Physiological and Genetic Analyses of Inbred Mouse Strains with a Type I Iodothyronine 5′ Deiodinase Deficiency

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Abstract

Inbred mouse strains differ in their capacity to deiodinate iododioxin and iodothyronines, with strains segregating into high or low activity groups. Metabolism of iododioxin occurs via the type I iodothyronine 5′deiodinase (5′DI), one of two enzymes that metabolize thyroxine (T_4) to 3,5,3′-triiodothyronine (T_3). Recombinant inbred strains derived from crosses between high and low activity strains exhibit segregation characteristic of a single allele difference. Hepatic and renal 5′DI mRNA in a high (C57BL/6J) and low (C3H/HeJ) strain paralleled enzyme activity and concentration, in agreement with a recent report. 5′DI-deficient mice had twofold higher serum free T_4 but normal free T_3 and thyrotropin. Brown adipose tissue 5′DII was invariant between the two strains. Southern analyses using a 5′DI probe identified a restriction fragment length variant that segregated with 5′DI activity in 33 of 35 recombinant inbred strains derived from four different pairs of high and low activity parental strains. Recombination frequencies using previously mapped loci allowed assignment of the 5′DI gene to mouse chromosome 4 and identified its approximate chromosomal position. We propose the symbol *Dio1* to denote the mouse 5′DI gene. Conserved linkage between this segment of mouse chromosome 4 and human HSA1p predicts this location for human *Dio1*. (*J. Clin. Invest.* 1993. 92:1517–1528.) Key words: chromosomal mapping • genetics • mice • thyroxine • type I iodothyronine deiodinase

Introduction

Thyroxine (T_4)[1] must be deiodinated to 3,5,3′-triiodothyronine (T_3) in order to be fully active under physiological conditions. Two enzymes catalyzing this reaction have been identified, the types I and II iodothyronine 5′ deiodinases (5′DI and 5′DII). Recently one of these, the type I enzyme, has been cloned from rat and human liver (1, 2). The enzyme contains selenocysteine in its catalytic site and mutation of the selenocysteine to cysteine leads to a marked decrease in the catalytic efficiency of the rat enzyme (3). Experimental selenium deficiency leads to an increase in serum T_4 and a decrease in serum T_3 concentration in association with a reduction in hepatic and renal 5′DI activity (4, 5). This occurs as a consequence of compensatory increases in thyroid-stimulating hormone (thyrotropin; TSH) secretion owing to the sensitivity of the hypothalamic-pituitary feedback system to circulating T_3 (6, 7). Type I deiodination follows ping-pong, bisubstrate kinetics with a requirement for reduced thiol as second substrate (3). 6-*n*-propylthiouracil (PTU) is a specific inhibitor of the 5′DI and has been postulated to form a stable dead-end complex between the selenolyl iodide intermediate of the first half-reaction and the sulfhydryl group of the thiourea compound (3). In thyroidectomized rats given replacement T_4, PTU inhibition of 5′DI causes marked hypothyroidism in the liver, which derives most of its T_3 from plasma T_3 (8).

There are only a few examples of thyroid hormone resistance in humans which could potentially be attributed to a deficiency of 5′DI (9, 10). Until recently, no animal models had been identified. Accordingly, it is difficult to develop an uncomplicated model of 5′DI deficiency, because both PTU administration and selenium deficiency could have other biochemical or physiological effects in the intact animal. We noted in studies of [^{125}I]2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin (iododioxin, [^{125}I]Cl_3DpD) metabolism, that inbred mouse strains segregated into groups exhibiting high or low hepatic iododioxin deiodinase activity. In particular, hepatic deiodination of iododioxin was ~ 20-fold lower in C3H/HeJ (C3H) than in C57BL/6J (B6) mouse strains. Subsequent studies showed that the differences in enzyme activity with respect to iododioxin were also found when [^{125}I]T_4 was used as substrate. Thus, it appeared that iododioxin was a substrate for the 5′DI and that this enzyme was expressed at different levels in these two strains. We initiated the present collaborative study (*a*) to identify the mechanism by which 5′DI deficiency occurred, (*b*) to explore the effects of deiodinase deficiency on serum thyroid hormones, TSH, and type II deiodinase activity, and (*c*) to analyze the inheritance of 5′DI deficiency in recombinant inbred (RI) mouse strains by restriction fragment length variant (RFLV) analysis.

Methods

Materials. Inbred, RI, and mutant strains of mice were from either the Research or Animal Resources colonies of the Jackson Laboratory, Bar

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1. *Abbreviations used in this paper:* BAT, brown adipose tissue; $BrAcT_3$, *N*-bromoacetyl-L-3,5,3′-triiodothyronine; 5′DI, type I 5′ deiodinase; 5′DII, type II 5′ deiodinase; I-Cl_3DpD, 2-iodo-3,7,8-trichlorodibenzo-p-dioxin; PTU, *6-n*--propylthiouracil; RFLV, restriction fragment length variant; RI, recombinant inbred; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone (thyrotropin).

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Harbor, ME. Mice used as source of tissues for deiodinase assays were usually ~ 7-wk-old males, though males and females of different ages produced similar results. [^{125}I]T_4, T_3, and 3,3',5'-triiodothyronine (reverse T_3, rT_3) were from Dupont-New England Nuclear (Boston, MA). [^{125}I]Cl_3DpD (2,176 Ci/mmol) was synthesized and purified as previously described (11). RNA reagents were of molecular biology grade. All other materials were of reagent grade.

Preparation of microsomes. Mice were killed by cervical dislocation, and the livers were removed and homogenized with 9 vol of MDEN buffer (25 mM Mops, 1 mM DTT, 1 mM EDTA, and 0.02% sodium azide), and centrifuged at 10,000 *g* for 20 min. The supernatant fraction was made 10 mM in EDTA and centrifuged at 100,000 *g* for 1 h. The microsomal fraction was resuspended (equivalent to 1 g of liver/ml) in 0.25 M sucrose and stored at −80°C until use.

[^{125}I]Cl_3DpD deiodinase assays. The standard assay consisted of a total incubation volume of 400 μl containing 9.5 μmol phosphate buffer (pH 7.4), 3 μmol EDTA, 6.7 μmol DTT, 300 μg bovine serum albumin, 12.5 μg liver microsomal protein, and 10 μl of DMSO containing the substrate (1 pmol of radiolabeled [^{125}I]Cl_3DpD, ~ 4.83×10^{-5} dpm plus unlabeled compound to give the desired concentration). The reaction was incubated at 37°C in a shaker-water bath for 15 min unless otherwise indicated, terminated by the addition of 50 μl 50% TCA, and placed on ice. The protein precipitate was removed by centrifugation, the supernatant was decanted, and 50 μl of a solution containing 50% NH_4OH, 1 mg $Na_2S_2O_5$, and 0.1 μg NaI was added. The solution was extracted with 2 ml of mixed hexanes and after freezing in a dry ice-ethanol bath, the organic layer removed by decanting. Extraction with hexanes was repeated twice more. The remaining aqueous soluble radiation was quantified by gamma-scintillation spectrometry (Minaxi 5000; United Technologies-Packard, Des Plaines, IL).

Type I 5' deiodination of T_4. The standard assay consisted of a total volume of 200 μl containing 4.75 μmol of Mops (pH 7.3 at 37°C), 0.6 μmol EDTA, 4 μmol DTT, 250 μg liver microsomal protein, and 10 μl of 50% methanol/aqueous phosphate buffer containing 1.6 pmol [^{125}I]-T_4, (290,000 dpm) plus unlabeled T_4, to give the necessary concentration as indicated. The reaction was incubated at 37°C in a shaker-water bath for 1 h, and terminated by addition of 200 μl of 10% TCA and 10 μg carrier NaI. After centrifugation to remove the precipitate, the supernatant was decanted and passed over an AG 50W-X cation exchange column (BioRad Laboratories, Richmond, CA) preequilibrated and washed with 8 M acetic acid. The radiolabel in the eluate was quantified by gamma-scintillation spectrometry.

Type I 5' deiodination of rT_3. Reactions contained 4–40 μg liver or kidney microsomal protein, 2 nM [^{125}I]rT_3, varying concentrations of unlabeled rT_3, and 5 or 10 mM DTT in 100 mM potassium phosphate, 1 mM EDTA, pH 6.9 (PE buffer), in a reaction volume of 300 μl. Reactions were incubated at 37°C for 15 min to 1 h, and processed as described previously (3). All reactions were performed in duplicate.

Type II deiodinase assays. Reactions contained 2 nM [^{125}I]T_4, varying concentrations of unlabeled T_4, 40 mM DTT, 1 mM PTU, and 10 μg brown adipose tissue (BAT) microsomal protein, 150 μg brain microsomal protein, or 50–60 μg pituitary sonicate protein in PE buffer. Brain deiodinase assays also contained 1 μM T_3 to saturate type III (inner ring) deiodinase activity. BAT reactions were incubated at 37°C for 15 min to 1 h; brain and pituitary reactions were incubated for 2 h. All reactions were performed in duplicate.

Product identification and quantitation. [^{125}I]Cl_3DpD, [^{125}I]T_4, the radiolabeled products of their respective deiodination, and Na ^{125}I were examined by thin-layer chromatography on LK5DF silica gel plates (Whatman Inc., Clifton, NJ) with developing solvent of *N*-butanol saturated with NH_4OH. Iodine and T_4 were visualized by staining with ferric chloride-ferricyanide and arsenite (12). All compounds were radiolabeled and visualized by autoradiography. [^{125}I]Cl_3DpD had an R_f of 0.87, thyroxine had an R_f of 0.39, and the radiolabeled products of incubation of both substrates cochromatographed with free $^{125}I^-$ with an R_f of 0.51. The recovery of $^{125}I^-$ added to the I-Cl_3DpD deiodination incubation (hexane extraction) was > 90%, and similar near-quantitative recovery of $^{125}I^-$ was observed in the T_4 deiodination assay. Correction for [^{125}I]Cl_3DpD and [^{125}I]T_4 not separated from the product $^{125}I^-$ was made by subtraction of zero time incubation blanks. Both assays yielded linear formation of product with time and with microsomal protein under the conditions employed.

Affinity labeling. Bromoacetyl [^{125}I]T_3 (BrAc [^{125}I]T_3) was synthesized from bromoacetylchloride and [^{125}I]T_3 as described previously (2, 13), and the iodinated product was purified by chromatography on LH-20 Sephadex. Affinity-labeling reactions contained 30 μg of liver or kidney microsomal protein, 0.05 μCi BrAc [^{125}I]T_3 and 10 mM DTT in PE buffer in a final volume of 50 μl. Labeling was at ambient temperature for 10 min. and was terminated by addition of an equal volume of electrophoresis sample buffer containing 2.5% SDS and 0.5 M 2-mercaptoethanol. Labeled proteins were analyzed by SDS-PAGE and autoradiography. Quantitation was performed both by densitometry (Computing Densitometer; Molecular Dynamics, Sunnyvale, CA) and by excision and counting of the ~ 27-kD 5'DI bands, with closely agreeing results. Specific incorporation into deiodinase protein was determined by subtraction of nonspecific from total incorporation (13). Nonspecific incorporation was determined by quantitating the corresponding region from parallel gel lanes in which labeling of the 27-kD protein was completely inhibited by addition of excess unlabeled $BrAcT_3$.

Northern blot analysis. RNA was isolated from liver and kidney by homogenizing tissues in guanidinium thiocyanate and centrifugation through cesium trifluoroacetate (Pharmacia Inc., Piscataway, NJ). Poly A+ RNA was prepared as described previously (14). RNA samples (5 μg) in 50% formamide and 1.3% formaldehyde were heated at 70°C for 5 min and electrophoresed on a 1.1% agarose gel containing 20 mM Mops (pH 7.0), 5 mM sodium acetate, 1 mM EDTA, and 1.3% formaldehyde. RNA was blotted to GeneScreen Plus (DuPont Co., Wilmington, DE), UV cross-linked, and probed with a rat 5'DI cRNA (1), and the blot was washed at high stringency, with the final wash being in 0.1× SSC, 0.1% SDS at 65°C. The blot was stripped by boiling and reprobed with a mouse β-actin cRNA. Quantitation of the blot was by densitometry.

Measurements of thyroid hormones and TSH. Serum T_3 and T_4 concentrations were quantitated by radioimmunoassays as previously described (15, 16). The free fractions of T_3 and T_4 were determined by equilibrium dialysis in duplicate samples, and free hormone concentrations were estimated by multiplication of the free fraction by total hormone concentration. Serum TSH was measured by radioimmunoassay using materials provided by the Hormone Distribution Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The antigen was mLH/TSH AFP5171.8MP. The antisera, GP-9 8/9/91 (A. F. Parlow, UCLA/Harbor, Torrance, CA), was used at a final concentration of 1:32,000. Rat TSH (NIDDK-RTSH-I-9, AFP-7308C) was used as ^{125}I-labeled antigen in the displacement assay. In sera from mice which had received 200 ng thyrotropin-releasing hormone (TRH) intraperitoneally 30 min before killing to increase serum TSH, the displacement curves of the mouse standard and five serial dilutions of mouse sera (3–50 μl serum) were parallel. However, 25 and 50 μl of sera from non–TRH-treated C3H and B6 mice did not cause tracer displacement parallel to the standard. TSH concentrations estimated using 50 μl of sera were systematically lower than those estimated using 25 μl. The displacement of [^{125}I]rTSH with both volumes was in the midportion of the standard displacement curve and was therefore not explained by a lack of sensitivity. We interpreted these results as indicating the presence of a matrix effect of mouse sera to displace [^{125}I]rTSH. This effect was not observed in the presence of high concentrations of endogenous TSH after TRH stimulation. This was confirmed by assay of sera from mice which had received 20 μg T_3 per day for 3 d. After this treatment, serum TSH was ~ 130 ng "mTSH"/ml. While these results were significantly lower than the estimated TSH concentrations in euthyroid mice, they were inappropriately elevated. There was no difference between the sera of B6 and C3H

mice in this respect. Accordingly, we expressed all TSH concentrations based on the displacement using 25 μl serum, recognizing that this is an overestimate of the authentic basal TSH concentration, though not of TRH-stimulated TSH.

Genomic DNA and Southern hybridization. Genomic DNAs were isolated from various mouse strains and Southern blots were prepared as previously described (17). Plasmid probes were labeled with [^{32}P]-dCTP by the random hexamer labeling method (18). The resulting probes, which had a specific activity of $> 3 \times 10^8$ cpm/μg, were used in hybridization to Southern blots as described previously (19).

Isozyme typing. Mice were typed for the kidney isozyme hexose-6-phosphate dehydrogenase (*Gpd-1*) by the Genetic Quality Control Service of the Jackson Laboratory under the supervision of Dr. Richard Fox.

Probes. The full-length rat type I deiodinase cDNA plasmid clone in Bluescript KS (1) was used to probe Southern blots. Other probes used are described in Table X (q.v.).

Statistical analysis. Estimated recombination frequencies (r) between gene pairs were obtained from the analysis of RI strain data using the equation $r = R/(4 - 6R)$, where R is the ratio of the number of RI strains with recombinant genotypes to the total number of RI strains typed (20). Confidence limits of recombination frequencies obtained from RI data were calculated as described by Silver (21). Confidence limits of recombination frequencies from testcross progeny were obtained from a table of binomial confidence intervals.

Results

Deiodination of [^{125}I]Cl_3DpD. [^{125}ICl_3DpD, a radiolabeled isosteric analogue of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) differing only by the substitution of an iodine atom for a chlorine atom, was synthesized to study pharmacokinetics (11). To our surprise, [^{125}I]Cl_3DpD was rapidly eliminated from the mouse, while TCDD is known to have a whole body half-life of 2 wk (22). This suggested that the iodine rendered the molecule subject to metabolism. Several pathways have been described for deiodination of iodinated xenobiotics (23–25). To explore this, we incubated [^{125}I]Cl_3DpD with glutathione, DTT, or NADPH with the whole homogenate, cytosolic, or microsomal fraction of mouse liver, and examined the unextractable, aqueous radioactivity ($^{125}I^-$) formed. Liver homogenate in the presence of glutathione or DTT produced substantial deiodination, whereas modest activity was found when the liver cytosol was incubated with glutathione. The majority of the deiodinating activity was found in the hepatic microsomal fraction when incubated with DTT, but not when incubated with an NADPH-generating system.

The deiodination of [^{125}I]Cl_3DpD by liver microsomes is dependent on DTT, increased with time of incubation and amount of microsomal protein, and has a pH optimum of 7.2. This activity was highest in microsomes from liver, appreciable in kidney, and trace activity was found in intestines (data not shown). The above characteristics suggest the enzyme involved is the type I iodothyronine deiodinase, the high capacity, low affinity deiodinase that metabolizes T_4 to T_3, and that has been shown to metabolize a number of other iodinated xenobiotics. From a study of substrate concentration versus reaction velocity, we found that [^{125}I]Cl_3DpD deiodination by liver microsomes from B6 mice had a V_{max} of 18 pmol I^-/12.5 mg protein/15 min and a K_m of 265 nM at 5 mM DTT. [^{125}I]-Cl_3DpD deiodination is competitively inhibited by T_4 and rT_3 (Fig. 1). Interestingly, inhibition by TCDD is noncompetitive, with a reduction in V_{max} but little effect on K_m (Fig. 2).

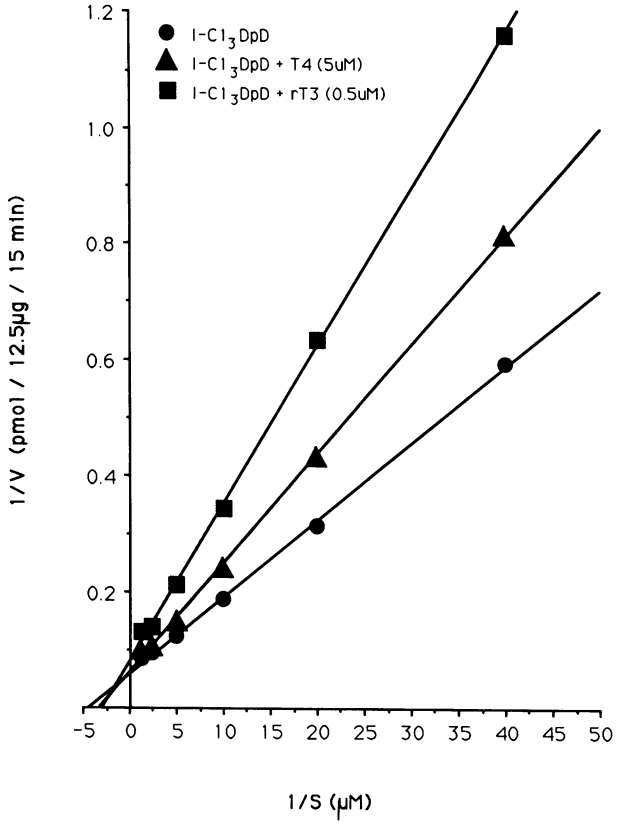

Figure 1. Kinetics of [^{125}I]Cl_3DpD deiodination by liver microsomes and inhibition by rT_3 and T_4. Double reciprocal plot of rate of deiodination versus [^{125}I]Cl_3DpD concentration in the absence (●) or presence of T4 (▲) or rT3 (■). Deiodinase activity was determined as described in Methods.

During the course of these studies, a large difference was noted in [^{125}I]Cl_3DpD deiodinase activity in liver microsomes from B6 versus C3H mice. Analysis of substrate versus reaction velocity indicated that B6 has a V_{max} > 20-fold greater than C3H, and the hybrid B6C3F_1/J mouse has an intermediate value. The Michaelis constants for [^{125}I]Cl_3DpD are similar for B6, C3H, and B6C3F_1 hybrids. In light of these results, we screened a variety of inbred strains of mice for hepatic microsomal deiodination of [^{125}I]Cl_3DpD (Table I). The strains with the lowest V_{max} are C3H, CBA/J (not shown), and SJL/J, while B6, SWR/J, and AKR/J have high activity. The K_m values are similar (less than twofold difference) in all strains. Comparison of deiodinase activity using [^{125}I]Cl_3DpD versus [^{125}I]T_4 showed an excellent correlation between the two substrates for all strains of mice tested (Table II).

Segregation of iodothyronine deiodinase activity in recombinant inbred mice. The strain distribution pattern permitted us to select recombinant inbred lines developed from parental strains in which one parent had high and one low deiodinase activity. Table III shows hepatic T_4 deiodinase activities in B6 and C3H mice and in a panel of recombinant inbred strains, BXH, derived from the cross of B6 and C3H. The recombinant strains exhibit a distinct segregation into either high or low activity categories.

Kinetic studies of 5'DI activities in B6 and C3H strains. Table IV shows results of kinetic analysis of liver and kidney

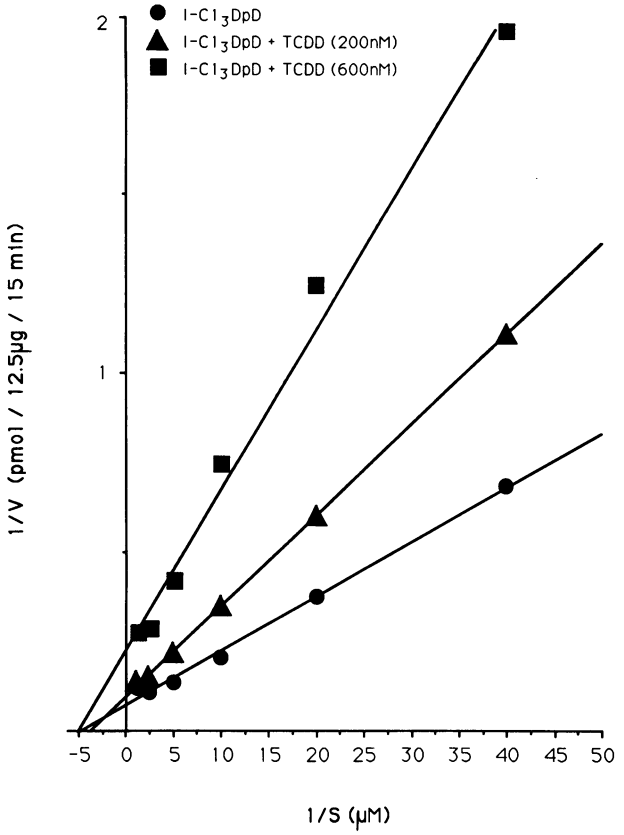

Figure 2. Kinetics of [^{125}I]Cl_3DpD deiodination by liver microsomes and inhibition by TCDD. Double reciprocal plot of rate of deiodination versus [^{125}I]Cl_3DpD concentration in the absence (●) or presence of 200 nM (▲) or 600 nM TCDD (■). Deiodinase activity was determined as described in Methods.

Table I. Strain Differences in [^{125}I]Cl_3DpD Deiodinase Activity

Strain	V_{max}	K_m
	pmol [^{125}I]Cl_3DpD/15 min per 12.5 µg	*nM*
A/J	14.97	215.6
AKR/J	36.91	254.6
BALB/cByJ	8.19	238.2
C3H/HeJ	0.66	241.5
C58/J	32.43	278.9
C57BL/6J	37.85	344.4
DBA/2J	27.07	293.3
MA/MyJ	8.13	259.4
NZB/BINJ	31.20	324.3
P/J	33.99	288.9
SJL/J	3.49	225.4
SM/J	27.37	254.5
SWR/J	45.05	310.8
129/J	15.19	209.6
B6C3F1/J	19.08	282.3

[^{125}I]Cl_3DpD-deiodinase activity was determined in hepatic microsomes as described in Methods at five substrate concentrations. Duplicate determinations and a zero time blank were performed at each concentration.

Table II. Correlation of [^{125}I]Cl_3DpD and T_4 Deiodinase Activities among Inbred Strains

Strain	[^{125}I]Cl_3DpD deiodinase	[^{125}I]T_4 deiodinase
	pmol/12.5 µg per 15 min	*pmol/100 µg per 2 h*
C3H/HeJ	0.249	0.539
BALB/cByJ	0.898	1.965
MA/MyJ	0.953	2.272
A/J	1.598	3.876
DBA/2J	2.073	4.888
NZB/BINJ	2.313	5.426
P/J	2.421	6.837
C57BL/6J	2.714	6.835
SWR/J	3.034	7.885
AKR/J	3.453	7.721

Hepatic microsomes were assayed for [^{125}I]Cl_3DpD deiodinase activity (25 nM I-Cl_3DpD) and [^{125}I]T_4 deiodinase activity (800 nM T_4) as described in Methods. Correlation coefficient R = 0.971.

specimens with respect to 5′-deiodination of the substrate, reverse T_3. K_m values for the two strains were indistinguishable. However, the V_{max} values in C3H were 8–24-fold lower in liver and 4–5-fold lower in kidney than in the B6 strain. The lower V_{max} in the C3H strain indicated either a difference in enzyme concentration or in intrinsic catalytic capacity. These two possibilities are distinguishable by quantitation of the enzyme. We therefore used affinity labeling with BrAc [^{125}I]T_3 (13) to compare the levels of the 5′DI enzyme in the two strains. Quantitation of label incorporated into liver and kidney microsomal 27-kD protein revealed a close agreement between covalent labeling of this protein and enzyme activity (see below). Thus, differences in enzyme concentration, rather than in catalytic capacity, account for the observed lower V_{max} in C3H mice.

Table III. T_4 Deiodination in C57BL/6J and C3H/HeJ Inbred and BXH RI Mouse Strains

Strain	V_{max}
	pmol T_4/h per 100 µg protein
C57BL/6J	87.0
C3H/HeJ	1.7
BXH- 2	2.4
3	59.2
4	8.3
6	68.5
7	74.1
8	72.6
9	5.9
10	15.3
11	8.9
14	77.0
19	4.5

Kinetic constants were derived from double-reciprocal plots. Assay conditions are as described in Methods for type I deiodination of T_4.

Table IV. Kinetics of Type I Iodothyronine 5' Deiodinase Activity in Liver and Kidney Microsomes of C57BL/6J and C3H/HeJ Mice

Strain	K_m		V_{max}	
	Kidney	Liver	Kidney	Liver
	μm		pmol rT_3/min per mg protein	
C57BL/6J	0.36	0.36	235.0	523.6
	0.82	0.66	219.5	581.4
C3H/HeJ	0.48	0.32	60.4	64.1
	0.62	0.36	40.7	25.1

Kinetic constants were derived from double-reciprocal plots. Assay conditions are as described in Methods for type I deiodination of rT_3. Data are the results of two independent experiments. For each experiment, tissues from three different mice were pooled to prepare the microsomes.

Studies of mRNA expression. Since differences in mRNA levels could explain the lower 5'DI levels in C3H mice, we performed Northern analyses of 5'DI mRNA in the two strains (Fig. 3). Quantitation of deiodinase RNA normalized to a β-actin RNA revealed B6/C3H mRNA ratios of 9.3 in liver and 2.3 in kidney. The mRNAs of both strains were ~ 2.1 kb. Slot blot analyses of tissues from three different animals of each strain showed similar differences, with the B6/C3H ratio approximately sevenfold for liver and twofold for kidney.

A comparison of 5'DI V_{max}, BrAc [^{125}I]T_3 labeling, and mRNA levels between these two strains shows that, for each strain, a close correlation exists between enzyme activity, deiodinase protein, and mRNA (Fig. 4). Thus, the 5'DI deficiency in the C3H mouse can be attributed to a decrease in the quantity of mRNA for this enzyme, presumably due either to altered mRNA transcription, or stability, or both.

Serum thyroid hormone concentrations and TSH in sera of B6 and C3H mice. While there was no difference in the concentrations of total or free T_3 in the two mouse strains, the serum total and free T_4 concentrations in the C3H mice were approximately twice those of the B6 strain (Table V). The basal and TRH-stimulated TSH concentrations were also not different in the two strains of mice. As indicated in Methods, the results for the basal TSH concentrations, but not the TRH-stimulated values, are confounded by displacement of [^{125}I]-rTSH from the antibody by both B6 and C3H sera. This activity is not affected by doses of exogenous T_3 which suppress TSH. While the estimated basal TSH concentrations are therefore artifactually elevated, there was no difference in TSH concentrations in the C3H and B6 inbred strains using 25, 50, or 100 ml of serum in the radioimmunoassay.

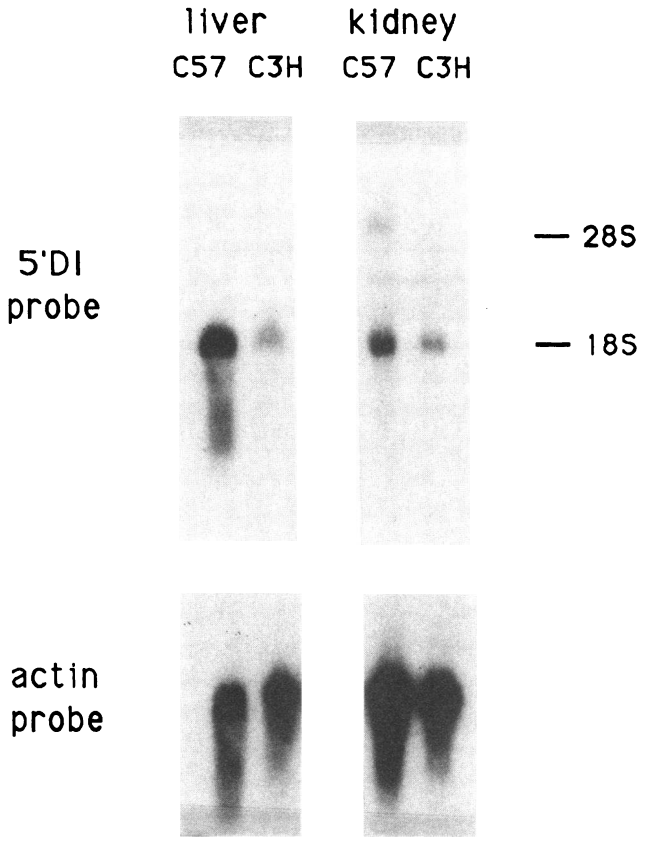

Figure 3. Northern analysis of type I deiodinase mRNA in C57BL/6J and C3H/HeJ liver and kidney. RNA blots were prepared as described in Methods, and probed with rat type I deiodinase cRNA (*upper panels*), then stripped and reprobed with mouse β-actin cRNA (*lower panels*).

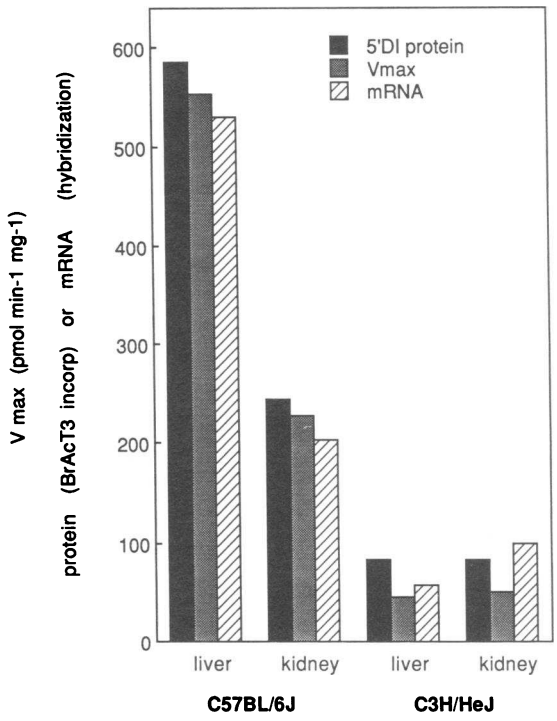

Figure 4. Quantitation of type I iodothyronine deiodinase protein, enzyme activity, and RNA in C57BL/6J and C3H/HeJ liver and kidney. Relative amounts of deiodinase protein were determined by quantitation of [^{125}I]BrAcT_3 label incorporated into 27-kD deiodinase protein, as described in Methods, and are expressed as arbitrary densitometric units. Activity levels are the means of the values in Table IV and are expressed as V_{max} in pmol min^{-1} mg^{-1}. Type I deiodinase RNA was quantitated by densitometric scanning of the autoradiogram in Fig. 3, and is expressed in arbitrary units.

Table V. Thyroid Hormone and TSH Concentrations in Sera from C3H/HeJ and C57BL/6J Mice

A. Total and free thyroid hormone concentrations

Mice	T_3	Percent free T_3	Serum-free T_3	T_4	Percent free T_4	Serum-free T_4
	ng/dl		*pg/dl*	*μg/dl*		*ng/dl*
C57BL/6J	65.7±12.6	0.35±0.04	23.7±2.7	2.36±0.46	0.062±0.005	0.13±0.01
C3H/HeJ	61.1±8.6 (ns)	0.38±0.04 (ns)	23.6±3.26 (ns)	3.76±0.88*	0.069±0.006 (ns)	0.27±0.04*

Values are the mean±SE from determinations of 6–10 mice. ns, not significant at $P < 0.05$. * $P < 0.001$.

B. Basal and TRH-stimulated serum TSH concentrations

	Basal (*n*)	After TRH (*n*)[§]
	ng of mLH/TSH/ml[‡]	
C57BL/6J	169±18 (8)	704±21 (6)
C3H/HeJ	167±4 (9)	869±80 (6)

[‡] Mean±SE. TSH concentrations calculated based on radioimmunoassay of 25 μl of serum. [§] TRH 200 ng intraperitoneally, 30 min before sampling.

Studies of Type II deiodinase. A second enzyme, the PTU-resistant 5′DII, also catalyzes conversion of T_4 to T_3. Based on a number of biochemical studies, it is thought that this enzyme is the product of a different gene from that of the type I enzyme and furthermore, that it is not a selenoprotein (26, 27). We analyzed 5′DII enzyme activity in pituitary and brain of C3H and B6 mice. The 5′DII activities in these two organs were approximately half of those in the B6 strains (Table VI). Because 5′DII activity is inhibited by T_4 (28), this difference can be attributed to the twofold higher serum T_4 in the C3H strain. To test further the hypothesis that the 5′DII gene is not affected in the C3H strain, mice were exposed to 4°C overnight and 5′DII activity measured in brown fat (29) (Table VI). These results showed no difference in this enzyme activity between the two strains.

Deiodinase gene restriction fragment variants and genetic mapping. A panel of 15 inbred strains, including progenitors of RI strains were screened for TaqI, EcoRI, PvuII, HindIII, and SstI RFLVs. Variants were detected with TaqI, EcoRI, and PvuII, identifying four distinct haplotypes (Table VII and Fig.

Table VI. Type II Deiodinase Activities in Pituitary, Brain, and Brown Adipose Tissue of Euthyroid C57BL/6J and C3H/HeJ Mice

A. Pituitary and brain

Strain	Pituitary	Brain
	fmol T_4/hr per mg protein	
C57BL/6J	19.96±3.75*	1.542±0.156[‡]
C3H/HeJ	10.09±1.33	0.658±0.139

Reaction conditions are as described in Methods for type II deiodinase assays. Data are given as the mean±SE of six different mice of each strain. * Significantly different from C3H, $P < 0.02$. [‡] Significantly different from C3H, $P < 0.005$.

B. Brown adipose tissue from cold exposed mice

Strain	K_m	V_{max}
	μM	*pmol T_4/min per mg protein*
C57BL/6J	0.0051	1.46
	0.0029	0.58
C3H/HeJ	0.0098	1.62
	0.0032	0.42

Kinetic constants were derived from double reciprocal plots from two experiments. For each experiment, tissues from three mice were pooled.

Table VII. RFLVs Associated with the Dio1 Locus among Inbred Mouse Strains

Haplotype	Restriction fragment sizes			Inbred strains
	TaqI	*Eco*RI	*Pvu*II	
	kb			
a	12.0	4.3	3.1	BALB/cBy, C3H/HeJ, PL/J, SJL/J
	4.0	4.1	2.6	
	2.9	3.8	2.3	
		3.4	1.7	
b	12.0	4.3	3.1	AKR/J, B10.D2 (58N)/Sn,
	3.8	4.1	2.6	C57BL/6J, C57BR/cdJ, C57L/J,
	2.9	3.8	2.3	C58/J, DBA/2J, NZB/BINJ,
		3.2	1.7	SWR/J
c	8.4	*	4.2	129/J
	3.6		3.1	
	3.4		2.6	
d	8.4	7.0	3.1	CAST/Ei
	3.6	5.3	2.6	
	2.6	4.3	2.3	
	2.1	4.1	1.7	
		3.8		
		3.2		

* Strain not typed.

5). A subtle TaqI difference between the normal activity B6 strain versus the low activity strains BALB/cBy (BALB) and C3H provided an opportunity to define the inheritance of the deiodinase structural gene relative to the deiodinase activity variant in BXH (from cross of B6 and C3H, Table III) and CXB (cross of BALB/cBy and B6) RI strains. In order to increase the resolution of the TaqI variants, B6 and C3H DNAs were double digested with TaqI and other restriction enzymes and analyzed by Southern blotting. Double digests with TaqI and SstI yielded a common fragment of 2.8 kb and a variable fragment of either 2.5 kb (B6) or 2.6 kb (BALB and C3H). This difference was employed to type the CXB and BXH RI strains (data not shown). The SWXJ (cross of SWR/J and SJL/J) RI strains were typed with respect to an EcoRI variant. A TaqI RFLV was used to type the NX129 (cross of NZB/BINJ and 129/J) RI strains. All of the RI strain typings (RFLVs and activity variants described in the previous section) are summarized in Table VIII. There was complete concordance between the deiodinase RFLV and high or low deiodinase activity in 21 informative BXH and CXB RI strains. In the SWXJ RI strains, there was also concordance in all but two strains, SWXJ-11 and 12. The single SWXJ-12 mouse typed for the *Dio1* RFLV appeared to be heterozygous. Of the two SWXJ-12 mice tested for deiodinase activity, one was clearly intermediate, while the other appeared to belong to the low activity class. In SWXJ-11, the single mouse tested for the RFLV was homozygous for the SJL/J allele, while the deiodinase activities of the two tested mice were intermediate. Since the SWXJ RI strains were not highly inbred at the time they were tested, residual heterozygosity might explain these anomalous results. We attempted to clarify this matter by testing two additional mice of both SWXJ-11 and SWXJ-12 for deiodinase activity and by typing the same mice for the deiodinase RFLV. Age and sex matched progenitor mice were analyzed simultaneously. Both strains were clearly intermediate in activity, and both were homozygous for the SJL/J deiodinase RFLV. The correct interpretation of the SWXJ-11 and -12 strains is unclear. The fact that these two strains exhibit an intermediate deiodinase activity suggests that activity is not strictly under the control of a single gene in this particular cross. Thus, one could postulate that there are one or more additional genes that can affect enzyme activity. Since the existence of other loci is not evident in the other strains analyzed, it is possible that there are two genes affecting activity on chromosome 4 and that one of the low activity alleles has become separated from the deiodinase RFLV in SWXJ-11 and -12. However, the general pattern emerging from the analysis of the RI strains derived from high and low activity strains is that enzyme activity is closely associated with the deiodinase RFLV.

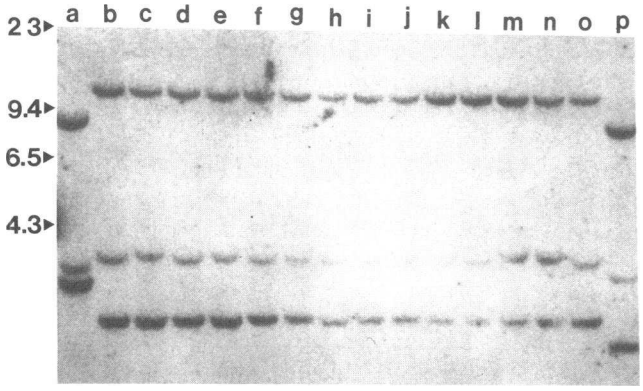

Figure 5. Southern blot showing distribution of *Dio1* TaqI RFLVs among inbred strains. Blot hybridized with rat 5′DI cDNA probe. Lanes (mouse strains): *a*, 129/J; *b*, AKR/J; *c*, BALB/cByJ; *d*, B10.D2(58N)Sn; *e*, C3H/HeJ; *f*, C57BR/cdJ male; *g*, C57BR/cdJ female; *h*, C57BL/6J; *i*, C57L/J; *j*, C58/J; *k*, DBA/2J; *l*, NZB/BINJ; *m*, PL/J; *n*, SJL/J; *o*, SWR/J; *p*, CAST/Ei. Size markers shown at left are HindIII-digested λ phage DNA.

Table VIII. Inheritance of Dio1 Alleles in RI Strains

Dio1 RFLV haplotype*	Deiodinase phenotype	RI strains (progenitors)
a	H	(C3H/HeJ), BXH-2, 4, 9, 10, 11, 19
b	B	(C57BL/6J), BXH-3, 6, 7, 8, 12, 14
a	C	(BALB/cBy), CXB-D, G, H, I, O, P
b	B	(C57BL/6J), CXB-E, J, K, L, M, N, Q
a	J	(SJL/J), SWXJ-3, 7, 13, 14
b	SW	(SWR/J), SWXJ-1, 2, 4, 5, 6, 8, 9, 10
a	SW/J	SWXJ-11, 12
a	J	(SJL/J), BXJ-1, 2
b	B	(C57BL/6J)
b	n.d.	(NZB/BINJ), NX129-1, 5, 7, 10, 17, 19
c	n.d.	(129/J), NX129- 2, 12

* The BXH and CXB RI strains were scored for the *Taq*I RFLV at the *Dio1* locus by analyzing blots of genomic DNAs digested with both *Taq*I and *Sst*I. The BXH, BXJ, SWXJ, and the CXB D through K RI strains were also scored with respect to the deiodinase activity variant. The SWXJ and BXJ RI strains were scored with respect to the *Eco*RI RFLV. The NX129 RI strains were scored with respect to the *Taq*I RFLV. n.d., not determined.

We propose the locus symbol *Dio1* to denote the type I deiodinase gene of the mouse, with haplotypes *a* through *d* based on the RFLVs described in Table VII. It is interesting that low activity strains are found only in the *a* haplotype. The segregation of *Dio1* in the CXB and BXH RI strains is discordant in 2 of 19 strains with both of endogenous murine leukemia virus genomes designated *Pmv-19* and *Pmv-23* (30), which map near the middle of chromosome 4 (Table IX). These data suggested that *Dio1* might map to this same region. The estimated recombination frequency (and 95% confidence interval) between *Dio1* and either *Pmv-19* or *Pmv-23* is 0.031 (0.0033–0.16). Table IX shows the strain distribution patterns for several chromosome 4 loci. The placement of *Dio1* relative to *Pmv-23* and *Pmv-19* shown in Table IX is arbitrary. Furthermore, neither *Pmv-19* nor *Pmv-23* have been firmly positioned within the chromosome 4 linkage group.

In order to more precisely map *Dio1*, we utilized an intersubspecific test cross [(CAST/Ei × MEV)F1 hybrids crossed to either BXD-32 or SWR/J] involving the MEV linkage testing stock crossed to an inbred derivative of the related *Mus* subspecies, *Mus castaneus* (19). This test cross had been typed for many DNA RFLVs and biochemical markers and has been used to map several genes (31, 32). A *Dio1* TaqI RFLV was identified and scored in 90 test-cross progeny. Other chromo-

Table IX. Inheritance of Dio1 and Other Chromosome 4 Loci in the CXB and BXH RI Strains

	CXB								BXH												
Locus	D	E	G	H	I	J	K	Reference	2	3	4	6	7	8	9	10	11	12	14	19	Reference
Ifa	C[a]	B[a]	C	C	C	C	B	(50)	H[a]	B[a]	H	B	B	B	H	B	B	H	B	H	(53)
																	x	x			
Pmv-23	C	B	C	C	C	C	B	(30)	H	B	H	B	B	B	H	B	H	B	B	H	(30)
						x										x					
Dio1	C	B	C	C	C	B	B		H	B	H	B	B	B	H	H	H	B	B	H	
						x	x														
Pmv-19	C	B	C	C	C	C	C	(30)	H	B	H	B	B	B	H	H	H	B	B	H	(30)
	x						x					x									
Scl	B	B	C	C	C	C	B	(51)	H	B	H	H	B	B	H	H	H	B	B	H	(51)
													x			x			x		
Mpmv-19	B	B	C	C	C	C	B	(52)	H	B	H	H	H	B	H	B	H	B	H	H	(52)

The letters B, C, and H are used as generic symbols for alleles inherited from the C57BL/6 (By or J substrains), BALB/cBy, and C3H/HeJ progenitor strains, respectively.

some 4 RFLV loci scored in the cross and the probes used are shown in Table X. The results shown in Table XI demonstrate unequivocal evidence for chromosome 4 linkage and indicate the following gene order (centromere to telomere) and recombination frequencies (±standard errors): *Tsha* 0.151±0.037 – *Mup-1* – 0.169±0.040 – *Dio1* – 0.033±0.019 – *Glut-1* – *0.071±0.028* – *Lck* – 0.191±0.043 – *Gpd-1*. These data confirm the assignment of *Dio1* to chromosome 4 and places it ~ 3 centimorgans proximal to the glucose transporter-1 (*Glut-1*) locus. The closest proximal marker, *Mup-1*, shows only loose linkage with *Dio1*. In general, the map distances among *Mtv-17, Glut-1, Lck,* and *Gpd-1* agree well with the consensus map.

Relationship to the obesity-diabetes syndrome mutation. Because of the profound consequences of thyroid status on activity and basal metabolic rate and their similar map positions, we considered the possibility that the recessive mutant gene diabetes (gene symbol, *db;* for review, see reference 33) might be an allele at the *Dio1* locus. This was tested by looking for a dissociation between the *db* mutation and its associated *Dio1* RFLV haplotype. Since the original *db* mutation arose on a C57BL/KsJ, and had been transferred by successive rounds of backcrossing and intercrossing to the C3H.SW congenic strain background, we examined the two parental strains and the C3H.SW/Lt-*db* (N9, F8 generation) congenic strain by Southern blotting. The results showed that C57BL/KsJ was homozygous for $Dio1^b$, and both C3H.SW and C3H.SW-*db* were homozygous for $Dio1^a$ (data not shown). Thus, at some point in the transfer of *db* to its new strain background or its subsequent propagation by brother-sister mating, a crossover must have occurred and become fixed in the congenic strain. Discounting the possibility of a rare intragenic recombination, this result excludes *Dio1* as a candidate gene for *db*.

Discussion

The close correlation between the levels of iododioxin and iodothyronine deiodinase activities in inbred strains of mice, in conjunction with the biochemical properties, cofactor requirements, and subcellular fractionation behavior, indicated that for both classes of substrate the activity could be attributed to 5′DI. Kinetic studies demonstrate that iodothyronines are competitive inhibitors of iododioxin deiodination (Fig. 1), further evidence that both compounds are substrates for the same enzyme. The segregation of 5′DI activity from progenitor mouse strains into RI strains exhibiting either high or low hepatic 5′DI activities allowed us to investigate the mechanism, genetics, and physiological function of the mouse 5′DI. For detailed studies of the etiology of the difference in 5′DI activity, the B6 (high) and C3H (low) inbred strains were examined in detail as representatives of the two phenotypes. The data presented in Tables III and IV demonstrate that the difference in 5′DI activities with respect to T_4 and rT_3 can be attributed to a difference in the apparent V_{max} of the hepatic and kidney 5′DI enzyme, with no difference in K_m. The results in Table III show that this difference segregates in the BXH RI strains, which have activi-

Table X. Probes and Genetic Variants Used for Mapping Deiodinase

Locus symbol	Locus name	Clone (species)	Restriction enzyme	Reference
Tsha	thyroid stimulating hormone, a subunit	pTSHa-1 (mouse)	*Bam*HI	(54)
Mup-1	major urinary protein-1	pLivS-1 (mouse)	*Pvu*II	(55)
Dio1	type I deiodinase	G-21 (rat)	*Taq*I	(1)
Glut-1	glucose transporter-1	phGT2-1 (human)	*Nsi*I	(56)
Lck	lymphocyte protein tyrosine kinase	NT18 (mouse)	*Pvu*II	(57)

Table XI. Linkage of Dio1 to Genetic Markers on Chromosome 4 in CAST/Ei × MEV Test Cross

Loci						Number of progeny
Tsha	*Mup-1*	*Dio1*	*Glut-1*	*Lck*	*Gpd-1*	
C*	C	C	C	C	C	19
M*	M	M	M	M	M	17
C	M	M	M	M	M	3
M	C	C	C	C	C	5
C	C	M	M	M	M	9
M	M	C	C	C	C	6
C	C	C	M	M	M	1
C	C	C	C	M	M	1
M	M	M	M	C	C	3
C	C	C	C	C	M	5
M	M	M	M	M	C	5
C	M	M	M	M	C	1
M	C	C	C	C	M	1
C	M	M	M	C	C	1
M	C	C	C	M	M	1
C	C	C	M	M	C	1
C	C	C	C	—	C	3
M	M	—	M	M	M	2
M	—	M	M	M	C	1
M	M	—	M	M	C	1
C	C	—	C	C	M	1
C	C	C	C	—	M	3
M	M	M	M	—	C	1
C	C	C	M	—	M	1
C	M	—	M	—	M	1
—	C	C	C	—	M	1
C	M	M	M	—	C	1
					Total	95

Recombination frequencies

Interval	Recombinants/total	Recombination frequency±SE	95% confidence limits
Tsha-Mup-1	14/93	0.151±0.037	0.085–0.24
Mup-1-Dio1	15/89	0.169±0.040	0.098–0.26
Dio1-Glut-1	3/90	0.033±0.019	0.0069–0.094
Glu1-Lck-1	6/84	0.071±0.028	0.027–0.15
Lck-1-Gpd-1	16/84	0.191±0.043	0.11–0.29

* Alleles inherited from the CAST/Ei and MEV strains are denoted by the letters C and M, respectively.

ties characteristic of either the B6 or C3H parental strains. The difference in 5′DI is greater in the liver (8–24-fold) than in the kidney (4–5-fold). The lower activity in C3H versus B6 and the differences between the RI strains are due to lower content of a kinetically normal 5′DI. The Northern blot studies (Fig. 3) illustrate that the difference in 5′DI activities can be explained by a difference in 5′DI mRNA levels. The data in Fig. 4 show the close correlation between 5′DI activity, BrAc [^{125}I]T_3-labeled 27-kD protein, and 5′DI mRNA levels in liver and kidney of the two strains. These results indicate that the low activity in C3H mice is not due to impaired translation or the production of an altered gene product, but to a decrease either in transcription rate or mRNA stability.

Given the clear segregation of the phenotypes in the BXH crosses, it seemed likely that this difference would be found in the 5′DI gene itself. We therefore undertook genetic mapping studies to localize this defect, as a first step towards determining the specific nature of the mutation. The *Dio1* locus has been mapped to a position ~ 3 centimorgans proximal to the *Glut-1* locus. This provides a position of ~ 49 cM from the most centromeric marker in the current consensus linkage map (34), placing it close to the complement 8-beta gene (C8b). It is also apparently close to the obese-diabetes syndrome mutant gene *db*. The latter gene has been the focus of intense interest as a model for human obesity and type II diabetes, and recently there has been a concerted effort to isolate the *db* gene by positional cloning. The possibility that *db* might be a mutant allele at the *Dio1* locus was considered, but disproved based on the observation of a crossover event in a *db* congenic strain. Direct measurement of the deiodinase activity of liver homogenates of *db/db* and control mice yielded indistinguishable measurements, providing further indication of the distinctness of the two loci. Although the phenotype of a mouse severely deficient in deiodinase activity can only be a matter of speculation, manifestations of thyroid insufficiency might be anticipated. In this regard, such a mouse might resemble the two known thyroid-deficient mice, hypothyroid (*hyt*) and congenital goiter (*cog*). The syndromes presented by these mutant mice do not include marked obesity or hyperglycemia. No hypothyroid mutant has yet been identified that maps to this region of chromosome 4 for which *Dio1* can be considered a candidate gene. If such a mutant is subsequently identified and mapped to chromosome 4, the possible association with *Dio1* should be apparent. The position of *Dio1* in chromosome 4 of the mouse allows the prediction that the human homolog of *Dio1* will map to HSA1p (34). The segment of chromosome 4 homologous to HSA1p extends proximally from *Jun* (position 44) to *Skv* (position 77) distally, and therefore represents one of the largest uninterrupted regions of conserved linkage between the two species. The placement of *Dio1* within the chromosome 4 linkage map and the association of the *a* haplotype with the low activity phenotype will provide a valuable reference point for further mapping in RI strains.

What are the physiological consequences of a 5–10-fold difference in 5′DI activity and what compensatory mechanisms occur in the intact animal? Data in Table V demonstrate that relative to the B6 mice, C3H strains have elevated serum total and free T_4 but normal serum-free T_3 and TSH concentrations. Quantitation of free hormones is required to establish this difference, since thyroid hormone binding abnormalities have been described in certain mouse strains, e.g., *db/db, ob/ob,* and dystrophic animals (16, 35). The fact that serum-free T_3 concentrations are normal in C3H animals would lead to a euthyroid state in those peripheral tissues (heart, liver, kidney, skeletal muscle) which depend predominantly on the plasma T_3 for their intracellular hormone (36, 37). This can account for the euthyroid phenotype of the C3H strain which shows none of the manifestations of hypothyroidism found in genetically hypothyroid mice such as those with the *hyt* or *cog* mutations. In the iodine-sufficient rat, 65–75% of the circulating T_3 derives from a PTU-sensitive T_4 5′-deiodination process, catalyzed by the 5′DI in liver and kidney (15, 37, 38). The re-

mainder originates from a combination of direct thyroidal T_3 secretion and from PTU-insensitive T_4 to T_3 conversion (5'DII). A similar analysis has not been performed in the mouse but it seems unlikely that this situation would be very different. The normal concentration of free T_3 in sera of C3H mice presumably is due to the fact that, while the fractional conversion of T_4 to T_3 per day would be reduced, the higher serum-free T_4 concentration permits normal daily T_3 production. We speculate that T_4 production rates could well be normal since the higher concentrations and reduced fractional clearance could balance each other. A reduction in inner ring deiodination of T_3, also a function of the 5'DI enzyme, could also contribute to maintenance of normal circulating T_3 concentrations.

An intriguing physiological question is how the hypothalamic-pituitary thyroid axis recognizes the requirement for higher serum T_4 concentrations. In the rat, the degree of saturation of nuclear T_3 receptors in the pituitary correlates most accurately with the rate of TSH secretion (6, 7, 39). There are two sources of pituitary nuclear T_3, that derived directly from the plasma and T_3 derived from intrapituitary T_4 to T_3 conversion via the type II, PTU-insensitive mechanism (36, 37). The 5'DII enzyme differs from 5'DI in its lower V_{max} and K_m, and its very low sensitivity to inhibition by PTU or gold (27, 36). Recent studies have suggested that 5'DII does not contain selenocysteine (26, 27), and this activity is expressed in rat brown adipose tissue, which does not express 5'DI (1). The level of 5'DII is reduced when the serum T_4 concentration is increased. This regulation probably occurs post-translationally by accelerating the degradation of the 5'DII (40). Thus, in hypothyroid animals, type II deiodinase activity increases, and after T_4 injections, this activity falls (41, 42).

It would therefore be anticipated that in C3H mice, the higher serum-free T_4 concentration would cause a decrease in the activity of 5'DII, and this prediction was born out by the data presented in Table VI. In both cerebral cortex and in pituitary, 5'DII activity was twofold lower in C3H than in B6 mice. A similar phenomenon has been identified in rats made selenium deficient (4, 43). However, recent studies have shown that in hypothyroid animals, selenium deficiency does not reduce 5'DII activity (44). Thus, in both the C3H strain and in intact selenium-deficient animals, the elevation in serum T_4 can explain the decrease in 5'DII activity in brain and pituitary. This is further confirmed by the finding of normal 5'DII activity in brown adipose tissue of C3H mice (Table VI), an observation which also demonstrates that 5'DII is not the product of the *Dio1* gene. Since the 50% decrease in 5'DII activity in the brain and pituitary is balanced by the elevation in serum T_4 concentration, one would predict that the T_3 contribution from this source to pituitary nuclear T_3 would be quite similar to that of the B6 animals. Therefore TSH secretion would be the same. The data in Table V show that TSH concentrations are not different in the two strains. Given all of these internal compensatory changes, one would expect a euthyroid state in the C3H strain, which is what has been observed.

After our submission of this manuscript, Schoenmakers et al. (45) reported 5'DI activities, mRNA, and serum hormone levels in C3H and B6 mice, initiating their studies after informal discussions with one of us (A.P.). In contrast to our findings, they observed highly variable hepatic 5'DI activities in C3H mice, though the mean was ~ 18% of that in the B6 strain. They also found that the levels of 5'DI mRNA and BrAc [^{125}I]T_3-labeled 28-kD protein were proportionately reduced in C3H animals with low 5'DI activity. However, again in contrast to our results, serum total T_4 concentrations were not significantly different between C3H and B6 strains, presumably due to the heterogeneity in 5'DI activities. We have no explanation for the disparate 5'DI results in this study, since we have not observed, in over 40 C3H mice, 5'DI activity or mRNA levels which overlap with the range in B6 animals. Neither 5'DII activities, free hormone concentrations, or serum TSH results were reported by Schoenmakers et al., though serum rT_3 was significantly higher in the C3H than in the B6 mice, consistent with the reduced 5'DI activity in this strain.

A putative Type I deiodinase defect has been described in one family and in one individual which may be analogous to the 5'DI differences seen in the C3H and other low 5'DI strains (9, 10). In these individuals, serum total and free T_4 are elevated, and serum T_3 and TSH are normal. These patients are also clinically euthyroid, indicating that the predominant manifestation of this defect is in the elevated serum-free T_4. The predicted location of the *Dio1* gene on human chromosome 1 may aid in characterization of such defects as due to alterations in the 5'DI gene. Furthermore, it is conceivable that the relatively broad normal range of serum free T_4 concentrations found in humans could be explained by differences in the concentration or function of the 5'DI enzyme. Such differences would be compensated internally and be reflected only in variable concentrations of free T_4, all associated with a normal serum free T_3 and TSH.

In addition to the allelic differences in type I deiodinase described herein, a number of other genetic differences between the C3H, B6, and BALB inbred strains have been described. C3H exhibits ~ 20–50-fold higher susceptibility than B6 to chemically induced hepatocarcinogenesis (46). This difference appears to be due to increased responsiveness of C3H liver cells to c-Ha-ras gene mutations. However, variations in tumor formation do not segregate with 5'DI, as BALB mice fall into the low tumor susceptibility category, like B6, whereas for *Dio1*, BALB segregates with C3H. Differences in atherosclerotic plaque formation and plasma HDL levels in response to a high fat diet have also been studied extensively, with B6 exhibiting the highest susceptibility and lowest HDL, BALB intermediate (plaque formation and HDL), and C3H the most resistant, with the highest HDL. The lack of clear segregation in BALB mice suggests that the plaque formation differences are due to multigenic effects, and allelic differences correlating with atherosclerosis susceptibility have been described in apolipoprotein II on chromosome 1 (47, 48), and in a newly identified gene, Ath-2 (49). Whether or not the 5'DI polymorphism also contributes to plaque formation is unknown.

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