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THE HEPATIC BIOSYNTHESIS OF RAT THYROXINE BINDING GLOBULIN (TBG) : DEMONSTRATION, ONTOGENESIS, AND UP-REGULATION IN EXPERIMENTAL HYPOTHYROIDISM

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Using a human thyroxine binding globulin (TBG) cDNA probe, we demonstrate that rat liver contains two TBG mRNA species of different length, consisting of about 1.8 Kb and 2.4 Kb respectively. Slot blot analysis of the hepatic mRNAs from rats of different age reveals a fair correlation between the developmental trend of the messengers and that of the TBG circulating levels. Finally Northern blot and slot blot studies demonstrate that the increase of serum TBG induced in adults by thyroidectomy actually reflects an enhanced hepatic biosynthesis of the protein. ©1990 Academic Press, Inc.

We have recently demonstrated in the rat serum a hitherto unrecognized major thyroxine binding protein, with binding and structural properties close to those of the human thyroxine binding globulin (TBG), and with a striking ontogenic profile, characterized by a high transient increase during post-natal development (1-3). Here we use a TBG complementary DNA probe of human origin (4) to demonstrate the presence of TBG mRNA in the rat liver. We also analyze the ontogenic variations of the rat liver TBG mRNAs, to determine whether the ontogenesis of the serum TBG is correlated with that of its hepatic transcription. Finally, we investigate at mRNA level the effect of thyroidectomy on the adult TBG to further clarify the mechanism by which serum TBG increases in experimental hypothyroidism (5-7).

MATERIAL AND METHODS

Tissues. Normal human liver was a gift from Professor Olivier Bernard (Inserm Unity n° 56, Kremlin-Bicêtre, France). Different age rats and thyroidectomized adult rats (Sprague Dawley, CD strain) were from Charles River, France. Thyroidectomy was performed at 5 weeks and the operated animals were studied at 8 weeks. The studies were carried out on mixed sexes up to 6 days after birth and on males afterwards.

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Northern blot analysis of TBG mRNAs

Preparation of total and polyadenylated [Poly (A)] RNAs. Total cellular RNA was extracted with phenol from human liver and from livers of 6 days, 2 months and 2 months old thyroidectomized rats and precipitated with LiCl 2M. Rat poly (A) RNAs were purified from total RNA by oligo (dT)-cellulose chromatography (8).

Electrophoresis and transfer of the RNAs. RNAs were denaturated in a pH 7.4 buffer, containing 6.5 mM Na H_2 PO_4, 8% formaldehyde and 0.6% formamide, for 10 min. at 65°C and separated by electrophoresis through 1% agarose gel. Transfer onto nylon membrane (Zeta-Probe, Biorad, Richmond, California) was followed by fixation of the RNAs at 80°C for 2 hours.

Preparation of the hybridization probe. The λcTBG 8 clone containing human TBG cDNA (4) was purified, digested with Eco RI endonuclease (Boehringer, Mannheim) and run on a 0.8% agarose gel. The insert (1.2 Kb) was excised and labelled with [^{32}P] αdCTP by random priming (Multiprime kit, Amersham Int. G.B.)

Hybridization. The membranes carrying the RNAs were incubated for 4 h. at 42°C in a pre-hybridization solution containing 4 x SSEP (600 mM NaCl, 40 mM Na H_2 PO_4, 4 mM EDTA), 50% formamide 1% SDS (sodium dodecyl sulfate), 0,5% Blotto (10% powder milk of the Regilait brand, 0.1% NaN_3) and 0.5 mg/ml herring sperm DNA. The hybridization step was performed at 42°C for 16 h. in a solution consisting of the pre-hybridization milieu with the admixture of 5% Dextran sulfate and of the labelled probe (500.000 cpm/ml). The membranes were washed for 20 min. in 0.5 x SSC (75 mM NaCl, 7.5 mM sodium citrate, pH 7)-0.1 x SDS at room temperature, then for 30 min in 0.1 x SSC-0.1 x SDS at 47°C. Finally they were exposed to Kodak X-OMAT-AR film at -80°C (9) with two intensifying screens. Quantitation of signal intensities was performed by densitometric scan with a dual wavelength TLC scanner CS 930 (Shimatzu Corp. Kyoto, Japan).

Slot Blot analysis of TBG mRNAs

For each studied liver tissue 20 µg of total RNA and serial dilutions (down to 2.5 µg) were resuspended in 10 x SSC - 28% formaldehyde, denatured at 65°C for 10 min., applied onto Zeta-Probe membranes and fixed at 80°C for 2 h. The pre-hybridization and hybridization steps were then performed as described for the Northern Blot analysis (9).

RESULTS

Northern blot demonstration of TBG mRNA in immature and adult rat liver

As shown in Fig. 1, Northern blot analysis using human TBG cDNA as hybridization probe and total human liver RNA as reference (lane 1), reveals with the polyadenylated RNAs of the 6-day

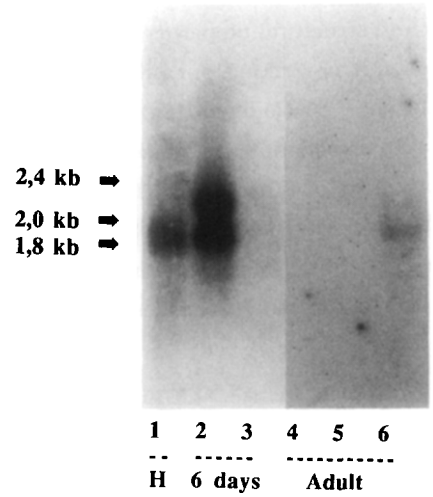

Fig. 1 : Northern Blot analysis of TBG mRNA in rat liver.
Lane 1 : Normal human liver : total RNA, 10 µg . Lanes 2 and 3, liver of 6 days rat : poly(A) RNA, 2 µg (Lane 2) ; total RNA, 20 µg (Lane 3). Lanes 4-6, liver of adult (60 days rat : poly(A) RNA, 2 µg (Lane 4) ; total RNA, 20 µg (Lane 5) ; poly(A) RNA, 20 µg (Lane 6).

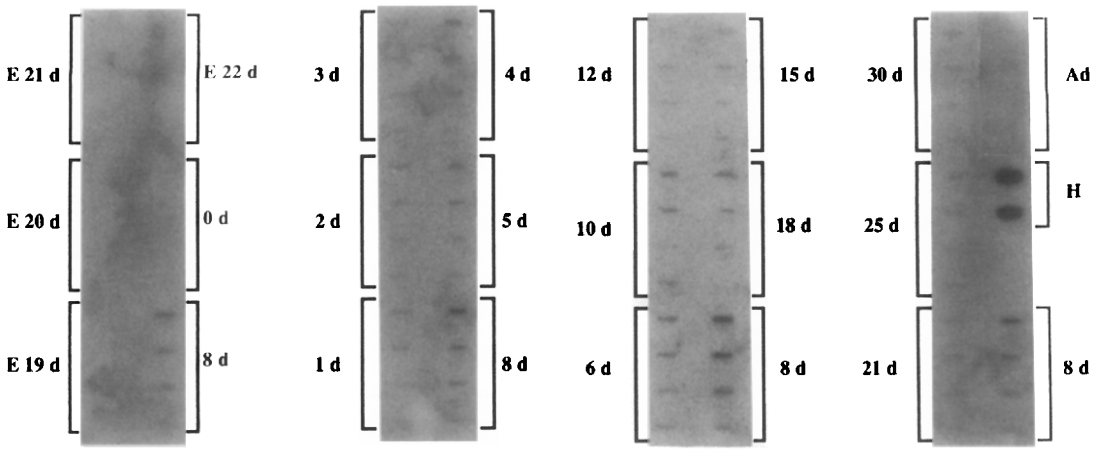

Fig. 2 : Slot Blot analysis of ontogenic variations of TBG mRNA in the rat liver.
20 µg, 10 µg, 5 µg and 2.5 µg of total RNA are applied for each studied age, from 19 day embryo (E 19 d) to 60 days adult (Ad).
On each membrane, the total RNA from 8 days rats [age corresponding to the serum peak of TBG (1)] is applied as intra- and inter-membrane control. Total Human RNA (H) is applied as 20 µg and 10 µg samples.

rat liver (2 µg/lane, lane 2) two TBG mRNA species, one approximately 1.8 kilobases and the other about 2.4 Kb in length. With the total RNA of the immature rat (lane 3), at 20 µg/lane, no image is visible. When the adult rat liver is studied, no labelling is discernible whether total RNA (20 µg, lane 4) or poly (A) RNAs (2 µg, lane 5) are applied ; however, increase of the adult poly (A) RNA sample to 20 µg /lane (lane 6) allows visualization of the 1.8 mRNA band. The densitometric scan of the autoradiograph demonstrates for the signal obtained with the 6-days liver (lane 2) that the shorter mRNA represents the more abundant species (57%).

These results demonstrate the hepatic biosynthesis of rat TBG and the existence of two mRNA species of the protein. The differences of labelling observed with the 6 days and the adult RNAs clearly indicate the higher concentration of TBG messengers in the pups. The high labelling seen in the immature liver contrasting with the hardly visible one in the adult prevents any reliable quantitative densitometric comparison of the two signals.

Slot blot analysis of ontogenic variations of TBG mRNAs in the rat liver

The labelling of the TBG mRNAs at different ages from 19-day embryos to 60 days old rats is shown in Fig. 2. There is no visible signal in the embryo, even at the highest concentration of RNA (20 µg). A slight signal appears at 1 day, when it is manifest only for the highest RNA concentration. Maximum density, visible with all four RNA concentrations, down to 2.5 µg RNA, is at 6-8 days, then the signal progressively decreases. In rats older than 30 days no labelling is seen.

The ontogeny of TBG hepatic biosynthesis correlates fairly well with that of serum TBG activities measured by equilibrium and non-equilibrium binding techniques (1-3), demonstrating that the post-natal peak of serum TBG actually reflects a postnatal surge of the biosynthesis of the protein.

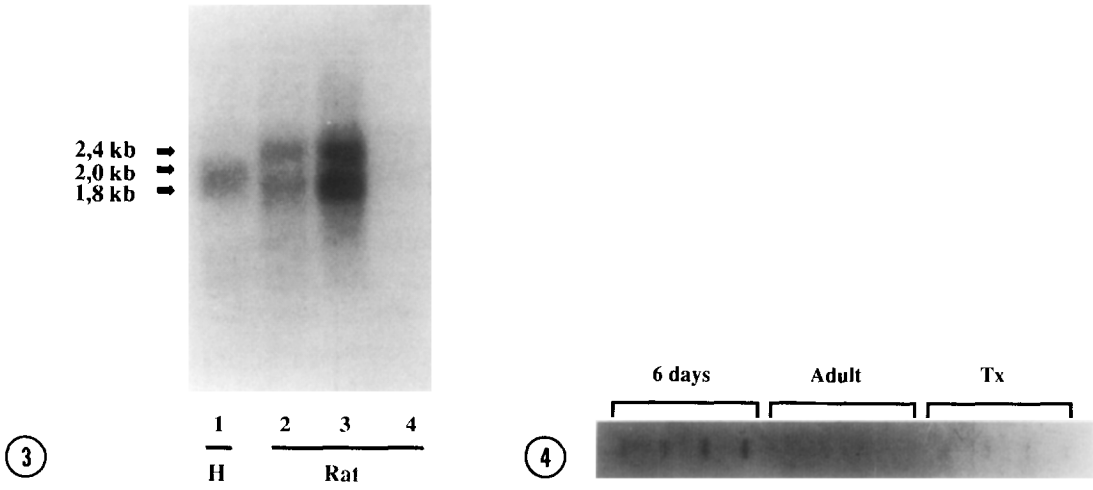

Fig. 3 : Northern Blot analysis of TBG mRNA in thyroidectomized adult rat liver. Lane 1 : normal human liver : total RNA, 10 µg. Rat liver : poly(A) RNA from : lane 2 : thyroidectomized (Tx) 60 days adult ; lane 3 : 6 days pups ; lane 4 : 60 days normal adult. In lanes 2, 3 and 4, poly (A) RNA are applied as 5 µg samples.

Fig. 4 : Slot Blot analysis of TBG mRNA in liver of thyroidectomized 60 days adult rat (Tx) compared to 6 days immature and to 60 days adult rats. For each studied case, 4 concentrations (2.5, 5, 10, 20 µg) of total RNA are applied.

Northern and slot blot analysis of TBG biosynthesis in adult thyroidectomized rat

Northern blot analysis (Fig. 3) clearly reveals both TBG mRNA species in the liver of thyroidectomized adult rat (lane 2), contrasting with the undetectable signal in the normal adult of the same age (lane 4). Densitometric scan evidences in the 6 days rat and in the hypothyroid adult the same proportion between the 1.8 Kb and 2.4 Kb TBG mRNAs, i.e. about 57% of the shorter species, while the total area of the TBG mRNA peak is about 2.5 times higher in the 6 days rat than in the hypothyroid adult. Thus the amount of TBG mRNAs in the thyroidectomised adult appears intermediate between that of the pups and that of the normal adult (no visible signal). This gradation is further confirmed by the slot blot analysis shown in Fig. 4.

These results clearly indicate that the increase of serum TBG found in hypothyroid adults (5-7) is due to an enhanced expression of the TBG gene.

DISCUSSION

We demonstrate the biosynthesis of thyroxine binding globulin by the rat liver and evidence the presence of two rat TBG mRNA species, a smaller and apparently more abundant messenger of about 1.8 Kb, and a longer one of approximately 2.4 Kb. Our demonstration is obtained through successful hybridization of the rodent liver RNAs with a TBG cDNA probe of human origin : this authenticates the rat TBG as a genuine member of the " TBG " family, an identity we had previously assigned to the rat thyroid hormone carrier on the basis of analogies of binding properties, electrophoretic migration and structural microheterogeneity, with the human TBG (1-3).

The rodent TBG appears similar to the human TBG (10) in that it has two different size transcripts, the 1.8 Kb mRNA being shared by rat and man TBGs, whereas the rat 2.4 Kb mRNA is larger than the 2.0 Kb human counterpart. This suggests that the 3'-untranslated DNA sequence, which has been shown to account for the size difference between the two human transcripts (10), may be somewhat different in the rat. In addition, similarly to what has been suggested for the human TBG mRNAs, the existence of two messengers for TBG in the rat could arise by alternative processing and polyadenylation at two different sites. Isolation ans sequencing analysis of rat TBG cDNA is needed to further clarify these points.

The present studies unambiguously establish that the striking ontogenic pattern of the rat serum TBG binding activities, with its characteristic high transient post-natal surge, reflects throughout development the hepatic levels of TBG messengers. Moreover, our results demonstrate that the increase of adult circulating TBG levels induced by experimental hypothyroidism (5-7) likewise results from an enhanced biosynthetic process. The evidence that the development-regulation as well as the thyroid hormone-regulation of the rat TBG occur at gene expression level is consistent with the possibility that the ontogenic variations of the protein carrier are controlled by the ontogenic variations of its hormone ligands. Thus it may be envisaged that the well-documented physiologic " hypothyroidism " of the neonates (11) is crucially involved in the neonatal rise of TBG.

Rat TBG is a novel example of a protein down-regulated by the thyroid hormones. There are only a few known instances of rat proteins undergoing this type of control, including the pituitary peptide TSH, the c-erb A α thyroid hormone receptor and the β myosin heavy chain (12, 13, 14). The fact that TBG regulation by the thyroid hormones is analogous to that of TSH, which is a key factor in the feedback control of thyroid hormone secretion, is of particular interest. Similarly to TSH, rat TBG appears both as index and regulator of thyroid hormone activities. Specifically, as an index it may represent an adequate measure of hormone action at protein synthesis level, while as a regulator its role may be important for the distribution to targets of the circulating hormones, particularly during definite developmental stages or thyroid disorders.

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