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Research Article

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Cloning of the Mammalian Type II Iodothyronine Deiodinase

A Selenoprotein Differentially Expressed and Regulated in Human and Rat Brain and Other Tissues

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Abstract

The deiodination of thyroid hormones in extrathyroidal tissues plays an important role in modulating thyroid hormone action. The type II deiodinase (DII) converts thyroxine to the active hormone 3,5,3′-triiodothyronine, and in the rat is expressed in the brain, pituitary gland, and brown adipose tissue (BAT). Complementary DNAs (cDNAs) for the types I and III deiodinases (DI and DIII, respectively) have been isolated and shown to code for selenoproteins. However, information concerning the structure of the mammalian DII remains limited, and the pattern of its expression in human tissues is undefined. We report herein the identification and characterization of rat and human DII cDNAs. Both code for selenoproteins and exhibit limited regions of homology with the DI and DIII. In the rat pituitary and BAT, DII mRNA levels are altered more than 10-fold by changes in the thyroid hormone status of the animal. Northern analysis of RNA derived from human tissues reveals expression of DII transcripts in heart, skeletal muscle, placenta, fetal brain, and several regions of the adult brain. These studies demonstrate that: (*a*) the rat and human DII are selenoproteins, (*b*) DII expression in the rat is regulated, at least in part, at the pretranslational level in some tissues, and (*c*) DII is likely to be of considerable physiologic importance in thyroid hormone economy in the human fetus and adult. (*J. Clin. Invest.* 1996. 98:405–417.) Key words: thyroid • selenium • metabolism • development • fetus

Introduction

In some mammalian tissues, such as the developing brain, the anterior pituitary gland, and brown adipose tissue (BAT)[1], where thyroid hormone appears to have particularly important regulatory effects, a relatively high proportion of the receptor-bound 3,5,3′-triiodothyronine (T_3) is produced within the tissue itself, rather than being derived from plasma (1–4). The expression in these tissues of the type II iodothyronine deiodinase (DII), which catalyzes deiodination of thyroxine (T_4) exclusively on the outer ring (5′-position) to yield T_3, strongly suggests that this enzyme is responsible for this "local" production of T_3 and is thus important in influencing thyroid hormone action in these tissues (5). In addition, DII activity is markedly elevated in the hypothyroid state and appears to be responsible for catalyzing the production of a large proportion of the circulating T_3 under such conditions (6). For example, in neonatal rats, circulating T_3 levels are not reduced when the type I deiodinase (DI) is inhibited by 6-*n*-propyl-2-thiouracil (PTU), a specific inhibitor of this enzyme (2). Thus, DII is of critical importance for both the local generation of T_3 in selective tissues and systemic thyroid hormone homeostasis.

Complementary DNAs for the DI and type III deiodinase (DIII) have recently been isolated and demonstrated to contain in-frame TGA codons that code for selenocysteine (7–10). The catalytic properties and tissue patterns of expression of these selenoproteins differ from those of the DII. Thus, DI appears to play an important role in converting T_4 to T_3 within the thyroid gland itself (11, 12), and, unlike DII, is expressed in the liver and kidney and is capable of inner ring (3- or 5-position) deiodination of sulfated thyroid hormone conjugates (13). DIII functions as an inner ring deiodinase to convert T_4 and T_3 to inactive metabolites (3,3′,5′-triiodothyronine [rT_3] and 3,3′-diiodothyronine, respectively). Its expression in placenta and several fetal tissues during early development suggests that it plays a role in preventing premature exposure of developing tissues to adult levels of thyroid hormones (14). The DII also is present in several fetal and neonatal tissues (15) and is essential for providing the brain with appropriate levels of T_3 during the critical period of development (16).

Isolating cDNAs for DII has been problematic. Our attempts at screening appropriate cDNA libraries under reduced stringency conditions using DI and DIII cDNAs as probes has proved unsuccessful (Croteau, W., M. Schneider, and D.L. St. Germain, unpublished data). Furthermore, unlike the DI and DIII, the DII is poorly expressed in *Xenopus laevis* oocytes after the injection of poly(A)$^+$ RNA from DII-containing tissues (17), thus rendering this expression system untenable as a tool for screening cDNA libraries. However, using a PCR-based strategy, we have recently cloned a cDNA (designated RC5′DII) for the DII of the amphibian species *Rana catesbeiana* (Davey et al., reference 18). In the present studies, we used this amphibian DII cDNA to identify homologous rat and human cDNAs. We have demonstrated that these cDNAs code for selenoproteins with DII activity. The predominant mRNA species for these mammalian enzymes are 6–8 kb in size and are highly expressed in a number of tissues including the fetal and adult brain of rats and humans, and in human heart, placenta, and skeletal muscle.

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1. *Abbreviations used in this paper:* 5-D, 5-deiodinase; 5′-D, 5′-deiodinase; BAT, brown adipose tissue; DI, type I deiodinase; DII, type II deiodinase; DIII, type III deiodinase; PTU, 6-*n*-propyl-2-thiouracil; rT_3, 3,3′,5′-triiodothyronine; SECIS, selenocysteine insertion sequence; T_3, 3,5,3′-triiodothyronine.

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Methods

RNA preparation and Northern analysis. RNA was prepared as previously described (19) from BAT of male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) (150–175 g) exposed to cold (4°C) for 24 h. Poly(A)$^+$ RNA was isolated by one or two cycles of chromatography over oligo(dT)-cellulose (Collaborative Biomedical Products, Bedford, MA). RNA from other rat tissues used for Northern analysis was prepared by the same methods. RNA blots of human tissues were purchased from Clontech (Palo Alto, CA) and contained ~ 2 μg of poly(A)$^+$ RNA per lane according to the supplier's specifications. Hybridization and washing of Northern blots were performed as previously described for rat tissues (20), or according to the supplier's instructions for blots of human tissues, except that final washes were performed at 42 or 60°C instead of 50°C. In some experiments, rats were rendered hypothyroid by the inclusion of 0.05% methimazole in their drinking water for 25–30 d, or hyperthyroid by a single daily subcutaneous injection of 50 μg T_3/100 g body wt for 4 d before killing. After hybridization with the specific DII probes identified in these studies, blots were stripped and reprobed with a mouse, rat, or human β-actin probe. Hybridization signals were quantified by densitometric measurements of scanned computer images of the autoradiographs using the IPLab Gel program (Signal Analytics Corp., Vienna, VA) on a Macintosh computer. In all cases, signals obtained using the DII probes were normalized using the signals generated with a β-actin probe.

cDNA probe preparation. cDNA probes for Northern analysis and library screening were prepared by PCR using the appropriate cDNAs as templates and gene-specific sense and antisense primers that flanked the regions of interest. After amplification, the PCR reaction products were separated on a low melt agarose gel stained with ethidium bromide, and the appropriate band was then excised and purified using a QIAquick Gel Extraction Kit (Qiagen Inc., Chatsworth, CA). The PCR product was then labeled with ^{32}P using the Oligolabeling Kit from Pharmacia LKB Biotechnology Inc. (Piscataway, NJ).

cDNA library construction and screening. Poly(A)$^+$ RNA from BAT of cold-exposed rats was used to prepare a cDNA library in the Uni-Zap XR Vector according to the kit manufacturer's instructions (Stratagene Inc., La Jolla, CA). First-strand cDNA synthesis was primed by the oligo-(dT) linker provided in the kit. Screening of the library was performed using plaque hybridization under low stringency conditions according to the methods of Lees et al. (21). The first 305 nucleotides of the coding region of the RC5′DII were used as a probe. (This region of the deiodinase proteins is the least conserved among the three enzyme subtypes. Thus, the use of the 5′ portion of the coding region made it less likely that DI and DIII cDNAs would be identified during the screening process.) Positive plaques were detected by autoradiography and purified by additional rounds of screening using the hybridization conditions described above. cDNA inserts were sequenced on both strands using vector and gene-specific primers and an automated sequencing system with fluorescent dye terminators (Applied Biosystems Inc., Foster City, CA).

Expression studies in X. laevis oocytes and COS-7 cells. Stage 5-6 *X. laevis* oocytes were isolated and each microinjected as previously described (19) with 50 ng of in vitro–synthesized, capped RNA transcripts prepared using the MEGAscript kit (Ambion Inc., Austin, TX). After injection, oocytes were incubated for 3–4 d in Barth's medium (for determination of 5-deiodinase [5D] activity) or L-15 medium (for determination of 5′-deiodinase [5′D] activity), and then harvested. Membrane fractions were prepared as described previously (22).

For expression in COS-7 cells, rat and human DII cDNAs and the G21 rat DI cDNA (kindly provided by Drs. M. Berry and P.R. Larsen, Brigham and Women's Hospital, Boston, MA) were subcloned into the pcDNA3 mammalian expression vector (Invitrogen Corp., San Diego, CA). Cells were grown and maintained in DME supplemented with 10% iron-supplemented calf serum (Sigma Chemical Co., St. Louis, MO). Cells (10^7 in Hepes-buffered saline) were transfected with 22 μg of plasmid DNA using an Electroporator (Bio-Rad Laboratories, Hercules, CA), and then maintained in culture medium for 48 h before harvesting. After aspiration of the medium, cell monolayers were washed twice with phosphate-buffered saline, and then scraped from the dish, pelleted, and sonicated in 0.25 M sucrose, 0.02 M Tris/HCl, pH 7.4.

5′D and 5D activity were determined in oocyte membrane preparations or COS-7 cell sonicates according to published methods (17, 22). For the 5′D assay, the reaction buffer contained 1 mM EDTA. In kinetic studies, 5′D activity was determined using either 0.5–16 nM [^{125}I]rT_3 or 0.5–12 nM [^{125}I]T_4 with 20 mM dithiothreitol as cofactor. Kinetic constants were determined from double reciprocal plots. ^{125}I-labeled iodothyronines used as substrates were obtained from Du Pont de Nemours (Boston, MA) and purified by chromatography using Sephadex LH-20 (Sigma Chemical Co.) before use. In other experiments, the deiodinase activities in oocyte membrane preparations or COS-7 cell sonicates were determined in the absence or presence of PTU (10–900 μM) or aurothioglucose (0.01–10 μM). 5′D activity was measured using 1.5 nM [^{125}I]rT_3 as substrate and 20 mM dithiothreitol as cofactor.

Protein concentrations were determined by the method of Bradford (23) with reagents obtained from Bio-Rad Laboratories.

Reverse transcriptase-PCR assay. Coupled reverse transcription and PCR amplification was used to determine the presence of DII transcripts in selected adult rat tissues including BAT from a hypothyroid animal, and liver and white fat from a normal animal. Reactions used the Access RT-PCR System (Promega Corp., Madison, WI) with poly(A)$^+$ RNA (1 μg) as a template. Reaction conditions were as specified by the manufacturer except that 30 or 35 cycles were used in the PCR. Specific oligonucleotide primers derived from the coding region of the rat DII sequence (sense: ACTCGGTCATTCTGCTCAAG; antisense: TTCAAAGGCTACCCCATAAG) were used to prime first-strand cDNA synthesis, and then the amplification of a predicted 590-bp PCR product. Reaction mixtures lacking reverse transcriptase or an RNA template were used as controls. Products were then separated on a 1.0% agarose gel, transferred to a nylon membrane (Magna Charge Micron Separations, Inc., Westboro, MA), and hybridized with a radiolabeled nested oligonucleotide rat DII probe (AATGCCACCTTCTTGACTTT). After washing, the blot was exposed to x-ray film for 12 h. The signals were quantified using a phosphoimager (Molecular Dynamics, Inc., Sunnyvale, CA).

Preparation of chimeric DII/DIII cDNAs. Chimeric cDNAs were constructed by splicing part or all of the 3′-untranslated region of the full length rat DIII cDNA (rNS43-1), which contains an active selenocysteine insertion sequence (SECIS element), to the 3′ end of the coding regions of the rat BAT 1-1 or human Z44085 DII cDNAs (Fig. 1). The 3′-untranslated portion of the rNS43-1 was amplified by PCR using sense primers that included, at their 5′ ends, 19 or 20 nucleotides of the rat or human DII cDNA, and the antisense pBlueScript M13 forward primer. Conditions for these PCR reactions varied, but generally included 28 to 35 cycles of amplification involving 94°C for 1 min, 53 or 57°C for 45 sec, and 72°C for 1.5 min, and a final 10-min extension period. PCR reaction products were purified as described above for cDNA probe preparation.

For construction of the rBAT 1-1 coding region/rat DIII SECIS chimera cDNA (Fig. 1 *A*), the rNS43-1 3′-untranslated region was amplified with primers 1 and 2, and the PCR product was then digested with XbaI and ApaI. This was then subcloned into the rat BAT 1-1 DII pcDNA3 construct that had been truncated in the 5′-untranslated region at a BamHI site and, after relegation, digested with XbaI and ApaI to remove the 3′-untranslated region of the rat DII clone. This construct retained the entire coding region and stop codon of the rat DII clone, although the stop codon was changed from TAG to TAA. The 5′-truncated Bat 1-1 clone was used for this construct because of the possibility that a shorter 5′-untranslated region would allow higher levels of expression in *X. laevis* oocytes.

For constructing the hZ44085 coding region/rat DIII SECIS chimera cDNA, convenient restriction sites near the 3′ end of the human

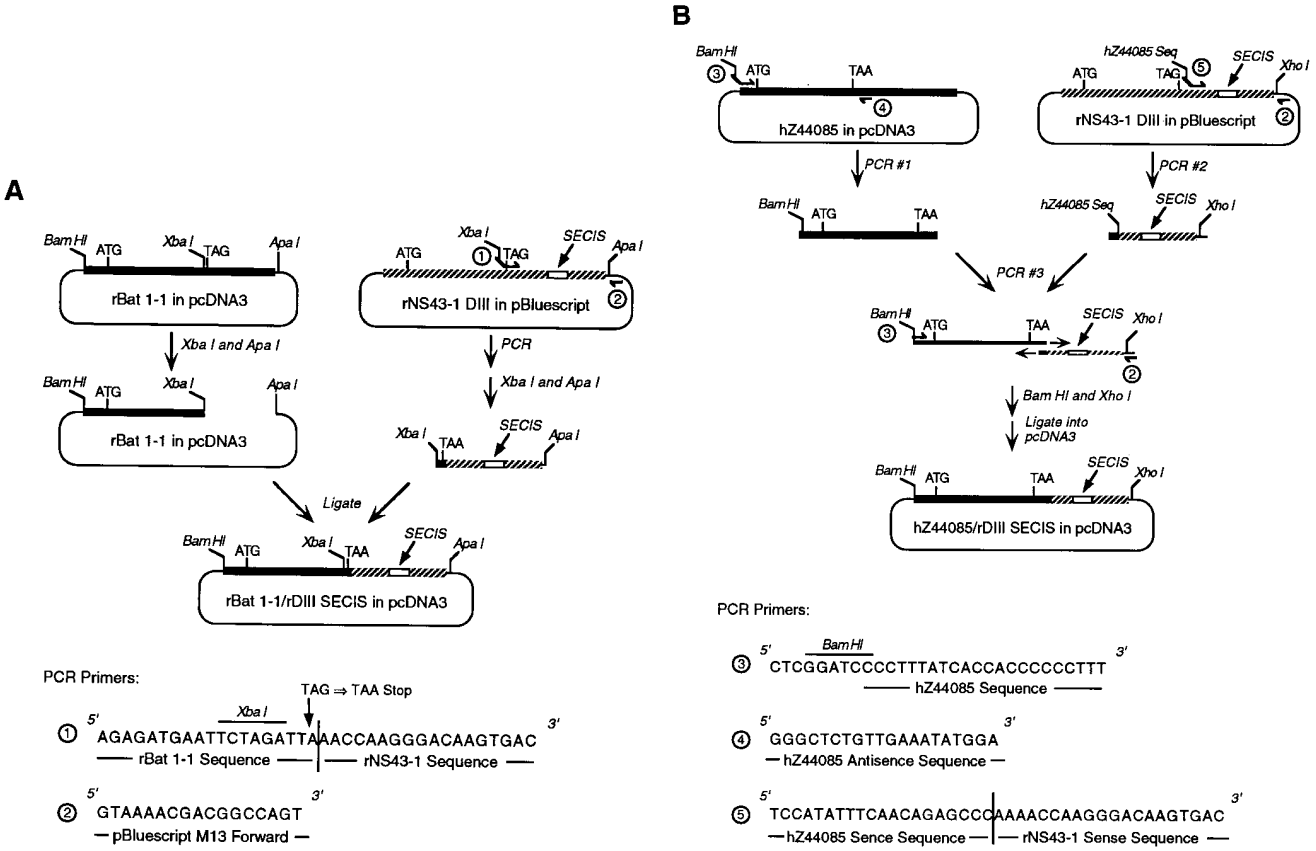

Figure 1. Schematic diagram outlining the strategy for construction of (*A*) the rBAT 1-1/rDIII SECIS and (*B*) the hZ44085/rDIII SECIS chimeric cDNAs. Sequences of the oligonucleotides used as PCR primers are shown in the lower part of each figure. Primer 2 was used in both schemes. See text for detailed description of methodology.

DII coding region were not available, hence an overlap extension PCR method (24) was used (Fig. 1 *B*). The coding region of the human Z44085 DII cDNA was amplified using sense primer 3, whose sequence was derived from the 5′-untranslated region of the clone (with a BamHI restriction site included near the 5′ end of the oligonucleotide), and an antisense primer 4, located in the 3′-untranslated region at 70 nucleotides 3′ to the TAA stop codon. The rNS43-1 3′-untranslated region for this construct was amplified with primer 5, that contains hZ44085 sequences at its 5′ end, and primer 2. After gel purification, these PCR products were spliced together in an overlap extension PCR reaction to yield the full length chimeric hZ44085/rat DIII SECIS cDNA, which was then digested with BamHI and XhoI and cloned into the pcDNA3 vector. Conditions of these PCR reactions were the same as those noted above for amplification of the 3′-untranslated portion of the rNS43-1. Critical regions of the chimeric cDNAs were sequenced to insure the accuracy of the construction methods.

Other methods. Kinetic constants were determined by linear regression analysis of double reciprocal plots. Results are reported as the mean±SEM. Statistical analysis used Student's *t* test with the Bonferroni correction applied for multiple comparisons (25). The evolutionary tree diagram was produced with the GeneWorks program (IntelliGenetics, Mountain View, CA) using the unweighted pair group method with arithmetic mean.

Results

Previous studies employing actinomycin D have provided indirect evidence that the adrenergically mediated cold-induced stimulation of DII activity in rat BAT results from transcriptional activation and an increase in DII mRNA levels (26, 27). Thus, as an initial step in isolating a cDNA for the rat DII, we constructed a cDNA library from poly(A)$^+$ RNA derived from the BAT of rats exposed to 4°C for 24 h. Screening the library by plaque filter hybridization at reduced stringency with a 305-bp fragment from the coding region of the RC5′DII resulted in the isolation of 13 clones containing two different cDNA inserts. The clone containing the largest cDNA (1.9 kb, designated rBAT 1-1) was sequenced on both strands (Fig. 2 *A*). rBAT 1-1 contains an open reading frame of 798 nucleotides that includes two in-frame TGA codons. The first (cDNA nucleotide 951, codon 130) is located in a region that exhibits high homology with the regions in RC5′DII, DI, and DIII cDNAs that contain a TGA triplet that codes for selenocysteine. The second TGA triplet (codon 262) is located four codons upstream from an unambiguous TAG stop codon and is not present in any other deiodinase cDNA previously isolated. The other, smaller cDNA isolated from the BAT library was also sequenced and found to be identical to portions of the BAT 1-1 clone, including the presence of both TGA codons, thus confirming their presence in the rat DII homolog. The 798-nucleotide open reading frame of the rBAT 1-1 cDNA is predicted to code for a protein of 266 amino acids of 29.8 kD. If translation stopped at the second TGA codon, rather than the downstream TAG, a slightly smaller protein would result.

A search of GenBank revealed a DNA sequence (acces-

A

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CCCAAACAACCCTTGCGCTTACAGCACAGTTAAGAAATTGGCAGTGTAAGAAGCCAGACT   60
GCGTTTAAAGGAACATGAAAAGCTAAAAGAAATTTTTACCATCTCTGCAGTCTCATAGGT  120
GCCTGGAAATGAAAGTAGAACTTCCTGTCTTTAACGGACTCTGACAGGGGTAAACTGGAT  180
TAGGGACTGGCACGCCAGGGTGTTGTTGTTGTTGTTGTTGTTTNGTTGTTGTTGTTG    240
CTGGGAGTGGTGGTGTTTTGTTTTTTTTTTTTAACCATCTTAAATCCTGGAAAAAAAATG  300
CATCTCGGAATTGAATGAATTGATGGGTCCACTCCAACTGCTGGGCTGCAGAGACTGAAC  360
TTGGTCTTTCCATTTCTGCTTCTTTCAAAGAAGAGACAAGTTGGGATCCCTTCTAATTTA  420
GCTTTTCTCCCTCTCCCTCACCCCCCACCCCCACTCCCAACCTTCCCCTTGGTCCCCCAC  480
TTCTCTACCACCACCTTCCTTTGCAAAAAGGGGGTGACCGGGGAAGCAGAGTGCACAGGA  540
GACTGACTGAGGAGGTAAAGAAGATGGGACTCCTCAGCGTAGACTTGCTGATCACTCTTC  600
                        M  G  L  L  S  V  D  L  L  I  T  L

AGATTCTGCCAGTCTTTTTCTCCAACTGCCTCTTCCTGGCGCTCTATGACTCGGTCATTC  660
Q  I  L  P  V  F  F  S  N  C  L  F  L  A  L  Y  D  S  V  I

TGCTCAAGCACGTGGCGCTGCTTCTCAGCCGCTCCAAGTCCACTCGCGGAGAGTGGAGGC  720
L  L  K  H  V  A  L  L  L  S  R  S  K  S  T  R  G  E  W  R

GCATGCTGACCTCAGAAGGACTACGCTGTGTCTGGAACAGCTTTCTCCTAGACGCCTACA  780
R  M  L  T  S  E  G  L  R  C  V  W  N  S  F  L  L  D  A  Y

AACAGGTTAAATTGGGTGAAGATGCTCCCAATTCCAGTGTGGTGCACGTGTCCAATCCTG  840
K  Q  V  K  L  G  E  D  A  P  N  S  S  V  V  H  V  S  N  P

AAGCAGGTAACAATTGTGCCTCAGAGAAGACGGCGGATGGGGCTGAATGCCACCTTCTTG  900
E  A  G  N  N  C  A  S  E  K  T  A  D  G  A  E  C  H  L  L

ACTTTGCCAGTGCAGAGCGCCCACTGGTGGTCAACTTTGGTTCAGCGACCTGACCACCTT  960
D  F  A  S  A  E  R  P  L  V  V  N  F  G  S  A  T  SeC  P  P

TTACTAGGCAACTGCCAGCCTTCCGCCAGTTGGTGGAAGAGTTCTCCTCGGTGGCTGACT 1020
F  T  R  Q  L  P  A  F  R  Q  L  V  E  E  F  S  S  V  A  D

TCCTGTTGGTATACATTGATGAGGCTCACCCTTCAGATGGCTGGGCAGTGCCTGGGGACT 1080
F  L  L  V  Y  I  D  E  A  H  P  S  D  G  W  A  V  P  G  D

CCTCTATGTCTTTTGAGGTTAAGAAGCACCGGAACCAAGAGGACCGATGTGCTGCAGCCC 1140
S  S  M  S  F  E  V  K  K  H  R  N  Q  E  D  R  C  A  A  A

ACCAGCTCCTGGAGCGTTTCTCCTTGCCGCCCCAGTGTCAAGTTGTGGCTGACCGCATGG 1200
H  Q  L  L  E  R  F  S  L  P  P  Q  C  Q  V  V  A  D  R  M

ACAATAATGCCAACGTAGCTTATGGGGTAGCCTTTGAACGTGTGTGCATCGTGCAGAGAC 1260
D  N  N  A  N  V  A  Y  G  V  A  F  E  R  V  C  I  V  Q  R

GGAAAATTGCTTACTTAGGAGGGAAGGGCCCCTTCAGCTATAACCTGCAAGAAGTCCGAA 1320
R  K  I  A  Y  L  G  G  K  G  P  F  S  Y  N  L  Q  E  V  R

GTTGGCTGGAGAAGAATTTCAGCAAGAGATGAATTCTAGATTAGCTGGATAAAAGTGTGA 1380
S  W  L  E  K  N  F  S  K  R  *  I  L  D  Stop

TTGTAATAGAGTTTATTATTTTAAAAGAATGTGTAAAAGTTGAGAACTGAACTGAATCTA 1440
TTATTTCAACGGAATCCCATCGCCTCACTGAAAGACGGGTCATACCTGTCAGAAGATCTT  1500
GAACCTCTTTAACATCTCAGTACTTCCTTTAGTACACAAACGGCATCTGGCTAAACAGCA  1560
GCCCTGTTACCTACCTTCAGAGCTAACTGGTCCAAAATGGAGGGCCAGAAACCCCGTTGC 1620
AGCCTTGTTCATTCTCGCTTGTGAAAGAAGTGATGCTGCCCTATGTGTGCTCTGGGACTA 1680
GAGGACAAAAGTTCCCAGAAAACCTATTGTTAAGGGAGCTACTACCTATGATCTGATTAA 1740
GTGTATTGTCTCAGTCAGCTTTAGTCACCAGAAGTAAACCCAACTGGAAAGTCCCCCCAA 1800
ATACATACTTATAATGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1860
AAAAAAAAAAAAAAAAAA                                            1879
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B

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Rana    MGlLSVDLLI TLQILPgFFS NCLFLALYDS VvLvKHVlLq LnRSKSshGq   50
Rat     .......... ......v... .......... .i.l...a.l .s....tr.e   50
Human   ..i....... ......v... .......... .i.l...a.l .s....tr.e   50

Rana    WRRMLTpEGL RCVWnSFLLD AYKQVKLGgD APNSnViHVT dknsssgkpg   100
Rat     ......s... .......... .........e. ....s.v..s npeagnncas   100
Human   ......s... ....k..... .........e. ....s.v..s steggdnsgn   100

Rana    tp-------- -CHLLDFASs ERPLVVNFGS ATXPPFisQL PAFskmVEEF   141
Rat     ---ektadga e........a .......... ......tr.. ...rql....   147
Human   gtqektadga t........p .......... ......ts.. ...rkl....   150

Rana    SaVADFLLVY IDEAHPSDGW AaPGiS--Sy EVKKHrNQED RCAAAnkLLE   189
Rat     .s........ .......... .v..d.sm.f .......... .....hq...   197
Human   .s........ .......... .i..d.sl.f .....q.... .....qq...   200

Rana    qySLPPQCqV VADcMDNNtN aAYGVsFERV CIVQRqKIvY LGGKGPFfYN   239
Rat     rf........ ...r....a. v....a.... .....r..a. .......s..   247
Human   rf......r. ...r....a. i....a.... ........a. .......s..   250

Rana    LQEVRqWLEl tfgkkaesgq tgtek                              264
Rat     .....s...k n.s.rxild                                     266
Human   .....h...k n.s.rxkktr lag                                273
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Figure 2. (*A*) Nucleotide and predicted amino acid sequence for the rBAT 1-1 DII cDNA. The first in-frame TGA codon is designated as coding for selenocysteine (*SeC*), whereas the second TGA codon is shown by an asterisk since it is uncertain if this triplet codes for selenocysteine or is read as a termination signal. An unambiguous TAG stop triplet is located four codons 3′ of the second TGA. (*B*) Comparison of the amino acid sequences deduced from the *R. catesbeiana* (RC5′DII), rat (rBAT 1-1), and human (hZ44085) DII cDNAs. X, selenocysteine; x, position of second TGA codon in rat and human cDNAs.

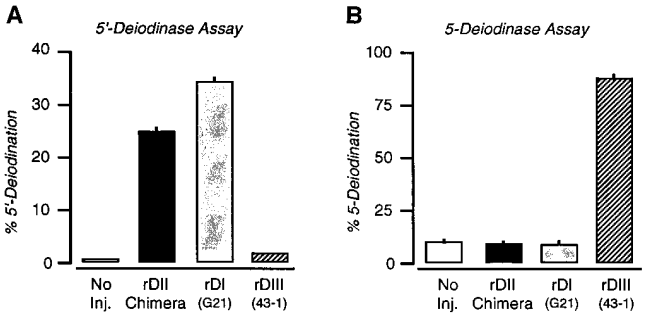

Figure 3. Expression of rat deiodinase cDNAs in *X. laevis* oocytes. Individual oocytes were injected with 50 ng of in vitro–synthesized RNA transcripts prepared using T3 or T7 RNA polymerase and, as template, either the rat Bat 1-1 DII/rDIII SECIS chimera cDNA, the rat G21 DI cDNA, or the rat NS43-1 DIII cDNA. (*A*) 5′-deiodinase or (*B*) 5-deiodinase activity was measured in oocyte membrane preparations. Because of high levels of activity, the G21 and NS43-1 membrane preparations were diluted 20-fold before the 5′-deiodinase or 5-deiodinase assays, respectively. For each group of oocytes, both assays were conducted on aliquots of the same membrane preparations using substrate concentrations of ~ 1.5 nM rT_3 (5′-deiodinase assay) or 1.0 nM T_3 (5-deiodinase assay).

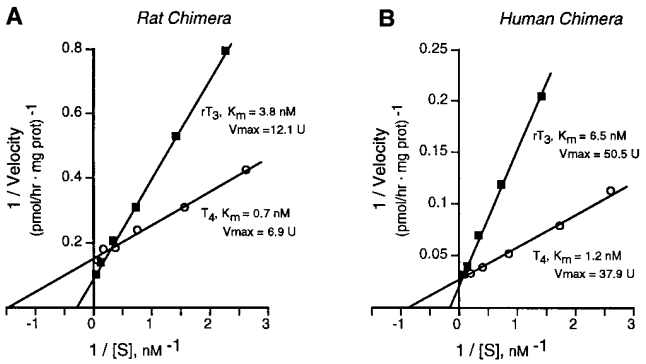

Figure 4. Kinetic analysis using T_4 or rT_3 as substrates of the protein products of the (*A*) rat Bat 1-1 DII/rDIII SECIS chimera and (*B*) human Z44085 DII/rDIII SECIS chimera as expressed in COS-7 cells. V_{max} values are expressed in units of activity where 1 U = 1 pmol/hr per mg protein.

sion Z44085) that is highly homologous (89% nucleotide identity) to a portion of the 5′-untranslated region and the beginning of the coding region of the rBAT 1-1 cDNA. The 320 nucleotides of the hZ44085 sequence were derived from the 5′ end of an expressed sequence tag isolated from a cDNA library prepared from the brain tissue of a 3-mo-old human infant. The high homology suggests that the Z44085 cDNA represents the human DII homolog. The hZ44085 cDNA was kindly provided by Genethon (Evry, France), and we sequenced it in its entirety. This cDNA is ~ 1.9 kb and contains an open reading frame of 819 nucleotides that codes for a protein of 30.0 kD (Fig. 2 *B*) that is highly homologous to those coded by the rBAT 1-1 and RC5′DII cDNAs (87 and 73% amino acid residue identity, respectively). Overall, 72% of the amino acid residues have been conserved among these three proteins. The hZ44085 cDNA contains two in-frame TGA codons in locations analogous to those found in rBAT 1-1. As is the case for the DI and DIII proteins, the amino-terminal portions of these three proteins contain a region of 42 amino acid residues that is highly hydrophobic and may represent a membrane-spanning domain (28). The complete sequences of the rBAT 1-1 and hZ44085 cDNAs have been submitted to GenBank/EMBL/DDBJ and have been assigned accession numbers U53505 and U53506, respectively.

Attempts to demonstrate functional deiodinase activity of the rBAT 1-1 and hZ44085 proteins by either (*a*) the injection of in vitro–synthesized RNA transcripts into *X. laevis* oocytes, or (b) transfection of the corresponding cDNAs into COS-7 cells were unsuccessful. Truncating the rBAT 1-1 cDNA of 405 nucleotides of the 5′-untranslated region at a BamHI site did not result in a cDNA that codes for a functional deiodinase. Examination of the 3′-untranslated region in both cDNAs suggested that they lack a classic stem-loop SECIS element that is necessary for the read through and translation of the TGA codon(s) into selenocysteine. Lacking such elements, one would predict that attempts at expression of these cDNAs would result in the formation of truncated, inactive proteins, as

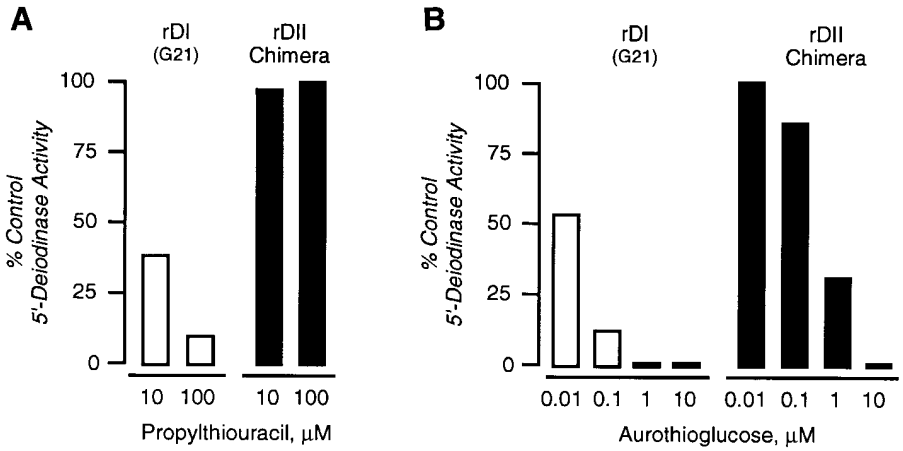

Figure 5. Sensitivity of the G21 rDI and the rBAT 1-1 DII/rDIII SECIS chimera deiodinases to the inhibitory effects of (*A*) PTU as determined in oocyte membrane preparations and (*B*) aurothioglucose as defined in COS-7 cell homogenates. Oocytes previously injected with RNA synthesized in vitro using the G21 rDI or rBAT 1-1 DII/rDIII SECIS chimera cDNAs as templates were harvested, membranes prepared, and then assayed for 5′-D activity in the presence of PTV. Control incubations were performed in aliquots of the same membrane preparations in the absence of inhibitors. For studies examining the effects of aurothioglucose, COS-7 cells were transfected with either the G21 or rBAT 1-1 DII/rDIII SECIS chimera and assays performed in the absence or presence of the inhibitor in cell sonicates. Entirely analogous results were obtained with the hZ44085/DIII SECIS chimera.

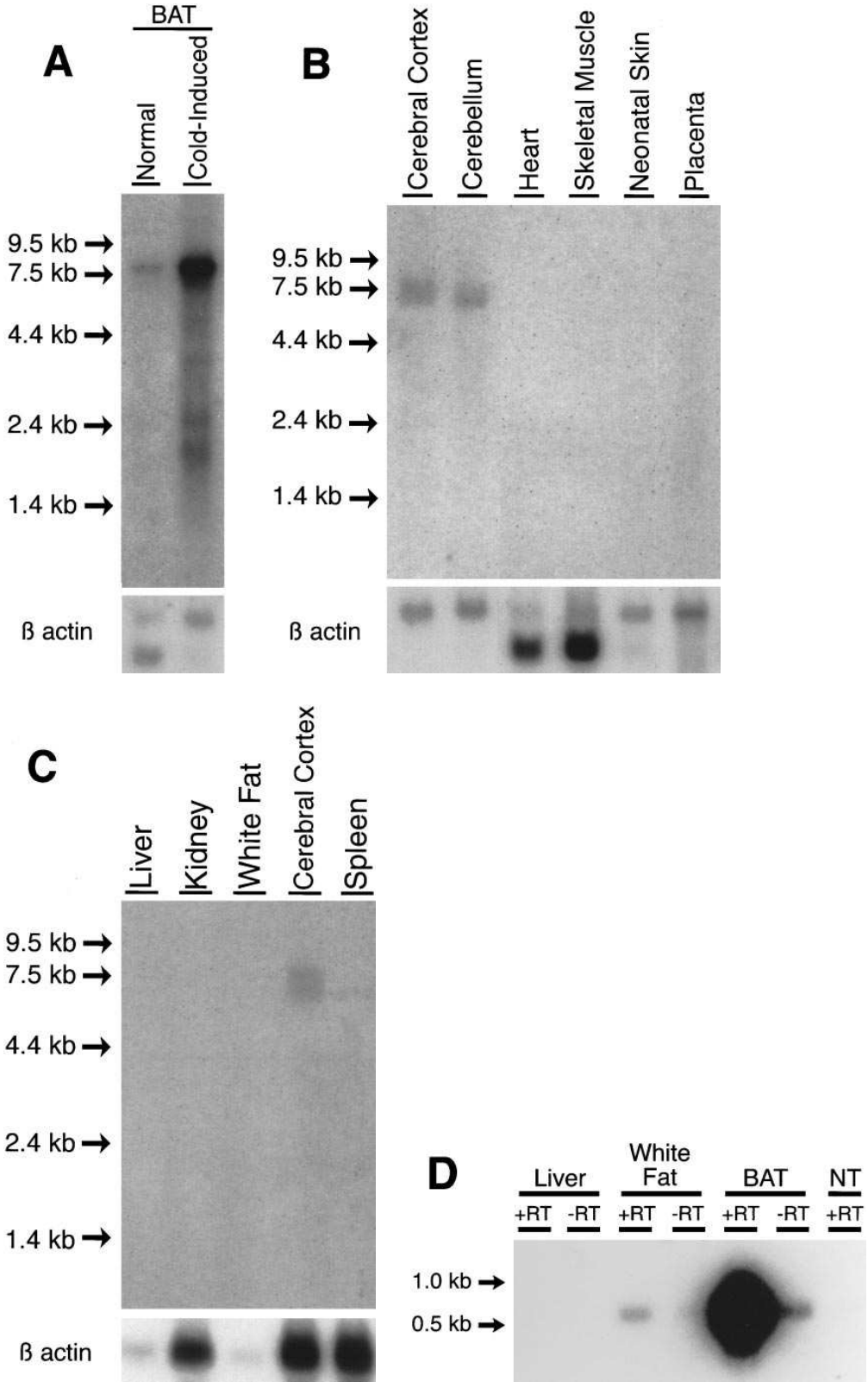

Figure 6. Expression of DII transcripts in rat tissues (*A–C*) by Northern analysis of poly(A)$^+$ RNA (6 μg/lane) from several tissues of euthyroid rats. A portion of the coding region between nucleotides 615–1238 of the rBAT 1-1 cDNA was used as a probe. The final wash step was for 1 h at 42°C in 0.1 × SSC, 0.1% SDS. Blots were then subjected to autoradiography for 1 wk. Identical patterns of hybridization were noted after a 1-h wash in the same solution at 60°C; however, the intensity of the signal was generally somewhat diminished. When using the actin probe, blots were washed in the same solution at 60°C and autoradiographed overnight. (*D*) Coupled reverse transcriptase/PCR assay using poly(A)$^+$ RNA from liver, white fat, and hypothyroid BAT as template. For each sample, reactions were performed with (*+RT*) and without (*−RT*) reverse transcriptase in the mixture. Also, a mixture that did not include RNA (no template) was run as an additional negative control. PCR products were separated on an agarose gel, transferred to a nylon membrane, and then probed with a radiolabeled, nested rat DII oligonucleotide.

has been demonstrated for other deiodinase cDNAs (7, 9). Thus, chimeric constructs were prepared by replacing part or all of the 3′-untranslated regions of the rBAT 1-1 and hZ44085 cDNAs, respectively, with the 3′-untranslated region of the rNS43-1 that contains a potent SECIS element (Moyer, B., and D.L. St. Germain, unpublished observations). In both chimeric constructs, the coding regions of the rBAT 1-1 and hZ44085 cDNAs remained intact.

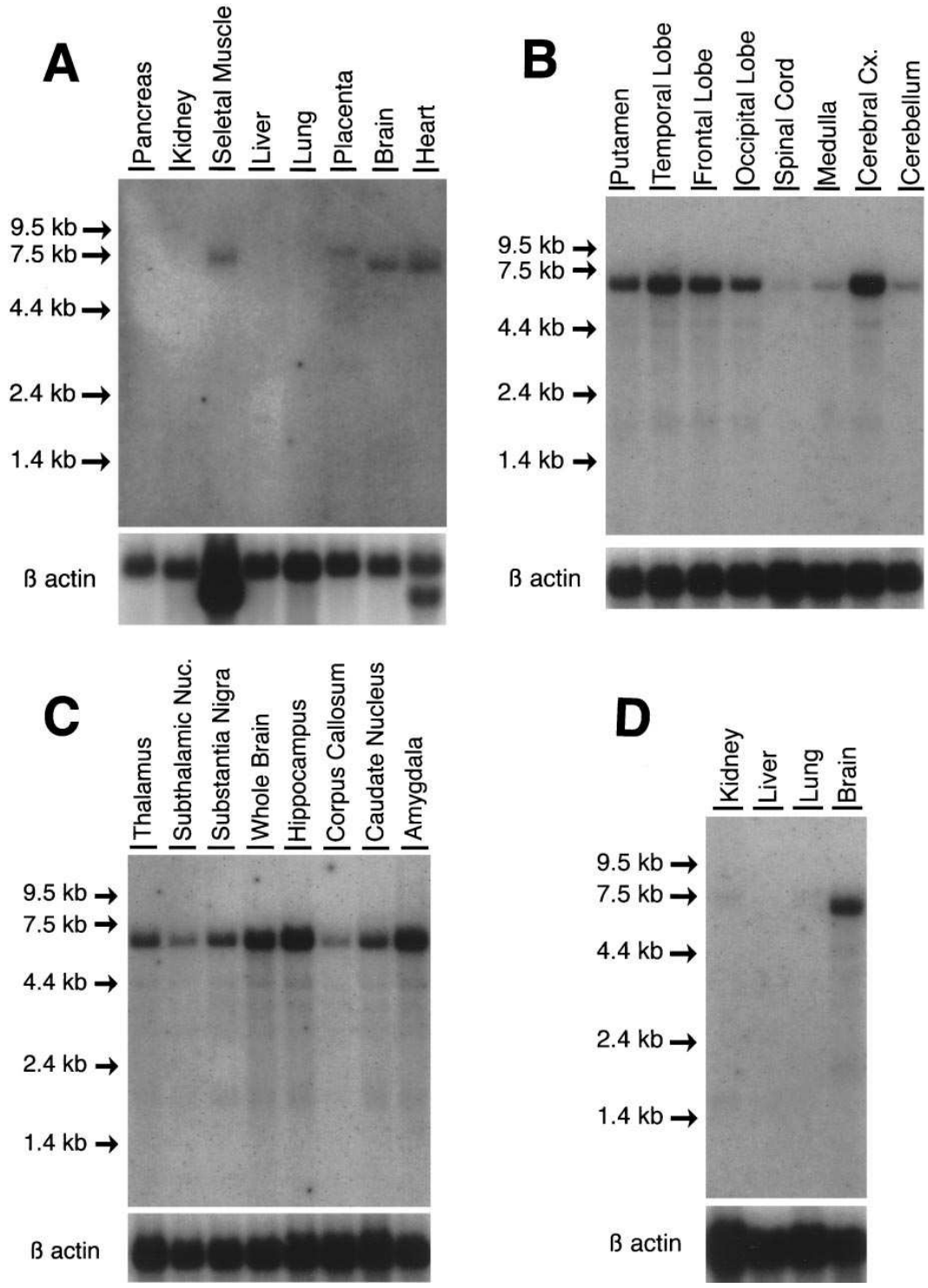

Figure 7. Expression of DII transcripts in human tissues. (*A–D*) Northern analysis of poly(A)$^+$ RNA (2 μg/lane) derived from several human tissues using a portion of the coding region of the hZ44084 cDNA (nucleotides 220–814) as probe. Final wash conditions were the same as those described in Fig. 6, and the blots were autoradiographed for 5–14 d. Washing at 60°C did not affect the patterns of hybridization in any of the blots. After stripping, blots were reprobed with a human actin probe.

As shown in Fig. 3, the injection into oocytes of RNA transcripts derived from the rBAT 1-1/rDIII SECIS chimera cDNA induced 5′-, but not 5-deiodinase activity. In the same experiment, the G21 and the rNS43-1 cDNAs induced high levels of 5′- or 5-deiodinase activity, respectively.

When examined in rat tissue homogenates, the DII exhibits several characteristic properties including: (*a*) K_m values in the nanomolar range for iodothyronine substrates; (*b*) the ability to efficiently 5′-deiodinate T_4 as well as rT_3; and (*c*) relative insensitivity to the inhibitory effects of PTU and gold compounds such as aurothioglucose (29). Using the oocyte and COS-7 cell expression systems, the functional properties of the proteins coded by the rat and human chimeric cDNA constructs were investigated. Kinetic analyses performed in sonicates of transfected COS-7 cells are shown in Fig. 4. Both enzymes showed saturable reaction kinetics with K_m values for T_4 and rT_3 of $\sim$ 1 and 5 nM, respectively. V_{max} values using rT_3 as substrate were 75 and 33% higher for the rat and hu-

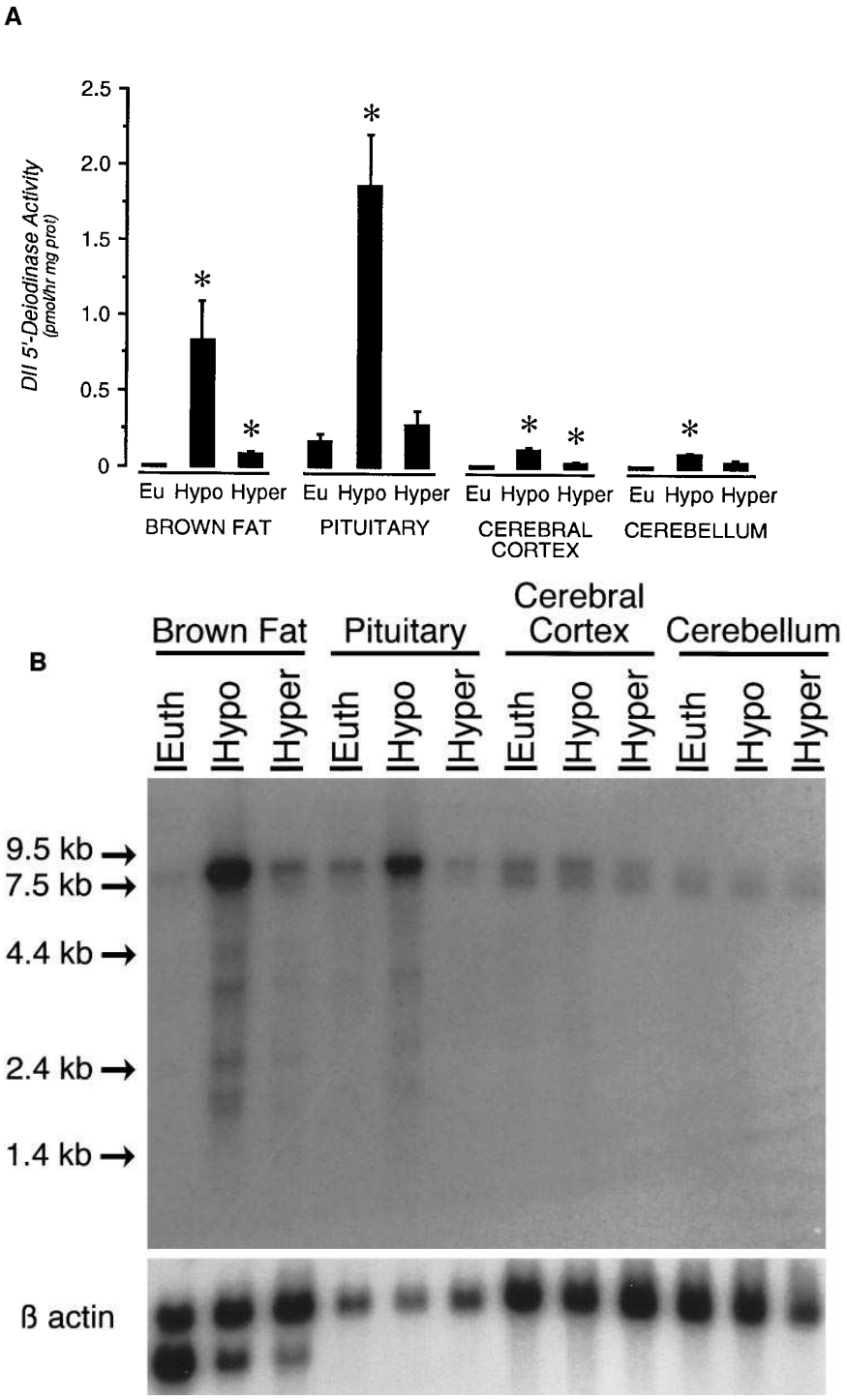

Figure 8. Effect of altered thyroid hormone status on DII (*A*) activity and (*B*) mRNA levels in the BAT, anterior pituitary gland, cerebral cortex, and cerebellum of rats. Rats were rendered hypo- and hyperthyroid as described in Methods. For determination of DII activity, individual tissues were harvested from three rats, homogenized, and assayed in the presence of 0.1 mM PTU using 1.5 nM rT_3 as substrate and 20 mM dithiothreitol as cofactor. Results represent the mean±SEM. $*P < 0.05$ vs euthyroid value. For Northern blot analysis, RNA was prepared from BAT and cerebral cortex of individual animals, from a pool of three cerebellums, and from a pool of 10 anterior pituitary glands. Approximately 10 μg (*brown fat*, *cerebral cortex*, and *cerebellum*) or 5 μg (*pituitary*) of poly(A)$^+$ RNA were loaded per lane on the gel.

man enzymes, respectively, when compared to values obtained with T_4.

The rBAT 1-1 deiodinase proved to be insensitive to inhibition by PTU and aurothioglucose. PTU, 100 μM, had no effect on 5′-deiodinase activity, whereas DI activity induced by the expression of the G21 cDNA was inhibited by ~ 90% (Fig. 5 *A*). The hZ44085 protein also proved completely insensitive to PTU at concentrations as high as 900 μM (data not shown). Although aurothioglucose did inhibit both the rBAT 1-1 and hZ44085 enzyme activity, a much higher concentration (> 10-fold) was required to achieve inhibition comparable to that observed with the DI (Fig. 5 *B*, and data not shown for the hZ44085 enzyme).

The expression of rBAT 1-1–associated mRNA was investigated in rat tissues by Northern analysis and coupled reverse transcription PCR. A relatively weak hybridization band of 7.5 kb was noted in RNA from normal BAT (Fig. 6 *A*). The abundance of this species was increased 17-fold in the sample of RNA from BAT of cold-exposed animals. (In a second experiment, a ninefold increase was noted, data not shown.) In addition, smaller and much less abundant species of ~ 4.0, 2.5, and 2.0 kb were also noted in this and other RNA samples from

this tissue. No hybridization signal was seen with RNA samples from rat heart, white fat, placenta, liver, kidney, neonatal skin, or skeletal muscle (Fig. 6, *B* and *C*). In adult rat cerebral cortex, two major hybridizing species of approximately equal abundance at 7–8 kb were observed (Figs. 6 *B* and 8 *B*, and below). A similar pattern was seen in the cerebellum, though the smaller species of the doublet appears somewhat more abundant in this tissue.

Although no hybridization signal was detected on Northern blots using RNA from rat liver and white fat (Fig. 6 *C*), the lesser β-actin control signal in these RNA samples suggested that this analysis was less than definitive. Hence, a coupled reverse transcription-PCR technique was used to examine whether DII transcripts were expressed in these tissues. As shown in Fig. 6 *D*, an abundant PCR product of the expected size (590 bp) was observed when RNA from BAT of a hypothyroid rat was used in the assay (positive control). This signal was markedly diminished in the absence of reverse transcriptase and was absent when the template was excluded from the reaction mixture. A faint signal, only 1/100th the intensity of that observed in BAT, was noted in white fat, whereas no signal was observed in this experiment when liver RNA was used as the template. In another experiment using a higher cycle number, a faint band was detected in liver (data not shown). Such a finding is likely of little physiologic significance and may correspond to the phenomenon of "illegitimate transcription" as defined by Chelly et al. (30).

The hZ44085 cDNA was used to probe RNA blots of human tissues. High levels of expression of a 6.6-kb transcript were noted in brain, skeletal muscle, and heart, with lesser amounts of a larger 7.5-kb species present in placenta and heart (Fig. 7 *A*). No signal was detectable in pancreas, kidney, liver, or lung. The distribution of hZ44085-associated transcripts in human brain was investigated further (Fig. 7, *B* and *C*). Again the predominant species observed was ~6.6 kb with transcripts of 4.4, 4.0, 3.2, and 1.9 kb also observed in some tissues. Transcripts appeared abundant throughout the neocortex as well as the putamen, amygdala, and hippocampus. Lesser degrees of expression were noted in the cerebellum, caudate nucleus, substantia nigra, thalamus, subthalamic nucleus, corpus callosum, medulla, and spinal cord.

Because of the known importance of thyroid hormones for brain development, an RNA blot of human fetal tissues (18–24 wk gestation according to the blot manufacturer, Clontech) was also probed with hZ44085 (Fig. 7 *D*). High levels of expression of a 6.6-kb species were detected in fetal brain, but not in fetal lung, liver, or kidney.

Previous studies have demonstrated that the regulation of DII activity is complex and appears to involve both pre- and posttranslational mechanisms in different tissues (29, 31). In particular, thyroid hormones exert important regulatory effects on DII activity as well as on the other deiodinases. Thus, an investigation of the effects of altered thyroid hormone status on the BAT 1-1–associated transcripts was of interest. For this study, DII activity was determined and RNA extracted from several tissues of normal (euthyroid), hypothyroid, and hyperthyroid adult rats. DII activity levels are shown in Fig. 8 *A*. As compared to the euthyroid state, hypothyroidism was associated with a significant increase in activity of 6-, 9-, 11-, and 118-fold in the cerebellum, cerebral cortex, anterior pituitary, and BAT, respectively. In addition, hyperthyroidism induced by T_3 administration was associated with a trend toward higher DII activity in all tissues, and this was statistically significant in BAT (11-fold increase in activity) and cerebral cortex (2-fold increase).

The Northern analysis of RNA samples from these rats is shown in Fig. 8 *B*. Comparison of the hybridization signals in the various tissues from the euthyroid animals demonstrates that rBAT 1-1–associated transcripts are most abundant in the anterior pituitary gland. Thus, the DII transcript levels in the cerebellum, cerebral cortex, and pituitary, when normalized for the actin mRNA signal in each tissue, are ~7-, 30-, and 50-fold higher, respectively, than those observed in euthyroid BAT. In pituitary and BAT RNA samples, hypothyroidism was associated with a marked increase (3.3- and 12-fold, respectively) in DII transcripts when compared to the levels in euthyroid samples. Furthermore, hyperthyroidism resulted in a 70% decrease in pituitary DII mRNA relative to the control samples. In contrast, altered thyroid status had little or no effect on the DII transcript levels in the cerebral cortex and cerebellum, where again two species between 7 and 8 kb were observed.

Discussion

The present studies demonstrate that the rBAT 1-1 and human hZ44085 code for mammalian DIIs. This conclusion is based on both the functional activity of the expressed proteins and the tissue patterns of expression of their associated mRNAs.

Both the rBAT 1-1 and hZ44085 cDNAs appear to lack functional SECIS elements in their 3′-untranslated regions. (Although it is theoretically possible that the SECIS element could be located in a missing portion of the 5′-untranslated region of these cDNAs, stem loop structures function very inefficiently as SECIS elements in this location, reference 32, and no native 5′-untranslated region SECIS elements have been described.) This explains the inability of these cDNAs to code for proteins with functional deiodinase activities; without an active SECIS element, translation likely terminates at the first TGA triplet located midway through the coding regions of these genes. However, by fashioning chimeric cDNAs in which the open reading frames of the rBAT 1-1 or the hZ44085 were fused to the SECIS-containing 3′-untranslated region of the rNS43-1 rat DIII cDNA, expression of functional deiodinases was obtained.

The properties of these expressed enzymes are entirely consistent with those of the endogenous DII previously defined in mammalian tissue homogenates (33). Thus, both enzymes manifest K_m values for T_4 and rT_3 in the low nanomolar range when using 20 mM dithiothreitol as cofactor. Furthermore, using the V_{max}/K_m ratio as an approximate indicator of catalytic efficiency, T_4 is the preferred substrate for both enzymes; values of this calculated parameter for T_4 are three- to fourfold higher than for rT_3. This finding contrasts sharply with the catalytic properties of the DI where rT_3 is the much preferred substrate, and T_4 is converted to T_3 with relatively poor efficiency (33). Also contrasting with the DI is the marked insensitivity of these expressed deiodinases to the inhibitory effects of PTU and gold compounds.

Several studies have provided indirect evidence that the mammalian DII is not a selenoprotein. Such evidence includes (*a*) the observation that DII activity in rat astrocytes is not diminished when the cells are cultured in selenium deficient medium (34); (*b*) the inability to label candidate DII proteins with [^{75}Se] (34); and (*c*) the insensitivity of the DII to PTU and gold

A

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          *   *    *           *        *       *
Rat DI    M--GL--SQL WL------WL KRLVIFLQ-- VALEVATGKV LMTLF-----   33
Rat DII   M--GLLSVDL LI------TL QILPVFFSNC LFLALYDSVI LLKHV-----   37
Rat DIII  MLRSLLLHSL RLCAQTASCL VLFPRFLGTA FMLWLLDFLC IRKHFLRRRH   50

                     *
Rat DI    PERVKQNILA MGQKTGMTRN PRFAPDNWVP TFFSIQYFWF VLKVRWQRLE   83
Rat DII   ALLLSRSKST RGEWRRMLTS EGLRCVWNSF LLDAYKQVKL GEDAPNSSVV   87
Rat DIII  PDHPEPEVEL NSEGEEMPPD DPPICVSDDN RLCTLASLKA VWHGQKLDFF   100

                          *          *      **** *** * *** *
Rat DI    DRAEYGGLAP NCTVVRLSGQ KCN-VWDFIQ GSRPLVLNFG SCTXPSFLLK   132
Rat DII   HVSNPEAGNN CASEKTADGA ECH-LLDFAS AERPLVVNFG SATXPPFTRQ   136
Rat DIII  KQAHEGGPAP NSEVVRPDGF QSQRILDYAQ GTRPLVLNFG SCTXPPFMAR   150

             *  **          ***    ** ***  ** *                **
Rat DI    FDQFKRLVDD FASTADFLII YIEEAHATDG WAFKNNVDIR Q----HRSLQ   178
Rat DII   LPAFRQLVEE FSSVADFLLV YIDEAHPSDG WAVPGDSSMS FEVKKHRNQE   186
Rat DIII  MSAFQRLVTK YQRDVDFLII YIEEAHPSDG WVTTDSPYVI PQ---HRSLE   197

          **  **  *          * *   * * * *       *     **       *   *
Rat DI    DRLRAAHLLL AR---SPQCP VVVDTMQNQS SQLYAALPER LYVIQEGRIC   225
Rat DII   DRCAAAHQLL ERFSLPPQCQ VVADRMDNNA NVAYGVAFER VCIVQRRKIA   236
Rat DIII  DRVSAARVLQ QG---APGCA LVLDTMANSS SSAYGAYFER LYVIQSGTIM   244

          * *  **  *    * *  **
Rat DI    YKGKPGPWNY NPEEVRAVLE KLCIP-PGHM P-QF                    257
Rat DII   YLGGKGPFSY NLQEVRSWLE KNFSK-RxIL D                      266
Rat DIII  YQGGRGPDGY QVSELRTWLE RYDEQLHGTR PRRL                    278
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B

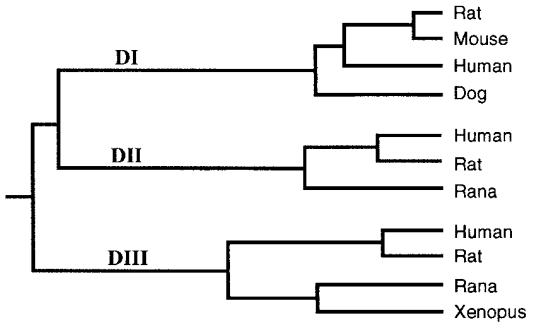

Figure 9. (*A*) A comparison of the amino acid sequence of the rat DI, DII, and DIII proteins as deduced from the cDNA nucleotide sequence. Asterisks represent amino acid residues that have been conserved identically between these three proteins. X, selenocysteine; x, position of second TGA codon in rat DII. (*B*) Evolutionary tree diagram based on the deduced amino acid sequences of 11 deiodinase cDNAs reported to date (7–10, 18, 71–75).

inhibitors as noted above (35). Furthermore, although DII activity is modestly diminished in the brain and anterior pituitary gland of selenium-deprived rats (36–38), Chanoine et al. (38) have provided convincing evidence that this results from an increased rate of posttranslational inactivation of the enzyme secondary to elevations in serum, and presumably tissue, T_4 levels rather than a selenium-induced decrease in enzyme synthesis.

The present study, however, demonstrates that the rat and human DIIs are selenoproteins that contain a selenocysteine within a region that is highly conserved with the other deiodinases (Fig. 9 *A*). This observation, along with prior site-directed mutagenesis studies (7, 9, 10, 18), demonstrates that this rare amino acid is essential for efficient catalysis of iodothyronines by all members of this enzyme family. The inability of Safran et al. (34) to affect DII activity in cultured rat astroglial cells by the manipulation of selenium concentrations in the medium may be related to the findings of others that the brain and pituitary gland, like the thyroid gland and other endocrine organs, are resistant to selenium depletion (39). That this property is maintained in vitro in primary cell culture was recently demonstrated by Beech et al. (40), who were unable to affect the activity of the DI in cultured human thyroid cells by selenium starvation. Furthermore, in whole animal studies, they observed that thyroidal DI activity was actually increased by 42% in rats on a selenium-deficient diet in spite of a modest (30%) decrease in thyroidal glutathione peroxidase activity, another selenoenzyme. Such results confirm the findings of others (41) indicating that there is a hierarchy of expression of different selenoproteins in a cell in the face of selenium deprivation.

Alternatively, the DII in cultured rat glial cells could lack selenocysteine and be coded by a mRNA that is unrelated to the rBAT 1-1. However, our observations that rBAT 1-1– and hZ44085-associated transcripts are highly expressed in the rat anterior pituitary gland and in the central nervous system of fetal and adult rats and humans strongly suggest that brain DII is coded by these genes. Furthermore, the predicted size of the rBAT 1-1 protein (29.8 kD) is virtually identical to a 29-kD protein identified by affinity labeling techniques in cultured glial cells and proposed by Safran and Leonard to be the DII (42).

In addition to the highly conserved regions surrounding the selenocysteine residue, other shared structural features of the deiodinases are apparent (Fig. 9 *A*). All three subclasses of deiodinase contain regions of strong hydrophobicity near their amino termini and this may represent membrane-spanning domains (28). Furthermore, two histidine residues demonstrated by Berry (43) to be essential for catalysis by the DI (residues 158 and 174 in the rat DI) are conserved in the DII and the DIII enzymes. The second in-frame TGA triplet is unique to the rat and human DII cDNAs. Whether these code for a second selenocysteine in these proteins or function in their more

traditional role as stop codons is uncertain. It appears likely, however, that a second selenocysteine is not required for DII activity since this second TGA codon is not present in the RC5′DII cDNA (Fig. 2 *B*). A comparison of the amino acid sequence of the 11 deiodinases for which cDNAs have been isolated to date is shown as an evolutionary tree diagram in Fig. 9 *B*. This analysis demonstrates that the DIs, DIIs, and DIIIs form three separate subfamilies, each approximately equally dissimilar to the other two. Furthermore, based on these limited data, the members within the DII subfamily appear more highly conserved across species lines than the DI or DIII.

The tissue expression of rBAT 1-1–associated transcripts is consistent with the known distribution of DII activity in the rat (33); hybridizing RNA species were observed in the anterior pituitary gland, cerebral cortex, cerebellum, and BAT. In BAT, where cold exposure induces DII activity (26), a 9–17-fold increase in abundance of a 7.5-kb band was noted in response to this stimulus. The large size of the predominant DII transcript observed in both the rat and human was unexpected given the much smaller size ($\sim$ 1.6–2.2 kb) of the DI (44) and DIII mRNAs (9, 10), and our prior observation that in *Rana catesbeiana* the predominant DII mRNA is 1.5 kb (18). Thus, the rBAT 1-1 and hZ44085 cDNAs, both $\sim$ 1.9 kb, are not full length with respect to the 6–8-kb DII mRNAs. This likely explains the lack of SECIS elements in their presumably truncated 3′-untranslated regions. Both cDNAs, however, do contain the entire coding region for the DII proteins. The finding that the rBAT 1-1 cDNA contains a long poly(A)$^+$ tail at its 3′ end suggests that it is derived from one of the smaller, less abundant RNA species observed on Northern blots of RNA from BAT of cold-exposed animals. Thus, either alternative RNA splicing or the use of alternative polyadenylation sites may be a feature of the processing of the mammalian DII mRNA.

Information on the distribution of the deiodinases in humans is limited (8, 45, 46). The present studies demonstrate that DII transcripts are more widely distributed in this species than in the rat. Thus, in addition to its expression in the central nervous systems, DII mRNA was also observed in the human placenta, heart, and skeletal muscle at levels comparable to those observed in the brain. In contrast, no hybridizing species could be detected with RNA from these nonneural tissues in rats, consistent with earlier reports that rat heart (47, 48) and skeletal muscle (49) contain only low levels of DI activity and no detectable DII activity. Rat placenta, however, does contain DII activity (46). Thus, our failure to detect DII mRNA by Northern analysis in rat placenta likely results from the relatively low abundance of this transcript.

The pattern of DII expression in the human fetus (Fig. 7 *D*) is of considerable interest given the prior report by Bernal and Pekonen (50) that T_3 and nuclear T_3 receptors are detectable in human fetal brain as early as 10 wk of gestation, long before T_3 becomes detectable in the serum (51). Thus, DII expression in the central nervous system early in gestation is likely of great importance for the generation of the T3 needed by this tissue. Of note, tissues such as the liver and lung, which do not express DII at this early stage of development, do not contain detectable T_3 (50), thus reinforcing the importance of this enzyme in regulating local T_3 tissue concentrations.

Northern analyses revealed that the expression of DII transcripts in the human adult brain is widespread and shows a general rostral–caudal distribution. This regional pattern of DII expression correlates closely with the relative abundance of thyroid hormone nuclear receptors in various regions of the adult rat central nervous system as defined by both saturation analysis (52) and T_3 receptor mRNA expression (53). Although the importance of thyroid hormone in the developing brain is unquestioned (54, 55), there continues to be controversy concerning the role of these hormones in adult brain function (56). Thus, the apparent coexpression of the DII and thyroid hormone receptors in several regions of the adult human brain provides strong evidence for a continuing important role of T_3 after the developmental period and reinforces clinical observations of altered central nervous system function in states of hypo- and hyperthyroidism (56–58). Of particular note are the relatively high levels of expression of DII transcripts in the hippocampus, amygdala, and basal ganglion, suggesting an important influence of thyroid hormones on memory and learned patterns of movement (59). Such effects might explain some of the cognitive dysfunction seen in adults with altered thyroid function as well as the movement disorders observed in neurologic cretinism (60).

To our knowledge, adult human heart and skeletal muscle have not been examined for the presence of DII activity. However, the expression of DII in these tissues, as suggested by the results of the Northern analysis, could be of considerable physiologic importance. For example, LoPresti et al. (61) have demonstrated that the serum T_3 level in euthyroid subjects is unaltered after 4 d by the administration of high doses of PTU, suggesting an important role for the DII in maintaining circulating T_3 concentrations in healthy humans. DII expression in skeletal muscle could provide a source for such T_3 production. In addition, several investigators have demonstrated that in rats, DII-containing tissues (predominantly the cerebral cortex and BAT) are able to maintain relatively normal tissue T_3 levels, and hence presumably a euthyroid state, over a wide range of plasma T_4 levels (62–64). Thus, human heart and skeletal muscle may also be relatively protected from the effects of hypothyroidism.

DII activity in rat tissues is markedly influenced by thyroid hormone status. For example, hypothyroidism is associated with marked elevations in DII activity (65, 66). In the central nervous system and anterior pituitary gland, posttranslational processes are important in mediating this effect (67, 68), whereas in BAT, multiple pre- and posttranslational mechanisms appear to influence DII activity in hypo- and hyperthyroid states (27, 31). The cloning of the rBAT 1-1 cDNA allowed us to investigate further these complex processes by focusing specifically on pretranslational mechanisms of regulation (Fig. 8). As expected from previous studies, the induction of hypothyroidism resulted in a marked increase in DII activity in all tissues studied. This was associated with a significant increase in DII mRNA expression in BAT and the pituitary, but little if any change in the cerebral cortex and cerebellum. It is thus apparent that in the brain, thyroid hormone regulates DII activity predominantly, if not exclusively, via posttranslational mechanisms as previously described (67). In BAT, Silva and Larsen (31) have demonstrated that the growth hormone deficiency that accompanies hypothyroidism stimulates DII activity, an effect that is blocked by actinomycin. Thus, the alterations in DII transcript levels in this tissue are likely due at least in part to a secondary effect of the thyroid hormone deficiency.

The significant increase in DII transcripts in the pituitary of hypothyroid animals demonstrates that pretranslational fac-

tors, in addition to the previously cited posttranslational effects of thyroid hormones, are responsible in part for the marked rise in DII activity in this tissue. Whether this increase represents a direct effect of thyroid hormone on DII mRNA levels is uncertain, but it seems plausible given that, unlike BAT, catecholamines and growth hormone have no demonstrable effect on pituitary DII activity (26, 31). A direct effect is also consistent with a recent study by Halperin et al. (69) who noted that T_3 was more potent than rT_3 in downregulating DII activity in cultured rat pituitary GC cells, an effect that they suggest is mediated by the nuclear thyroid hormone receptor. Alternatively, the increase in DII transcripts in hypothyroidism could be secondary to a relative increase in abundance of a pituitary cell type that expresses comparatively high DII levels. Indeed, the fraction of thyrotrophs has been observed to increase from 10.7% in the euthyroid rat pituitary gland to 34.4% in chronic hypothyroidism (70). Thyrotrophs, however, appear to contain less DII activity than other pituitary cell types (71). Thus an increase in the proportion of thyrotrophs is an unlikely explanation for our findings.

In our experiment, hyperthyroidism, as induced by 4 d of T_3 injections, was also associated with an increase in DII activity, though to a considerably lesser extent than that seen with hypothyroidism. Although not proven, this likely results primarily from thyroid-stimulating hormone suppression, leading to reduced serum and tissue T_4 levels, and hence decreased posttranslational inactivation of the DII protein (67). Such a phenomenon has been noted previously in BAT (31), but has not been investigated in other tissues expressing DII.

In summary, we have identified cDNAs for the rat and human DII and have demonstrated that they code for selenoproteins that manifest critical areas of homology with the other deiodinase subtypes. The expression of the DII in mammalian brain, pituitary, BAT, heart, skeletal muscle, and placenta highlights the importance of thyroid hormone in regulating metabolic activity in these tissues, and lends further support to the concept that the DII is a critical modulator of thyroid hormone action.

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