts (Fig. 2*A*, lane 2), whereas probes specific for rTRα2 detected 2.6- and 6-kb RNAs (Fig. 2*A*, lane 3). The 6-kb transcript is presumably a precursor mRNA (24). rTRβ probes hybridized to RNAs of ≈4.5 and ≈6.2 kb (Fig. 2*A*, lane 4), and the REV-ErbAα probe detected a 3-kb transcript (Fig. 2*B*).

Hybridization Histochemistry. rTRα1 and rTRα2 mRNAs were widely distributed and were found in a similar, if not identical, pattern in all regions studied (Fig. 3, Table 1). In addition, a probe common to rTRα transcripts shared the same hybridization pattern of probes specific to rTRα1 and rTRα2 (Fig. 4). The ratio of signal intensities produced by

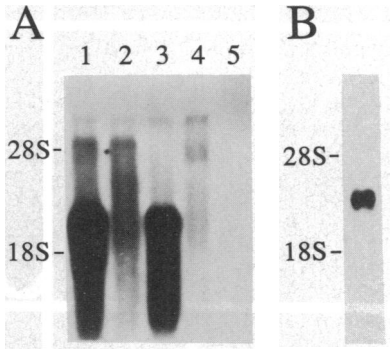

FIG. 2. Northern analysis of rat thyroid hormone receptor-related transcripts. Poly(A)$^+$-selected rat brain RNA (8 μg per lane) was subjected to electrophoresis in a 0.8% agarose/2.2 M formaldehyde gel (*A*) and a 1.0% agarose/2.2 M formaldehyde gel (*B*), transferred to GeneScreen, and hybridized with the same probes used for hybridization histochemistry. (*A*) Lane 1, rTRα1 and rTRα2 common probe; lane 2, rTRα1-specific probes; lane 3, rTRα2-specific probe; lane 4, rTRβ1 and rTRβ2 common probe; lane 5, messenger sense vasopressin control probe. (*B*) REV-ErbAα-specific probe. Positions of 18S [1.9 kilobases (kb)] and 28S (4.8 kb) rRNA are shown. Filters were apposed to film for 1 week at −70°C. Shorter exposures revealed more clearly 2.6-kb RNAs in lanes 1 and 3 of *A*.

these three probes appeared fairly constant. The common probe produced signals greater than the probe specific for rTRα2 mRNA, which, in turn, hybridized more intensely than the probes specific for rTRα1 transcripts. Since the distributions of rTRα1 and rTRα2 transcripts are indistinguishable at our level of resolution, they are summarized below under the heading rTRα. rTRβ transcripts had a more restricted distribution that overlapped with rTRα mRNAs. Since our β probes are complementary to both rTRβ1 and rTRβ2 cDNAs, distributions of the corresponding transcripts are collectively referred to as rTRβ.

Telencephalon. In the rostral telencephalon, very high levels of hybridization to rTRα mRNA were found in the internal granular layer of the olfactory bulb and the compact cell layer of the olfactory tubercle (Fig. 3). In striking contrast, rTRβ transcripts were at control levels in these regions. In the neocortex, rTRα transcripts were seen at moderate levels in layers II, V, and VI and at low levels in layers III and IV. rTRβ mRNA was seen in a different pattern with prominent labeling of layers IV and V and lesser labeling of the remaining layers. Both rTRα and rTRβ probes intensely labeled the pyramidal cell layers of the hippocampus and the granule cells of the dentate gyrus. rTRβ probes labeled the lateral septal nucleus prominently but were absent in the medial septal nucleus. Weak hybridization of rTRα probes was found in both the lateral and medial septal nuclei.

Diencephalon. rTRα transcripts were abundant in the supraoptic nuclei and were present in low to moderate amounts in the preoptic and suprachiasmatic nuclei (Table 1). In contrast, rTRβ mRNAs were detected at negligible to low levels in these nuclei. rTRβ probes labeled the parvocellular part of the paraventricular hypothalamic nucleus (PVN)

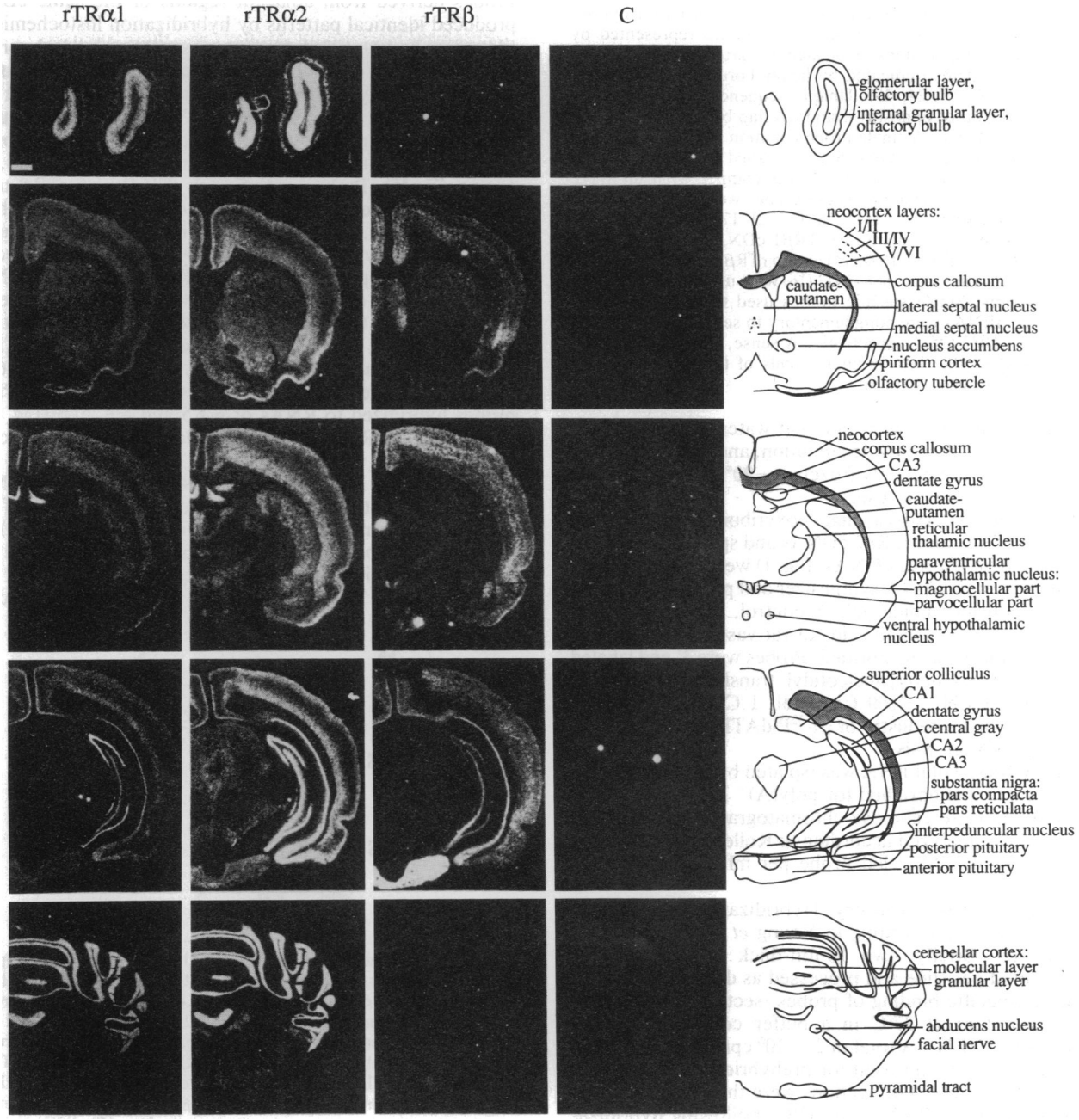

FIG. 3. Distribution of rTRα1, rTRα2, and rTRβ mRNAs in rat brain and pituitary. Immediately adjacent coronal sections from five brain regions were hybridized with ^{35}S-labeled probes for rTRα1, rTRα2, and rTRβ mRNAs and apposed to film for 8 weeks. A sense, control probe (C) was similarly used. Higher levels of hybridization appear whiter. Schematic representations of sections are shown on the right. Images in the second row from the top are derived from a different brain than those in other rows. (Bar = 1 mm.)

Table 1. Relative levels of thyroid hormone receptor mRNAs

Area	rTRα1	rTRα2	rTRβ
Telencephalon			
Olfactory bulb			
Internal granular layer	++	+++	−
Glomerular layer	+/++	++	−
Olfactory tubercle	++	+++	−
Piriform cortex	++	+++	++
Neocortex			
Layers I/II	+/++	++	+
Layers III/IV	+	+	++
Layers V/VI	+/++	++	+
Caudate/putamen	+/++	++	+
Globus pallidus	−	−	−
Hippocampus			
Dentate gyrus	+++	++++	++
CA1	++	++++	+++
CA3/CA4	++	++++	+++
Lateral septal nucleus	−/+	+	++
Medial septal nucleus	−/+	+/++	−
Diencephalon			
Reticular thalamic nucleus	+	+/++	+
Habenula	+	+/++	−/+
Hypothalamic nuclei			
Preoptic	+	++	−/+
Suprachiasmatic	+	++	−
Supraoptic	++	+++	−/+
Paraventricular			
Magnocellular	−/+	+	−/+
Parvocellular	−/+	+	+++
Brainstem			
Central gray	−/+	+/++	−
Dorsal raphe	+	+++	−
Solitary tract nucleus	++	+++	−
Dorsomotor vagal nucleus	++	+++	−
Hypoglossal nucleus	++	+++	−
Cerebellum			
Granular layer	+++	++++	−
Molecular layer	−	−	−
Purkinje cells	−	−	−
Pituitary			
Anterior	+	+/++	++++
Intermediate/posterior	−	−	−

Weak (+), moderate (++), strong (+++), and very strong (++++) film autoradiographic signals were determined from immediately adjacent coronal brain and pituitary sections identically apposed to film for 8 weeks. −, Signal was less than or equal to sense, control probe level. For neocortex, cerebellum, brainstem, and pituitary, localization of hybridization was confirmed by using emulsion-coated sections.

prominently and the lateral, magnocellular part of the PVN to a much lesser degree (Fig. 3). A low level and uniform pattern of hybridization was found for rTRα mRNAs throughout the PVN.

Brainstem. In the midbrain and pons, the dorsal raphe, pontine nuclei, superior colliculus, and central gray were labeled at moderate to high levels by rTRα probes but low to background levels by rTRβ probes (Fig. 3, Table 1). Similarly, in the medulla, rTRα transcripts were seen in several structures, including the hypoglossal, solitary tract, and motor vagal nuclei, whereas rTRβ mRNAs were seen at very low levels in these nuclei (Table 1).

Cerebellum. Intense hybridization to rTRα transcripts was seen in the granular layer of the cerebellum (Fig. 3). rTRβ mRNAs were not detected above background in this layer, and both rTRα and rTRβ transcripts were nearly undetectable in the molecular layer. At high magnification, Purkinje cells were found to be unlabeled by both rTRα and rTRβ probes (Table 1).

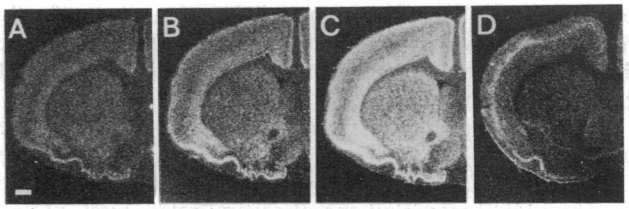

FIG. 4. Distribution of rat α T_3 receptor gene transcripts and REV-ErbAα mRNA in rostral telencephalon. Brain sections were hybridized with probes specific for rTRα1 (*A*), rTRα2 (*B*), and REV-ErbAα (*D*) mRNAs. A probe common to rTRα1 and rTRα2 transcripts was also used (*C*). Higher levels of hybridization appear whiter. Sections were apposed to film for 2 weeks. Sections in *A–C* are from the same brain. An equivalent section from a different brain is shown in *D*. Refer to the second row from the top of Fig. 3 for anatomy. (Bar = 1 mm.)

Pituitary. The anterior pituitary was more intensely labeled by rTRβ probes than any other structure studied (Fig. 3). rTRα transcripts were found at low to moderate levels in the anterior pituitary. Neither rTRα nor rTRβ mRNAs were seen in the intermediate or posterior lobes.

REV-ErbAα. As a control for nonspecific hybridization, a sense probe complementary to the rTRα2-specific probe was constructed. Unexpectedly, this probe produced a heterogeneous hybridization pattern. Lazar *et al.* (25) have recently characterized the transcript detected by our sense rTRα2 probe. They named the corresponding protein REV-ErbAα and showed that it shares homology with other members of the steroid hormone receptor superfamily. These findings prompted us to further compare the distribution of rTRα transcripts with those of REV-ErbAα. Fig. 4 shows that REV-ErbAα and rTRα mRNAs are found in distinct patterns, with REV-ErbAα probes hybridizing most intensely to a single band in the neocortex. In contrast to both rTRα and rTRβ mRNAs, REV-ErbAα transcripts were also found at high levels in the amygdala and at only moderate levels in the hippocampus and granular layer of the cerebellum (data not shown).

DISCUSSION

Our results show that rTRα1 and rTRα2 mRNAs are found in a wide and apparently identical distribution, with rTRα2 more abundant than rTRα1. We have also shown that rTRα1, rTRβ, and REV-ErbAα transcripts have distinct, partially overlapping distributions suggesting different functional roles for each of the corresponding gene products. Our β probes are common to rTRβ1 and rTRβ2 transcripts. Since rTRβ2 mRNAs are confined to the anterior pituitary, we have likely mapped rTRβ1 transcripts in brain and both rTRβ1 and rTRβ2 mRNAs in the pituitary.

T_3 feeds back on thyrotrophs in the anterior pituitary to control thyroid-stimulating hormone secretion (33). Similarly, T_3 regulates the expression of thyrotropin-releasing hormone in the parvocellular neurons of the paraventricular hypothalamic nucleus (PVN) (29, 34). This homeostatic role of T_3 prompted us to look for T_3 receptor transcripts in these locations. Extremely intense hybridization to rTRβ mRNA was seen in the anterior pituitary and parvocellular PVN, whereas rTRα1 transcripts were found at low and uniform levels in these areas. These results are consistent with rTRβ gene products mediating T_3 feedback regulation of thyrotropin-releasing hormone and thyroid-stimulating hormone.

Several different types of neurologic and psychiatric disturbances have been observed in adult humans with altered T_3 levels (10). The ataxia, tremor, and nystagmus associated with hypothyroidism are thought to be, in part, of cerebellar origin (35). The presence of rTRα1 transcripts in the cere-

bellum raises the intriguing possibility that a specific T_3 receptor subtype may mediate the pathogenesis of neurologic disorders in hypothyroidism through altered regulation of gene expression.

Alternative splicing of the rTRα gene produces multiple transcripts including rTRα1 and rTRα2 (21, 24). Unlike rTRα1, rTRα2 fails to regulate gene expression in response to T_3 (15); rTRα2 may play one of several roles. First, rTRα2 mRNA could merely be a transcriptional processing intermediate. Second, the recognition that rTRα1 and rTRα2 share identical DNA-binding domains and affinities for TREs has led to the more interesting suggestion that rTRα2 may modulate the action of functional T_3 receptors (21, 24). rTRα2 has been shown to diminish T_3-dependent induction of gene expression by rTRα1 and rTRβ (36). Koenig *et al.* (36) have speculated that rTRα2 may inhibit the action of rTRα1 and rTRβ in the brain, thereby contributing to the brain's apparent lack of response to T_3. The very close, overlapping distributions of rTRα1 and rTRα2 transcripts in every brain region examined support this idea.

Similarly, despite the different distribution patterns of rTRα2 and rTRβ mRNAs in the brain, no site was found with rTRβ transcripts in the complete absence of rTRα2 mRNAs. Therefore, rTRα2 could also modulate the action of rTRβ gene products. Alternatively, the apparent unresponsiveness of brain to T_3 may simply be for a lack of measurement of appropriate biochemical parameters. Last, rTRα2 may be activated by an as yet unidentified ligand.

The expression of rTRα2 may in turn be regulated by REV-ErbAα mRNA. The complementary regions of these two transcripts could potentially hybridize, thereby regulating each other's stability and translation (25). The high amounts of REV-ErbAα mRNA in a single band in the neocortex, a site with comparatively low levels of rTRα2 transcripts, make this scenario somewhat less plausible. Perhaps REV-ErbAα binds an unknown ligand.

Taken in aggregate, the distribution of rTRα1 and rTRβ mRNAs agrees with measurements of nuclear T_3 binding in different brain regions (5–7). ^{125}I-labeled T_3 binding sites in rat brain have also been identified by thaw mount film autoradiography (37). Since this approach identified sites of both T_3 processing and receptor binding, direct comparisons to receptor mRNA levels presented here are difficult to make. The use of antibodies specific to rTRα1, rTRα2, rTRβ1, rTRβ2, and REV-ErbAα should provide further insight into the function of each of these molecules.

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