We have quantitated in adult and developing rat tissues the molar concentrations of c-erbA $\alpha_1$- and $\beta_1$-mRNAs, which code for nuclear $T_3$-binding proteins, and c-erbA $\alpha_2$-mRNA, which is generated by alternate splicing of the $\alpha$ gene transcript and codes for a receptor variant which does not bind $T_3$. Comparison of the concentrations of c-erbA $\alpha_1$-mRNA, $\beta_1$-mRNA, or their sum to the $T_3$ nuclear binding capacity per mg of DNA in adult liver, kidney, heart, cerebellum, and cerebrospinal fluid and during the ontogeny of liver and brain shows that the $T_3$ binding capacity/c-erbA mRNA ratio is tissue-specific and related to developmental state. Administration of $T_3$ resulted in a 40-50% fall in the $\alpha_1$ signal of adult liver, kidney, and heart without changing either the $\beta_1$ signal or $T_3$ binding capacity. A 40-fold increase in rat brain $\beta_1$-mRNA occurred in the transition between the 19-day gestational fetus and the 10-day-old neonate. This corresponds to the period during which the $T_3$ content rises in brain and during which $T_3$ is known to influence central nervous system development. Our findings indicate that important translational or post-translational factors influence nuclear receptor levels. They show a striking rise in the brain $\beta_1$-mRNA is localized to the anterior pituitary of the rat (7), whereas the $\beta_1$-mRNA is widely distributed in several tissues (8). A high degree of homology exists between all the c-erbA proteins in the purported DNA binding region. In addition, the three c-erbA-s that code for $T_3$ binding proteins ($\alpha_1$, $\beta_1$, and $\beta_2$) show a high degree of homology in the $T_3$ binding region, particularly the last 40 amino acids which are completely absent from the nonbinding $\alpha_1$ variants.

The functional significance of the diverse proteins coded by the c-erbA genes has received considerable attention. In transient transfection experiments with cDNAs for the $T_3$ binding receptors, both $\alpha_1$ and $\beta_1$ c-erbA products show a capacity for facilitating the expression of specific target genes either in a $T_3$-dependent (9-11) or $T_3$-independent (12) manner. Recently, transient transfection of the cDNA coding for the nonbinding $\alpha_1$ variant has been shown to block the effect of a co-transfected $\alpha_1$ or $\beta_1$-cDNA in facilitating $T_3$ regulation of a third co-transfected reporter gene (13). This blockade has been ascribed to an ability of the $\alpha_1$ protein to compete with $\alpha_1$ and $\beta_1$ for the thyroid hormone response element situated in the 5'-flanking region of the reporter gene.

These findings clearly demonstrate the functional potential of the c-erbA products in transient transfection experiments. However, the role of these receptor forms in the intact animal remains less certain. A logical first step in deducing such function is to determine the relationship between mRNA levels and the $T_3$ nuclear binding capacity. Previous studies in this area had relied on the use of Northern blot analysis to estimate the levels of specific mRNAs. Whereas Northern blots allow comparison of the relative concentrations of the same mRNA in different tissues, this technique does not permit a quantitative comparison of signals generated in the same tissue by different probes hybridizing with distinctive mRNAs. Variation in the degrees of labeling of such probes and in the efficiency of mRNA transfer from agarose pose major obstacles.

Accordingly, we applied techniques which would allow us to measure in normal euthyroid rat tissues the mass of the specific c-erbA mRNA/mg of DNA and to compare the values with each other and the nuclear $T_3$ binding capacities. We further attempted to assess these relationships under circumstances associated with changing levels of receptors. Since $T_3$ lowers both the binding capacity (14) and c-erbA mRNA (15) content of cultured GH cells, we considered the possibility that the $T_3$ receptor content and c-erbA mRNAs in animal tissues might also be sensitive to hormonal state. Lastly, we examined these parameters in the neonatal development of liver and brain, processes which have been documented to be associated with changes in nuclear binding capacity (16).

The results of our studies highlight the importance of translational or post-translational determinants of tissue receptor levels. They show a striking rise in the brain $\beta_1$.

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**c-erbA mRNA Levels in Neonatal and Adult Rat Tissues**

mRNA between fetal day 19 and neonatal day 10, a period during which T₃ is known to exert important effects on central nervous system development. Our results suggest that the β-c-erbA mRNA plays a predominant role in mediating thyroid effects in adult rat tissue and in the tissues of the developing neonate.

**EXPERIMENTAL PROCEDURES**

**Animals**—Male Sprague-Dawley rats (150–200 g) were purchased from Bio-Lab (White Bear Lake, MN) and rendered hypothyroid by 3 weeks of treatment with 0.025% methimazole (Sigma) in their drinking water. Animals were made hyperthyroid by the intraperitoneal injections of 200 μg of T₃/100 g body weight every 48 h for 5 days. Rat fetuses and neonates were rendered hypothyroid by continuous feeding of the mothers' drinking water starting at 12 days of gestation and continuing throughout the period of study. Animals were killed by exsanguination under ether anesthesia, and the tissues were rapidly removed, frozen in liquid nitrogen, and stored at −80 °C until further use.

**Solution Hybridization**—Total RNA was extracted from various rat tissues using the method of Chirgwin et al. (17) and further purified by organic extraction and salt washings (18). The mass of c-erbA mRNA in adult cerebrum was measured by a modification of the solution hybridization assay (19), using [32P]UTP-labeled specific c RNA probes in conjunction with specific c-erbA mRNA standards.

**cDNA and mRNA Synthesis**—Linearized cDNA templates were transcribed as previously described (23). [32P]UTP and T7 or SP6 polymerase were used as indicated. Rat c-erbA α-cDNA was pGEM I (the α2) provided by Thompson et al. (4) was digested with HindIII and transcribed with T7 polymerase to generate the α₂ mRNA standard (2.1 kb) or digested with Sp6 and transcribed with SP6 polymerase in the presence of [32P]UTP to generate the α₂ specific cRNA probe. Rat α₂-cDNA in Bluescript (r-erbA α₂) from Lazar et al. (21) was linearized with HindIII and transcribed with T7 polymerase to generate β₂ mRNA (2.3 kb) and the noncoding orientation cut with PvuII and transcribed with T7 to produce a β₂-cRNA.

**Northern Gel Electrophoresis**—The combination of solution hybridization of the adult cerebrum sample in conjunction with Northern blot analyses of the test sample together with the adult cerebrum sample as a reference allowed us to quantify the molar amounts of each of the c-erbA mRNAs in all the tissues studied using the equation below:

\[
\text{fmol c-erbA mRNA tissue } y = \left( \frac{\text{OD tissue } y}{\text{OD adult cerebrum}} \right) \times \left( \frac{\text{fmol c-erbA mRNA adult cerebrum}}{\text{total RNA tissue } y} \right) \times \left( \frac{\text{mg total RNA tissue } y}{\text{mg DNA tissue } y} \right)
\]

where y is any tissue, A is the absorbance of specific cDNA hybridization bands on Northern blots.

**RESULTS**

**Tissue Distribution of c-erbA mRNAs in Euthyroid Rat:**

**Correlation with T₃ Binding Capacity**—A representative Northern blot of 20 μg of total brain cerebral RNA illustrates the characteristic sizes of the c-erbA mRNA species hybridized with the probes used in these studies (Fig. 1). The first lane was hybridized to a common α probe that anneals to both the 2.6-kb α₁-mRNAs (α₁-I and α₁-II; collectively referred to as α₁ in the present studies) and the 5.0-kb α₂-mRNA. After washing, the blot was rehybridized with the β₁ probe to yield the characteristic 6.0-kb c-erbA β₁ signal. The β₂-mRNA has previously been shown to be pituitary-specific and thus not present in brain (7).

Northern blot analyses have already demonstrated a heterogeneous distribution of c-erbA mRNAs in various rat tissues (8). The β₂ mRNA signal has been shown to be abundant in liver, whereas the levels of the α₂ signal are high in brain. We have overcome the quantitative limitations of Northern blot analysis by placing in each gel a reference sample of cerebral RNA, the c-erbA mRNA content of which had been predetermined by means of cRNA solution hybridization analysis. Measurements of the cRNA/DNA ratios of individual tissues allowed us to express the mass of each mRNA species per mg of DNA. Six rat tissues were analyzed: liver, heart, kidney, spleen, testis, and brain (Table I). The markedly lower
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T₃ nuclear binding capacity per mg of DNA previously observed in cerebellum as compared to cerebrum prompted us to divide the brain to allow analysis of these areas separately (28).

Cerebrum has the highest amount of α₁-mRNA (Column A), followed by cerebellum, kidney, heart, and finally liver. α₃-mRNA was virtually absent in spleen and was not detectable in testis. Cerebrum also had the highest level of the nonbinding α₂ variant mRNA (Column B), followed closely by the level in cerebellum. The levels of α₂-mRNA in cerebrum and cerebellum are higher by almost an order of magnitude than any other c-erbA mRNA measured in this series. Testis, which is devoid of both mRNAs that code for T₃ binding proteins (α₁ and β₁), does contain appreciable amounts of the α₂ variant. Cerebrum also contained the highest tissue content of β₂-mRNA (Column C), exceeding the liver content by some 3-fold. Whereas α₁- and α₂-mRNA levels in cerebrum and cerebellum are comparable, the β₂-mRNA levels in cerebellum are 25-fold lower than in the cerebrum. The level of the β₁-mRNA in spleen was also exceedingly low, 120-fold less than that in cerebrum, and comparable in magnitude to that of the α₂- and α₁-mRNAs in spleen. Thus, the expression of the α and β c-erbA genes differs markedly in various tissues and in different areas of the brain.

The sum of the masses of the mRNAs coding for T₃-binding proteins are listed in Column D. Cerebrum had the highest combined signal of all tissues. The fractional contribution of the β₂-mRNA to the sum of both binding forms is listed in Column E. The conventionally T₃-responsive tissues, liver, kidney, and heart show the highest proportion of β₂-mRNA: 82, 85, and 73%, respectively. In contrast, for spleen and cerebrum, the β₁ mRNAs represent only about 50% of the binding forms. In the cerebellum, the β₁-mRNA constitutes only 7% of the binding forms of mRNA. The relative tissue content of α₁ and α₂ is also determined by tissue-specific factors (Column F). In liver, the α₁ constitutes 48% of the sum of α₁ + α₂ mRNA whereas the corresponding value in testis is 0.

The binding capacities listed in Column G are consonant with previously published results (28, 29), with liver exhibiting the highest value and in declining order, cerebrum, heart, kidney, cerebellum, spleen, and testis. The last is devoid of measurable T₃ binding capacity. As in previous studies, the T₃ binding capacity/mg of DNA in cerebellum was 3-4-fold lower than that in cerebrum. The lack of any clear-cut correlation between T₃ binding capacity and the mass of c-erbA mRNA in a tissue is apparent from the ratio of binding capacity to the sum of the α and β mRNA (Column H = Column G/Column D). There is a 10-fold variation in this ratio among the tissues studied. A similar variation also occurs if the ratio of the binding capacity to the mass of either β₁-

### Table 1

| Tissue     | α₁ (fmol/mg DNA) | α₂ (fmol/mg DNA) | β₁ (fmol/mg DNA) | α₁ + β₁ (fmol/mg DNA) | β₁/(α₁ + β₁) | α₁/(α₁ + α₂) | Bₚmax (ng/mg DNA) | β₁/(α₁ + β₁) (F) | α₁/(α₁ + α₂) (F) | Bₚmax/(α₁ + β₁) | α₁ + β₁/Bₚmax (F) |
|------------|-----------------|-----------------|-----------------|------------------------|--------------|--------------|-----------------|-----------------|----------------|-----------------|-----------------|
| Liver      | 0.23 ± 0.05     | 0.25 ± 0.06     | 1.06 ± 0.4      | 1.29 ± 0.04            | 0.82 ± 0.05  | 0.48 ± 0.05  | 0.66 ± 0.05     | 0.51 ± 0.05     |
| Cerebrum   | 3.4 ± 0.47      | 31.0 ± 2.1      | 4.2 ± 0.28      | 7.60 ± 0.55            | 0.55 ± 0.10  | 0.10 ± 0.06  | 0.43 ± 0.02     | 0.06 ± 0.02     |
| Cerebellum | 2.43 ± 0.15     | 15.7 ± 1.9      | 0.17 ± 0.04     | 2.60 ± 0.07            | 0.13 ± 0.03  | 0.10 ± 0.06  | 0.16 ± 0.02     | 0.16 ± 0.02     |
| Kidney     | 0.52 ± 0.11     | 2.4 ± 0.8       | 2.5 ± 0.87      | 3.32 ± 0.85            | 0.18 ± 0.03  | 0.10 ± 0.06  | 0.38 ± 0.04     | 0.18 ± 0.02     |
| Heart      | 0.56 ± 0.12     | 1.29 ± 0.16     | 1.53 ± 0.39     | 2.09 ± 0.73            | 0.30 ± 0.03  | 0.18 ± 0.06  | 0.26 ± 0.03     | 0.26 ± 0.03     |
| Spleen     | 0.635 ± 0.013   | 0.554 ± 0.015   | 0.034 ± 0.01    | 0.069 ± 0.04            | 0.49 ± 0.03  | 0.39 ± 0.03  | 0.0023 ± 0.03   | 0.26 ± 0.03     |
| Testis     | 0               | 1.13 ± 0.29     | 0               | 0                      | 0             | 0             | 0               | 0               |

**Fig. 1.** Northern blot hybridization of rat brain RNA using c-erbA cDNA probes. Total RNA was extracted from euthyroid adult rat brain, electrophoresed in an agarose formaldehyde gel, electrotransferred to Zeta-bind paper, and hybridized to a nick-translated cDNA probe for c-erbA α₁ and α₂ (a) or a cDNA probe for c-erbA β₁ (β), as described in detail under “Experimental Procedures.”
or α₁-mRNA is calculated (calculations not shown). The results in Column H indicate the absence of a simple relationship between the amount of c-erbA mRNA in a tissue and T₃ binding capacity.

In an attempt to further define the physiologic roles of the various c-erbA mRNAs, we compared changes in the levels of the c-erbA mRNAs and T₃ binding capacity under circumstances that previously have been reported to alter the binding capacity: 1) thyroidal status and 2) neonatal brain and liver development.

**Effect of Thyroidal Status on the Levels of c-erbA mRNAs and T₃ Binding Capacity**—Previous studies designed to assess the effect of thyroidal state on the T₃ nuclear receptor content of tissues and cell lines have yielded conflicting results. Samuels et al. (14) have shown that addition of T₃ to the medium of GH cells lowered nuclear T₃ binding capacity to approximately one-half of the initial value. The fall in T₃ binding sites in GH cells is accompanied by a reduction in α₁, α₂, and β₂ mRNA, while β₁ mRNA content remained constant (7, 15).

In contrast, earlier studies in our laboratory showed that α₁ mRNA content of various c-erbA mRNAs, while β₁ mRNA content remained constant (7, 15). In parallel, earlier studies in our laboratory showed that in the absence of TB, T₃ nuclear binding capacity to approximately one-half of the initial value. The fall in T₃ binding sites in GH cells is accompanied by a reduction in α₁, α₂, and β₂ mRNA, while β₁ mRNA content remained constant (7, 15). In contrast, earlier studies in our laboratory showed that α₁ mRNA content of various c-erbA mRNAs, while β₁ mRNA content remained constant (7, 15).

The relatively low levels of both the α₁- and α₂-mRNAs remained constant during this period. The β₁ mRNA on the other hand fell by 60% from 19-day fetus to neonatal animals 4 days after birth. From day 4 to adult, the levels of the β₁-mRNA remained constant. During this same developmental period, the T₃ nuclear binding capacity began to increase, culminating in adult levels 5-fold the 19-day fetal level (Fig. 3B). The development of the liver is therefore associated with a 5-fold increase in T₃ binding without an increase in the levels of the c-erbA mRNA. These findings support the fact that no simple relationship exists between the c-erbA mRNAs and nuclear T₃ binding capacity of a tissue.

Fig. 4A illustrates the developmental pattern of the c-erbA mRNA levels in the livers of normal rats from fetal day 19 to the 2-month-old adult animal. The relatively low levels of both the α₁- and α₂-mRNAs remained constant during this period. The β₁ mRNA on the other hand fell by 60% from 19-day fetus to neonatal animals 4 days after birth. From day 4 to adult, the levels of the β₁-mRNA remained constant. During this same developmental period, the T₃ nuclear binding capacity began to increase, culminating in adult levels 5-fold the 19-day fetal level (Fig. 3B). The development of the liver is therefore associated with a 5-fold increase in T₃ binding without an increase in the levels of the c-erbA mRNA. These findings support the fact that no simple relationship exists between the c-erbA mRNAs and nuclear T₃ binding capacity of a tissue.

Fig. 4A illustrates the developmental pattern of the c-erbA mRNA levels in neonatal and adult rat tissues.
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FIG. 3. A, c-erbA mRNA levels in rat liver during ontogeny. Total RNA was extracted from the livers of rats during various stages of development, from 19-day gestational fetus to 2-month-old adult animals. The RNA was then analyzed for changes in the amounts of c-erbA α1 (○), α2 (■), and β1 (●) mRNA during development by Northern blots and solution hybridization as described under "Experimental Procedures." n = 4 animals per group for the neonatal day 10 and 15, as well as the adult animals, and n = 4 pools of 4-6 livers for the 19-day fetus and neonatal day 1, 4, and 6 animals (mean ± S.D.). B, nuclear T3 binding capacity in rat liver during ontogeny. Nuclear T3 binding capacity was measured in isolated whole nuclei of liver by Scatchard analysis as described under "Experimental Procedures." Nuclei were isolated from portions of the same pools of liver (4-6 rats per pool) used for c-erbA mRNA studies.

mRNAs in cerebrum during the same period. In the 19-day fetus, as in the adult, the predominant c-erbA mRNA is α1. The α2 mRNA is at all times approximately 10-fold higher than the α1 mRNA. Both α c-erbA mRNAs rise coordinately to a plateau value on day 4, a 4-fold rise from fetal levels. After day 10, both α mRNAs fall to an adult value 1.5-fold higher than that of the 19-day fetus.

The developmental patterns of the β1 mRNA contrast sharply with the pattern of the α1 and α2 mRNAs. The β1 mRNA in the 19-day fetus is barely detectable at levels less than 10% that of the α1. From these low fetal levels there is a sharp increase in the β1 mRNA with neonatal development. The rise in the β1 mRNA persists until day 10 when adult levels are achieved, 40 times the initial fetal values. As a result, β1 mRNA becomes the predominant T3 binding form in adult brain, some 3-4 times greater than the α1 mRNA.

Changes in the nuclear T3 binding capacity in cerebrum during this developmental period also demonstrates a dissociation between mRNA and binding capacity. The binding capacity rises 3-4-fold from the fetal value to reach a maximum on the 4th day after birth. Thereafter, the binding capacity falls to an adult value which is approximately 1.5 fold that of the fetus, in accordance with previously published data (16, 32). Although the developmental time course of the T3 binding capacity in rat cerebrum resembles that of the α1 mRNA, it differs sharply from the β1 mRNA as well as the sum of the α1 and β1-mRNA. Thus, unless one is prepared to assume that in the brain the β1-mRNA is not expressed as a functional nuclear T3-binding protein, these data like those from the developing liver do not support a simple proportional

FIG. 4. A, c-erbA mRNA levels in the developing rat brain. Total RNA was extracted from the brains of rats during various stages of development, from 19-day gestational fetus to 2-month-old adult animals. The RNA was analyzed for changes in the amounts of c-erbA α1 (○), α2 (■), and β1 (●) mRNA during development by Northern blots and solution hybridization as described under "Experimental Procedures." n = 4 animals per group for neonatal days 10 and 15, as well as the adult animals, and n = 4 pools of 4-6 brains for the 19-day fetus and neonatal day 1, 4, and 6 animals (mean ± S.D.). B, nuclear T3 binding capacity in the developing rat brain. Nuclear T3 binding capacity was measured in isolated whole nuclei of brain by Scatchard analysis as described under "Experimental Procedures." Nuclei were prepared from portions of the same pools of tissue used for the c-erbA mRNA studies.
erbA P1-mRNA levels were measured as described under "Experimental Procedures" in euthyroid and hypothyroid rat brain at various times during development by Northern blot analysis and solution hybridization. T3 levels in the cytosol were measured by immunoassay as described under "Experimental Procedures." Animals were rendered hypothyroid by addition of methimazole to the drinking water of the pregnant mothers from 12 days of gestation throughout the period of study. Cytosol T3 levels in hypothyroid brains were all at or below the level of detectability, 10 pg of T3/ml. For c-erbA β1 determinations, n = 4 animals per group for neonatal days 10 and 15, and n = 4 pools of 4-6 brains for the 19-day fetus and neonatal day 1, 4, and 6 animals (mean ± S.D.). For cytosol T3 determinations, n = 4 animals per group (mean ± S.D.). Control samples, open symbols; hypothyroid samples, closed symbols.

relationship between the level of the c-erbA mRNAs and T3 binding. The 40-fold rise in the β1-mRNA during the first 10 days of neonatal life takes on special significance in view of the fact that most of the developmental changes in the central nervous system induced by T3 take place in this interval (31). During this period of development, there is a well documented rise in the level of plasma T3 (16). The present studies indicate that this is reflected by a proportional increase in T3 in the brain cytosol (Fig. 5). To ascertain whether the increase in T3 initiated the rise in the β1-mRNA in hypothyroid animals were rendered hypothyroid by administering methimazole to the mothers at 12 days of gestation and continued throughout the neonatal development. Despite the fact that this procedure completely depleted plasma and cellular T3 in the neonates, the rise in the β1-mRNA was indistinguishable from that observed in untreated animals (Fig. 5). In a related experiment, administration of receptor-saturating doses of T3 to newborn rat pups did not accelerate the rise in β1-mRNA levels in the brain above that seen in the hypothyroid or euthyroid animal (data not shown). Therefore, the rise in β1-mRNA apparently represents a developmental phenomenon causally unrelated to the T3 content of the brain. 

DISCUSSION

The methods described in this manuscript have permitted for the first time a quantitative comparison of the levels of α2-, α1-, and β1-mRNAs in multiple tissues in adult and developing rat. These measurements have facilitated a comparison of the content of individual c-erbA mRNAs with nuclear T3 binding capacity as determined by isotopic displacement analysis in the same tissues.

Quantitation of the molar content of c-erbA mRNA has also made possible an estimate of the average number of molecules per cell. Such calculations, based on Avogadro’s number and the assumption of 8 pg of DNA per cell, indicate that in the average hepatic cell the number of c-erbA mRNA molecules is 1.1, 1.2, and 4.8 for α1, α2, and β1, respectively. These mRNAs are thus clearly in the low abundance class.

In contrast, we have estimated the number of receptor molecules per hepatic cell to be 4600 (33). Since the t1/2 of the receptor has been estimated to be about 4 h (34-36), we calculate that each mRNA molecule generates one receptor every 20 s. This is in general accord with published estimates of protein synthesis rates in eukaryotic cells (one molecule every 20-60 s) (37).

One of the major conclusions of this study is that there is no simple relationship between the content of α1- and β1-mRNA and the total T3 binding capacity. This is apparent from the 10-fold variation in the ratio of the binding capacity to the sum of α1- and β1-mRNAs in various tissues of the euthyroid rat and from the change in this ratio in developing rat liver and brain. Moreover, a selective 50-60% reduction of the α1-mRNA by thyroid hormone in liver, kidney, and heart results in no detectable changes in binding capacity. These findings supersede conclusions reached in our earlier studies based on heterologous cDNA probes which suggested a direct relationship between binding capacity and expression of the β gene (20).

The dissociation of binding capacity from mRNA suggests the operation of translational or post-translational factors in determining the expression of the c-erbA mRNAs as protein. Such factors could include different degrees of sequestration of the mRNA in cytosol, variation in the efficiency of translation at the ribosomal level, post-translational modification of the translational product, and alterations in the stability of the receptor protein. Although unlikely, the possibility has not been entirely excluded that the observed discrepancies are due to as yet unidentified receptor mRNA species.

Our studies emphasize that tissue and developmental factors determine both the expression of the c-erbA genes as mRNA and the various mRNAs as protein. The tissue-specific regulation of the α gene transcript is particularly instructive in this regard. At one extreme lies the testis which contains substantial quantities of α2-mRNA but no measurable amounts of α1-mRNA. At the other extreme, the gene products in liver are equally represented by the α1- and α2-mRNA species. The changes in total α gene expression observed in response to T3 administration in heart, liver, and kidney as well as the developmental changes noted in brain and liver development are characterized by a constant ratio of α1- to α2-mRNA. These findings indicate that, although T3 and developmental factors influence α gene expression, these factors do not influence the selective processing of the α gene transcript to the α1 and α2 gene.
fetal serum. In the present studies, we have also demonstrated that there is a parallel rise in the T_{3} tissue content of T_{3}. In the euthyreotic neonatal rat, the effects of thyroid hormone deficiency can be reversed if T_{3} treatment is started prior to day 15. Delays in starting T_{3} replacement past 15 days produce increasingly abnormal brain development. The clinical counterpart of this phenomenon is observed in human cretinism.

Our studies revealed that accompanying the increase in serum and brain T_{3} during the first 10 days of neonatal life was an unanticipated 40-fold increase in the level of β_{3}-mRNA. The rise in β_{3}-mRNA is not due to the rising level of T_{3}, since hypothryroid animals with undetectable cytotoxic brain T_{3} showed an identical increase in β_{3}-mRNA during this period. The coordinate increase in T_{3} concentrations, β_{3}-mRNA levels, and the well established functional and structural changes in the central nervous system during this time period suggest that the c-erbA β_{1} receptor is involved in the transduction of T_{3} effects on normal brain development. The target genes of T_{3} during this period may well be responsible for initiating the structural changes which occur during the early neonatal period. These genes may function only during a restricted time frame. This may account for the failure of T_{3} to reverse the structural damage of neonatal hypothyroidism in the rat if T_{3} is administered after neonatal day 15.

The finding that β_{3}-mRNA is the predominant mRNA in the conventionally thyroid-responsive tissues liver, kidney, and heart provides further suggestive evidence favoring a functional role of the β_{3} translational product. Furthermore, fetal rat brain, which is generally not believed to be responsive to T_{3}, is almost completely devoid of β_{3}-mRNA. Thus, the correlation of β_{3}-mRNA with thyroid response (rather than binding capacity) in certain tissues supports the suggestion that the β_{3}-mRNA product may play a central role in thyroid hormone action.

The potential role of the α_{3}-mRNA, which codes for a non-T_{3}-binding protein, has also received attention in the literature. Most recently, Koenig et al. (13) have shown by transfection experiments that the α_{3}-cDNA product can block the effects of co-transfected α_{1} and β_{1} in facilitating the regulation of reporter genes by T_{3}. These observations have prompted speculation that the high levels of α_{3} in brain account for the nonresponsive nature of this tissue to T_{3}.

Our findings provide the first quantitative assessment of the relative content of α_{1}, α_{3}, and β_{1} in cerebrum. The levels of α_{3} in adult cerebrum are 7-fold higher than those of β_{1}, and 9-fold higher than those of α_{1}-mRNA, thus lending credence to the possibility that the α_{3} product could block the accessibility of target genes to T_{3}-binding receptors. Such a view would necessarily be based on the unproved assumption that in cerebrum the α_{3} protein is greatly in excess of that coding for the β_{1} or α_{1} product.

The possibility that α_{3} protein under some circumstances stimulates constitutive gene expression rather than opposing the effects of β_{3} or α, deserves consideration. Although the gene for malic enzyme is regulated by T_{3} in many tissues it is not regulated by T_{3} in brain. The malic enzyme gene in brain is, nevertheless, highly expressed. Experiments in our laboratory have suggested that the level of malic enzyme mRNA in adult brain approaches that in the hyperthyroid liver (data not shown). It is possible that the level of expression of genes such as malic enzyme are rendered maximal by the presence of the β_{3}-mRNA product. The possibility should be seriously considered in light of previous findings by Samuels and coworkers (12) that transfection of α_{3} and β_{3}-cDNAs can result in both T_{3}-dependent and T_{3}-independent stimulation of gene expression. Further, our findings in tests demonstrate that this tissue contains substantial quantities of α_{3}-mRNA but is totally lacking α_{1}, and β_{3} mRNA and receptor demonstrable by displacement methods. Therefore, if α_{3} has any function in this tissue it is not that of blocking the binding of T_{3} receptors to putative thyroid hormone response elements of T_{3} target genes.

The possibility that the high concentrations of α_{3} in brain are responsible for preventing the regulation of many genes by T_{3}, either by blocking access to the T_{3} receptor complex or by maximal constitutive stimulation, presents a potential problem in understanding any direct effects of thyroid hormone effects on brain development. However, the present studies have clearly demonstrated that α and β gene products are differentially distributed in the brain. Whereas the relative α_{1} and α_{3}-mRNA content in cerebrum and cerebellum are roughly similar, the relative levels of β are much lower in the cerebellum. Examples of similar segregation of α and β gene products has also recently been demonstrated by the in situ hybridization studies of Bradley et al. (42). Such segregation would make it possible for certain cell groups rich in β and poor in α, to respond to T_{3}. A definitive resolution of many of the issues raised in this study will depend upon the development of specific antibodies for identifying the translational products of the α_{3}, α_{2}, and β_{3}-mRNA and the identification and quantification of specific brain genes which transduce the developmental effects of thyroid hormone.

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