Comparison of Iodothyronine 5′-Deiodinase and Other Thyroid-Hormone-dependent Enzyme Activities in the Cerebral Cortex of Hypothyroid Neonatal Rat

EVIDENCE FOR ADAPTATION TO HYPOTHYROIDISM

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ABSTRACT Recent studies have shown that ~75% of the nuclear 3,5,3′-triiodothyronine (T_3) present in adult rat cerebral cortex (Cx) derives from 5′-deiodination of thyroxine (T_4) within this tissue. The activity of iodothyronine 5′-deiodinase (I 5′D), the enzyme catalyzing T_4 to T_3 conversion, increases rapidly after thyroidectomy, suggesting that this could be a compensatory response to hypothyroxinemia. To evaluate this possibility during the period of central nervous system maturation, we studied several thyroid hormone-responsive enzymes (aspartic transaminase [AT], succinic dehydrogenase [S.D.], and Na/K ATPase) in the Cx of 2-, 3-, and 4-wk-old rats. The rats were made congenitally hypothyroid by placing 1, 2, 5, and 20 mg methimazole (MMI) in 100 ml of the mothers' drinking water from day 16 of gestation throughout the nursing period and to the litters after weaning. In addition, serum thyroid hormones, I 5′D, and, in some experiments, in vivo T_4 to T_3 conversion in Cx were measured in the same pups. Serum T_4 concentrations varied from <1 to 40 ng/ml and were generally inversely related to maternal MMI dose. Serum T_3 was less affected by MMI than was T_4. At 2 wk, decreases in AT, S.D., and ATPase were present in the 20-mg-MMI group but not in the 5-mg-MMI pups despite low serum T_4 (<10 ng/ml) in the latter. At 3 and 4 wk, both 5- and 20-mg-MMI groups had significant reductions in these cortical enzymes despite a normal serum T_3 in the 5-mg-MMI rats. Cortical I 5′D activity was 10-fold the control value in 5- and 20-mg-MMI animals at 2 wk but increased only three- to fivefold at 3 and 4 wk. I 5′D correlated inversely with serum T_4 ($r \geq 0.96$) at all ages, but the less marked elevation of this enzyme in 3- and 4-wk-old pups was not accompanied by an increase in serum T_4. Serum T_3 increased or remained the same between 2 and 3 wk. These results suggested that the 10-fold increase in I 5′D at 2 wk protected the 5-mg-MMI group from tissue hypothyroidism, but that the three- to fivefold increase at 3 and 4 wk could not. Injection of ~250 ng T_4/100 g body weight to 2-wk-old, 20-mg-MMI pups (one-sixth the normal T_4 production rate) led to both a 1.8-ng/g cortical tissue increment in cortical T_3 and a significant increase in AT at 24 h, compared with a 0.38-ng/g cortical tissue T_3 increment and no change in AT in euthyroid controls. The larger increment in T_3 of the 20-mg-MMI pups was due in great part to increased fractional T_4 to T_3 conversion. Although the latter resulted in greater serum T_3 concentrations, three-fourths of the newly formed T_3 in the cortex was generated *in situ*, and it was blocked by iopanoic acid as was the increase in AT. We conclude that 70–80% of the T_3 in the Cx of the neonatal rat is produced locally. Serum T_4 appears to serve both as a precursor for T_3 and as a critical signal for increases in cortical I 5′D. The increased I 5′D can result in normal or near-normal cerebrocortical T_3 concentrations despite marked reductions in serum T_4. This mechanism seems to be particularly effective around 2 wk of age when many thyroid-hormone-dependent maturational changes occur in the rat Cx.

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INTRODUCTION

The concept that thyroid hormones are essential for normal development of the central nervous system

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(CNS)[1] is well established (1). The lack of these hormones during critical periods of CNS maturation induces morphological, physiological, and biochemical abnormalities. The most striking morphological alteration is a reduction in the number of arborizations of the neurons in the cortex of cerebral hemispheres and cerebellum (2). These perturbations induced by thyroid hormone deficiency are paralleled biochemically by a decrease in the incorporation of glucose-carbon into glutamic acid in the so-called gamma aminobutyric acid shunt (3). Both aspartic transaminase (AT) and succinic dehydrogenase (S.D.), enzymes involved in these metabolic pathways, are known to be decreased in the brain of neonatal hypothyroid rats (4, 5), although a number of other enzymes not as closely related to this pathway have also been shown to be reduced (5).

We have recently found that *in situ* thyroxine (T_4) to triiodothyronine (T_3) conversion is the main source of T_3 in adult rat brain, accounting for 75 and 60% of the T_3 measurable in cerebral cortex (Cx) and cerebellar cell nuclei, respectively (6). Enzymatic 5′-deiodination of T_4 is demonstrable in homogenates of different regions of the brain (7). Even more interesting is that this enzyme activity has a maturational pattern that suggests that it may be of physiological relevance for the development of the brain (8), because the activity is increased between 10 and 30 d when most of the changes alluded to above take place (1–5). The finding of increased enzyme activity in adult (7, 9, 10), as well as in neonatal, hypothyroid rat cerebral homogenates (11) further supports that suggestion.

Earlier studies from our laboratory showed that the pituitary T_3 content is also heavily dependent on local T_4 to T_3 conversion (12). A fall in T_4 production resulting from a failing thyroid gland leads to pituitary thyrotropin (thyroid-stimulating hormone [TSH]) release, and the ensuing thyroid stimulation maintains plasma T_3 within normal limits until well advanced thyroid failure (for a review see 13). Studies of congenitally hypothyroid infants show that the level of serum T_3 is often normal, whereas that of T_4 and TSH are markedly altered (14).

It thus seems possible that the ultimate impact that thyroid hormone deficiency may have on brain development depends not only on the extent of the thyroid failure, but also on the success of the various compensatory mechanisms in the pituitary and in the brain itself. Because at birth both the CNS and hypothalamic-pituitary system of the rat are less mature than those in the human newborn (15, 16), the rat may be a suitable model for evaluating the impact that different degrees of thyroid failure may have on human brain development *in utero*. Accordingly, we have induced congenital hypothyroidism in rats by giving pregnant dams various concentrations of methimazole (MMI) in the drinking water from day 16 of gestational age (GA), i.e., before the thyroid of the fetus is functioning (15), throughout the nursing period. Because the most critical changes in brain development occur between 10 and 21 d postnatally (2, 3), the responses to hypothyroidism at 2 wk were compared with those at later ages.

METHODS

Rats and experimental protocols

Pregnant Sprague-Dawley rats were obtained from Zivic-Miller Laboratories (Allison Park, PA) on day 15 or 16 of gestation. They were housed in individual cages, and on day 16 of GA they were given tap water ad lib. with 0, 1, 2, 5, and 20 mg MMI/100 ml water (1-, 2-, 5-, and 20-mg-MMI groups). This regimen was maintained throughout the nursing period (up to 4 wk), at which time control rats and some of the litters receiving the lowest concentration of MMI had weaned, but were kept in the same cage with MMI-containing water. Within the first 24–48 h after birth, litters were made nine to eleven rats each by redistribution of the pups within dose groups; subsequently the number of rats per litter was kept as nearly equal as possible. Because several litters with the same treatment were run simultaneously, it was always possible to redistribute the pups and make up the groups from two to three litters for each experiment. However, due to a significant mortality rate (three to five rats/litter in 4 wk), and in order to examine the same litters through the 4-wk period, experimental groups were limited to four to five rats. Unless otherwise stated, the variables analyzed in a given experiment were all measured in the same pups. Within these MMI concentration ranges the dams did not restrict their food and water intake (40–70 ml/d). A number of pilot experiments were performed to determine whether the maternal thyroid status influenced either the nutritional condition or the thyroid status of the offspring. Mothers treated with the highest concentration of MMI received T_3 simultaneously (0.2 μg/ml) in drinking water containing 0.01% bovine serum albumin (BSA). At age 3 wk these pups weighed 29±4 g and their serum T_3 was 0.09±0.03 ng/ml as compared with 29±4 g and 0.16±0.08 ng/ml in the pups of mothers not receiving the hormone (n = 5). The mothers' serum T_3 concentrations were 0.40 and <0.03 ng/ml, respectively. In other experiments, the 20-mg-MMI-treated dams were implanted with an osmotic minipump (Alza Corp., Palo Alto, CA) to deliver ~500 ng T_3/100 g body weight (bw)/d; the minipump was replaced at 14 d as indicated by the manufacturer. At 3 wk, T_3-treated mothers had a serum T_3 of 2.5 ng/ml as compared with <0.03 in the T_3-untreated dams. The pups weighed 33±1 and 35±2 g and their serum T_3 concentrations were 0.16±0.06 and 0.17±0.08

[1] *Abbreviations used in this paper*: AT, aspartic transaminase; bw, body weight; CNS, central nervous system; Cx, cerebral cortex; DTT, dithiothreitol; GA, gestational age; α-GPD, α-glycerophosphate dehydrogenase; INT, iodonitrotetrazolium; IOP, iopanoic acid; I 5′D, iodothyronine 5′-deiodinase; MMI, methimazole; PTU, propylthiouracil; rT_3, reverse T_3; S.D., succinic dehydrogenase; TSH, thyrotropin (thyroid-stimulating hormone); T_4, thyroxine; T_3, triiodothyronine.

ng/ml, respectively ($n = 5$). Finally, the stomachs of the pups, usually full of milk, were removed at death and found to weigh 1.03±0.41 g ($n = 7$) and 1.15±0.7 g ($n = 8$) in controls and MMI-treated litters, respectively, at age 2 wk. These results indicated there was no significant effect of maternal thyroid status on the pups.

The pups were weighed only on the day of the experiment. They were killed either by decapitation or by aortic exsanguination if radioisotopes had been injected (see below). Cx was removed *in situ* with the aid of a fine spatula; it was usually contaminated with some white matter but basal ganglia were excluded. Between 600 mg (2 wk) and 900 mg (4 wk) of tissue was collected. Except occasionally at age 2 wk, there was no difference between the amount of tissue collected from control and from MMI-treated rats. The liver was removed almost *in toto* yielding between 0.7 and 1.5 g of tissue, depending on the age and on the thyroid status of the pups. Both tissues were immediately homogenized in four volumes of 0.32 M sucrose, 10 mM Hepes pH 7.0, and 1 mM dithiothreitol (DTT) (liver) or 10 mM DTT (cortex) (17). They were then either snap frozen in dry ice-acetone or kept in ice-cold water for assay within 1–2 h of death, as indicated below. All assays of a given age group were performed simultaneously.

Enzyme assays

Succinic dehydrogenase. Assay was performed in crude homogenate based on the method of Slater and Bonner (18) with some modifications. The total reaction volume was 3 ml with this final composition: 0.03 M Na succinate; 0.01 M KCN; 0.001 M $K_3Fe(CN)_6$; and 0.12 M potassium phosphate buffer, pH 7.2, containing 0.5% BSA. The tissue sample was added in 0.2-ml aliquots representing ~2–3 mg of protein or 40–60 μg of DNA. After the tubes were preincubated for 5 min at 37°C, prewarmed $K_3Fe(CN)_6$ was added quickly and the reaction started by the addition of Na succinate. The reaction was stopped by adding 2 ml of 10% ice-cold TCA at 1, 2, and 4 min. Blanks were made by adding the TCA before the $K_3Fe(CN)_6$ and Na succinate. The tubes were centrifuged at 2,000 *g* for 10 min and the optical density at 400 nm determined. Under these conditions, the reaction was linear with time and with homogenate concentration up to 5 mg protein/assay tube. Results are expressed in Δ optical density units per minute per milligram DNA (see below). In later experiments, S.D. was assayed using iodonitrotetrazolium (INT) as electron acceptor (19), which turned out to be simpler and faster. Total reaction volume was 0.27 ml: 100 μl of 0.12 M KPO_4, 0.5% BSA, pH 7.2; 50 μl of sample as a 1:10 dilution of crude homogenate in buffer (50–75 μg protein); 20 μl of 0.1 M KCN in water; and 50 μl of 4 mg/ml INT in water. After a preincubation of 5 min at 37°C the reaction was started by the addition of 50 μl of 0.15 M Na succinate, pH 7.2, in water. Only water was added to the blanks. The reaction was stopped at 15 min by adding 250 μl of 10% ice-cold TCA, followed by 2 ml of 95% ethanol. Tubes were centrifuged at ~2,000 *g* for 10 min and the optical density determined at 500 nm. The reaction was linear with time up to 20 min, provided the optical density was <0.600. The optical density of the blank tubes varied between 0.02 and 0.05, and the samples between 0.15 and 0.35. As to the effect of MMI-treatment, both methods gave the same results, i.e., the 20-mg-MMI pups showed 30–50% lower values than the controls (see Results).

Aspartic transaminase. The assay was performed using crude homogenates; the method used was based on that designed by Karmen (20), but slightly modified. The homogenate was pretreated with deoxycholate to release the latent enzyme (21). Usually 0.4 ml of one-fifth homogenate was incubated for 20 min at 37°C with 0.15 ml of 4.25% Na deoxycholate, and it was subsequently diluted 1:40 with the reaction buffer (0.01 M sodium phosphate, pH 7.4). The reaction mixture contained 4.2 ml of buffer; 0.6 ml of 0.2 M Na α-ketoglutarate, pH 7.4; 0.3 ml 500 U/ml malic dehydrogenase (in phosphate buffer); and 1.2 ml diluted homogenate. Sets of four tubes were immersed in a water bath at 30°C and preincubated for 5 min, and then 0.6 ml of 1 mg/ml NADH solution was added, rapidly followed by 1.5 ml of 0.2 M Na aspartate, pH 7.4, at 15-s intervals. Each tube was read at 340 nm for 3–4 min at 1-min intervals in a Gilson spectrophotometer (Gilson Medical Electronics, Inc., Middleton, WI) with an aspirating rapid sampler. Reaction velocity was linear with time (up to 5 min with euthyroid cortex homogenate and even longer in hypothyroid rat homogenates) and with protein concentration. Results are expressed as Δ optical density per minute per milligram DNA (see below).

Na/K-dependent ATPase. This was measured by the release of P from ATP in the presence and absence of 1 mM ouabain (22). Total assay volume was 0.25 ml and the reaction mixture contained 0.100 M Hepes, pH 7.4, 0.1 M NaCl, 0.02 M KCl, 6 mM ATP with 6 mM $MgCl_2$, and ±1 mM ouabain. 25 and 50 μl of crude homogenate diluted 1:15 in water (20–60 μg protein) were usually assayed. Incubation was for 30 min at 37°C. Results are expressed as micromoles of P released per minute per milligram DNA (see below).

Iodothyronine 5'-deiodination (I 5'D) activity. This was assayed by two methods in crude homogenates of cortex and liver: by T_4 to T_3 conversion and by 5'-^{125}I-reverse T_3 (rT_3) deiodination. Assay conditions, procedure, and product separation were recently described in detail (17). The T_4 concentration was 2 nM (cortex) or 100 nM (liver), along with ~100,000 cpm/tube of [^{125}I]T_4 (sp act ~4,200 μCi/μg). rT_3 concentration was also 2 nM. Assays were performed in the presence of 20 mM (cortex) or 1 mM (liver) DTT. 1 mM propylthiouracil (PTU) was added to the rT_3 deiodination assay because, in the cortex, the PTU-insensitive rT_3 5'-deiodination, at 2 nM rT_3, is indistinguishable from T_4 to T_3 conversion at 2 nM T_4 concentration (17, 23, 24). Results were expressed as either moles of T_3 formed or rT_3 deiodinated per hour per milligram DNA (cortex) or of protein (liver).

The Cx enzyme activities have been expressed on a DNA basis rather than on the more conventionally used protein basis. The rationale for this is that the Cx protein:DNA ratio is decreased in hypothyroid animals but it increases with age and body weight. Thus, protein:DNA ratio (milligram per milligram) in cortex homogenates of 2-wk-old 20-mg-MMI pups ranged from 30 to 70 for body weights of 18 to 30 g; the corresponding figures for controls were 40 to 90 for body weights between 38 and 48 g. On the other hand, the DNA content is fairly constant during the neonatal period in both controls and hypothyroid pups (4, 5). This method of expressing the results has been shown to be more sensitive (4, 5) and allows a better assessment of the enzyme on a per cell basis.

Hepatic mitochondrial α-glycerophosphate dehydrogenase (α-GPD). Liver homogenate was made 1:10 with 0.32 M sucrose and 1 mM $MgCl_2$, and then centrifuged at 800 *g* for 10 min. The supernatant was centrifuged at 10,000 *g* for 10 min and the pellet was washed once in 0.32 M sucrose and 1 mM $MgCl_2$. The pellet was resuspended in 2 ml of 0.05 M phosphate buffer, pH 7.4. The assay was performed

as modified by Gardner (25) and Okamura et al. (26) but using 0.05 M phosphate buffer instead of 3-(*N*-morpholino) propanesulfonic acid buffer as the latter authors did. The omission of phenazine methosulfate proposed in the original method by Lee and Lardy (27) reduces the amount of the colored product iodonitroformazan by 50–60% (25). Thus the optical density at 500 nm is reduced but the activity ratio of euthyroid to thyroidectomized is the same, i.e., ~10.

None of these enzyme activities was affected by adding 1 mM MMI in vitro, nor by preincubating the homogenate with that concentration of the drug. The highest concentration of MMI in the drinking water was 1.75 mM (20 mg/dl). Pilot experiments in adult thyroidectomized rats with undetectable serum T_3 and T_4 concentrations showed that MMI in the drinking water at 20 mg/100 ml for 2 mo did not affect the level of any of these enzymes. This indicates that the observed effects of MMI in the newborn rats are due to hypothyroidism and not nonspecific effects of the MMI.

In vivo studies

To assess the capacity of both the cortex and liver to generate T_3 from T_4 in vivo, small doses of radiolabeled T_4 were injected intravenously. In the first of these experiments ^{131}I-T_4, 600 ng/100 g bw (sp act ~5800 μCi/μg), was injected and animals were killed 16 h later. In the second experiment ^{125}I-T_4, 60 ng/rat (150–300 ng/100 g bw), was injected 24 h before death followed in 8 h by the injection of tracer [^{131}I]T_3 subcutaneously. The latter was used to estimate what fraction of the observed [^{125}I]T_3 in the tissues was accounted for by the plasma [^{125}I]T_3 (6). In these experiments, iopanoic acid (IOP) was given to some rats intraperitoneally before the injection of labeled T_4 (see Results). [^{125}I]T_3 and [^{131}I]T_3 in the serum were measured by a combined affinity and paper chromatography method described previously (28). Serum [^{125}I]T_4 was measured by descending paper chromatography of whole serum (29) or by TCA precipitation. Iodothyronines were extracted from crude homogenate in butanol saturated with 2 N HCl, usually 0.25 ml homogenate plus 1 ml of butanol HCl, which ensured >80% extraction. The extract was dried under N_2 and the iodothyronines resuspended in 50 μl ethanol: 2 N NH_4OH (99:1) containing 1 mg/ml T_4, T_3, and NaI. The suspension was applied to 3-mm Whatman paper (Whatman Inc., Clifton, NJ) and developed as described (29).

Other procedures

T_4, T_3, and TSH were assayed in serum by methods previously reported (30–32). T_4 and T_3 from hypothyroid rats were measured using hormone-stripped serum from hypothyroid rats in the standard curves. Hypothyroid rat serum decreases the slope of the displacement curve in such a way that samples with low T_3 values are overestimated if read using standard curves containing hormone-free serum from euthyroid rats.

Results are expressed as mean±SD or range. Statistical analyses included *t* tests, both paired and unpaired, and regression analyses. The latter were performed using a Hewlett-Packard 9815A calculator (Hewlett-Packard Co., Palo Alto, CA) by evaluating the best fit of the data to a number of linear and nonlinear mathematical models contained in the software from the manufacturer. This was done merely to facilitate the description of the data. The interpretation of the better fit to one model as compared with another is beyond the scope of this work. Similarly, we intend no implication as to whether the relationships implied by the formulas would predict the changes in y beyond the observed ranges of the variable x. As to the comparisons between the mean of a given variable obtained with a given dose of MMI and that of the control group within an experiment, i.e., at one particular age (Table I, Fig. 1), we used a per comparison approach (33) rather than a multiple comparison test (34). The rationale for this statistical treatment is that we do not attach any significance to each dose of MMI, which was arbitrarily chosen to induce graded hypothyroidism, but to the serum thyroid hormone concentrations induced by this treatment and their relationship to the biological responses analyzed. Because serum levels of T_3 and T_4 varied widely both within and between treatment groups, we further evaluated the data in terms of the individual hormone results, regardless of the MMI dose that induced them.

RESULTS

Effect of MMI given to the mothers on serum hormone levels of the offspring (Tables I and II and Fig. 1). Serum T_4 in control pups did not change with age, but serum T_3 increased between 2 and 3 wk to a plateau as has been described (15) (Table I). Such a pattern was observed also in the 1-mg-MMI group ($P < 0.01$), but was not evident in the pups exposed to higher concentrations of MMI. The various concentrations of MMI produced a wide spectrum of serum T_4 and T_3 concentrations even within a given group, probably reflecting individual variations in the MMI intake through the milk, the susceptibility of the thyroid to the drug, and the response of the pituitary thyroid axis, etc. (compare the standard deviations of MMI-treated pups with those of the control groups). The 1-mg-MMI group serum T_3 was not different from controls at any age, but the 5-mg-MMI group serum T_3 became significantly lower at 4 wk. At all ages, the 20-mg-MMI group had significantly lower serum T_3 concentrations than the controls. Serum T_4 was always decreased by MMI except in the 1-mg-MMI group at 2 wk, where there was an overlap with the controls. Fig. 1 shows the relationship between the individual serum concentrations of both iodothyronines and gives a better idea of the variation in the response to MMI. The data were best fit to a model where $[T_3] = a\,[T_4]^b$. At all ages, but especially at 2 and 3 wk, serum T_3 fell below the normal range only when T_4 was very low. At 3 wk and, to some extent, at 4 wk, supranormal levels of T_3 (>0.65 ng/ml) were often found with normal or low serum T_4 values.

Because of the short supply of serum, TSH was measured in a limited number of samples. Table II shows the results in samples from 2½- to 3-wk-old pups. The range of response was broad, as is evident from the standard deviation within each MMI-dose group. The 5-mg-MMI group showed the highest levels of TSH

TABLE I

Serum T_4 and T_3 Levels in Neonatal Rats with MMI-induced Congenital Hypothyroidism

	MMI*	Control		1		2		5		20	
	Age	T_4	T_3	T_4	T_3	T_4	T_3	T_4	T_3	T_4	T_3
	wk										
Exp. 1											
	2	36±3.8	0.41±0.10	27±8.0 <0.025‡	0.37±0.08 NS‡	—	—	2.6±3.2 <0.001	0.30±0.10 NS	0.9±0.7 <0.001	0.19±0.05 <0.010
	3	33±4.0	0.62±0.05§	29±2.6 NS	0.78±0.20§ NS	—	—	13.0±9.4 <0.01	0.58±0.25 NS	2.3±3.2 <0.001	0.36±0.18 <0.05
	4	37±2.2	0.65±0.03‖	21±4.3 <0.001	0.67±0.12§ NS	—	—	2.6±0.6 <0.001	0.32±0.04 <0.001	4.1±6.0 <0.001	0.25±0.05 <0.001
Exp. 2											
	2½	34±3.3	0.47±0.04	—	—	21±7.7 <0.025	0.69±0.20 <0.005	16±7.3 <0.005	0.41±0.18 NS	1.1±0.8 <0.001	0.16±0.04 <0.001

Data represent mean±SD.

* MMI was given to pregnant rats in drinking water from day 16 of gestation throughout the nursing period as described in Methods. The drug concentration is given as mg/100 ml. Each set of T_4 and T_3 values represents the mean±SD of four pups.

‡ P value when compared with age-matched controls.

§ $P < 0.01$ when compared with 2-wk-old rats receiving same treatment.

‖ $P < 0.005$ when compared with 2-wk-old rats receiving same treatment.

($P < 0.025$ compared with 20-mg-MMI group) and the same trend was shown by the 2-mg-MMI group. As shown in Fig. 1, many rats in these groups had elevated levels of T_3. However, the lack of measurements of T_4, T_3, and TSH in the same samples precludes further analysis.

Effect of different MMI regimens on neonatal Cx enzyme activities (Fig. 2). The enzyme activities were measured in tissue samples from the same pups discussed above. At 2 wk, AT, S.D., and ATPase activities were significantly reduced only in the 20-mg-MMI group. At 3 wk, reduced levels of these three enzyme activities were observed in the 5-mg-MMI group as well, and at 4 wk one of the enzymes, AT, was also reduced in the 1-mg-MMI group. Although all these differences from the controls reached statistical significance, the values found in the hypothyroid pups were seldom <60% of the euthyroid controls. The difference observed at 2 wk between the 20-mg-MMI group and the controls, although modest, was quite reproducible, as is evident in Table V (see below) and from several other subsequent experiments not shown.

I 5'D activity responded quite differently to MMI. At 2 wk, both 5- and 20-mg-MMI groups showed a striking 10–13-fold increase in the enzyme activity measured with either substrate, and there was no difference between these two groups. The 1-mg-MMI group showed a modest increase significant only when the enzyme was assayed with rT_3 as substrate. At 3 and 4 wk both 5- and 20-mg-MMI groups also showed comparable increases, but the activity was only three- to fivefold that of controls, although at 4 wk we measured only rT_3 5'-deiodination. The changes in 5'-deiodination of T_4 and PTU-insensitive rT_3 deiodination as assayed here were identical throughout, with the correlation coefficient of all pairs of such assays equal to 0.991.

Correlation between serum thyroid hormones and the observed changes in the various enzyme activities (Figs. 3, 4A–C). As indicated above, the various MMI

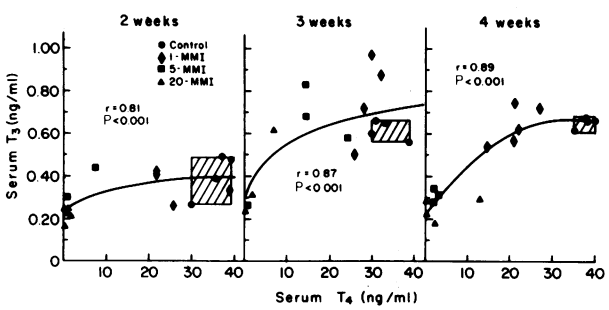

FIGURE 1 Serum T_4 and T_3 concentrations in neonatal rats with MMI-induced congenital hypothyroidism. MMI was given to the mothers in the drinking water from day 16 of gestation throughout the observation period. Each dot represents one individual rat and each symbol a different MMI concentration in milligrams/100 ml of drinking water. The square hatched area represents the control ranges for both variables. The coefficient of correlation corresponds to a power model.

TABLE II

Serum TSH Concentrations in 2½–3-Wk-Old Neonatal Rats with MMI-induced Congenital Hypothyroidism

MMI*	n	TSH‡
		μU/ml
Control	4	128±28
2	8	389±298
5	10	513±245§
20	6	245±73‖

Data represent mean±SD.

Most of these rats were littermates of those used for experiments 1 (3 wk) and 2 in Table I.

* MMI, methimazole concentration (mg/100 ml) in drinking water as described in Methods and Table I.

‡ TSH in microunits or RP1 standard.

§ $P < 0.01$ vs. control.

‖ $P < 0.025$ vs. 5-mg-MMI group.

doses induced a broad range of serum T_4 and T_3 concentrations, with great overlap in the responses to different doses of the drug. This allowed us to examine the relationships between serum thyroid hormones and enzyme activities rather than those between the latter and the doses of MMI. The correlation between I 5'D (using rT_3 as a substrate in the presence of 1 mM PTU) and serum levels of either T_4 or T_3 are shown in Fig. 3. A number of mathematical models for $y = f(x)$ were tested and the one that consistently gave the best fit was a parabolic model, where $y = a + bx + cx^2$. The correlation between I 5'D and serum T_4 is >0.96 in all cases. Three aspects of these data are of interest: (*a*) the *y*-intercept decreased between 2 and 3 wk with no further change between 3 and 4 wk; (*b*) the slope of the regression line for serum T_4 <30 ng/ml is significantly higher at 2 wk than at 3 or 4 wk; and (*c*) except for 1 pup, no supranormal I 5'D value was found if serum T_4 was normal. The correlation between I 5'D and serum T_3 (Fig. 3) was not as high as with T_4 (r = 0.66–0.92). Of the three characteristics noted for the I 5'D-T_4 correlations, only the first two are true for serum T_3. Elevated I 5'D enzyme activity levels were observed in pups with normal or even elevated serum T_3 concentrations.

The relationship of AT, S.D., and ATPase with serum T_4 and T_3 at each age is depicted in Fig. 4A–C. The parabolic model again gave the highest correlation coefficients. Although there were usually significant correlations between the AT and S.D., and serum levels of T_4 and T_3, the variation of ATPase was hardly, if at all, explained by the variation of serum

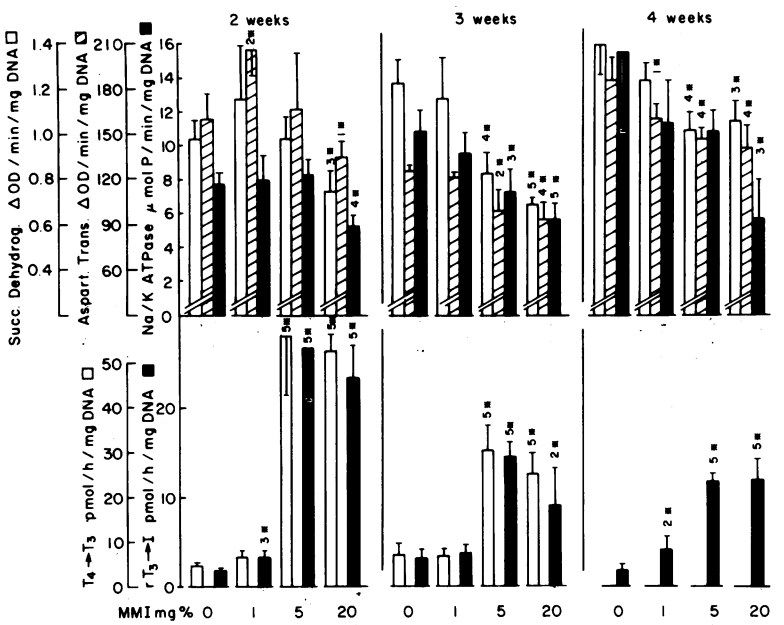

FIGURE 2 Effect of MMI-induced congenital hypothyroidism in various enzymes of the Cx of neonatal rats. Succ. Dehydrog., succinic dehydrogenase. These rats are the same as those whose serum values are shown in Table I, Exp. 1, and in Fig. 1. All enzyme activities were measured in crude homogenates as described in Methods. $T_4 \rightarrow T_3$ = T_4 to T_3 conversion as described in Methods. $rT_3 \rightarrow I$ = ^{125}I release from 5[^{125}I]rT_3 in the presence of 1 mM PTU as described in Methods. The brackets represent 1 SD. Statistical significance with respect to age-matched controls signaled by 1* ($P < 0.05$) to 5* ($P < 0.001$).

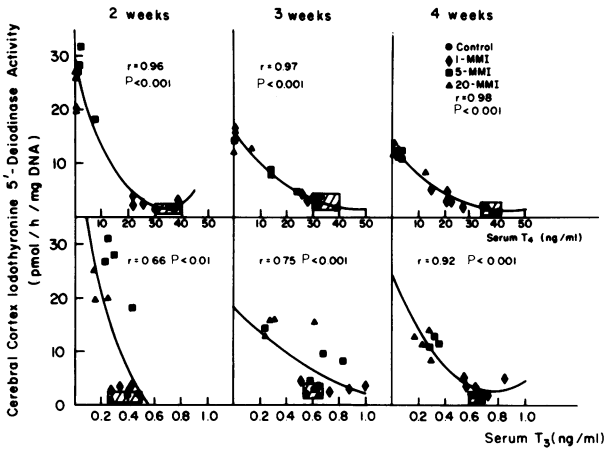

FIGURE 3 Relationship between I 5'D activity and serum T_4 and T_3 concentrations in neonatal rats with MMI-induced congenital hypothyroidism. Symbols and explanations as for Fig. 1. Correlation coefficients and regression lines correspond to a parabolic model $y = a + bx + cx^2$ as described in Results.

thyroid hormone levels. The most relevant aspects of these analyses are (*a*) the correlation coefficients are lower than those observed for I 5'D, indicating that other variables are responsible for a significant amount of the enzyme variability; (*b*) at every time point the correlation coefficient was higher for serum T_4 than for serum T_3; and (*c*) a completely different pattern was apparent at 2 wk when compared with the observations at 3 and 4 wk. At 2 wk, normal or even elevated enzyme levels were observed with subnormal T_4 levels (as low as 2.5 ng/ml), regardless of the serum T_3 concentration, although at this age it was normal in one-third of the MMI-treated pups. At 3 wk, and especially 4 wk, normal values of AT or S.D. were infrequent among the MMI-treated pups. In pups of these ages, a normal serum T_4 was a necessary, but not sufficient, condition for normal AT and S.D. activities, but normal values were observed independently of the serum T_3 level.

Comparison of T_4 to T_3 conversion activity response to hypothyroidism between Cx and liver (Fig. 5). This experiment was done in 17-d-old rats. Mean serum T_4 and T_3 concentrations are shown in Table I, Exp. 2. Although there was a very high inverse correlation between the I 5'D activity in the Cx and serum T_4, comparable to that shown in Fig. 2, in the liver the correlation was in the opposite direction and not as high. In liver, normal I 5'D activities were observed in the 2- and 5-mg-MMI group in spite of a reduced serum T_4. These rats (diamonds and squares identified with numbers in Fig. 5) often had elevated levels of serum T_3, whereas those with decreased enzyme activity had both a low serum T_4 and T_3. Consistent with the data in Fig. 2, a reduced serum T_4 was generally associated with an elevated I 5'D in the cortex. The regression line for T_3 approaches the normal range of I 5'D when the serum T_3 is well over the control range, i.e., between 0.6 and 0.8 ng/ml.

In vivo generation of T_3 from T_4 in MMI-treated neonatal rats (Tables III and IV). The data shown above suggest that the neonatal rat Cx has a high capacity to respond to hypothyroid levels of thyroid hormone by increasing I 5'D activity. This response could normalize the other cerebral enzymes in the face of markedly reduced serum T_4 levels. The experiments shown in Tables III and IV were intended to determine whether the elevated I 5'D activity found in vitro was a reflection of the in vivo capacity of Cx to generate T_3 from T_4. These experiments were performed in 2-wk-old pups, because at that time the response is maximal.

In the experiment shown in Table III, 600 ng of [^{131}I]T_4/100 g bw, approximately one-third of the daily production rate of T_4 for 2-wk-old rats (15), was given to euthyroid and 20-mg-MMI pups 16 h before death. IOP, an inhibitor of I 5'D, was also given to some pups to block in vivo T_3 formation. The cortex:serum ratio of [^{131}I]T_4 was greater in the euthyroid than in the 20-mg-MMI pups ($P < 0.001$), and in both it was significantly increased by IOP to similar values ($P < 0.001$). The cortex:serum ratio for [^{131}I]T_3 was increased threefold in 20-mg-MMI pups relative to controls ($P < 0.001$), and this difference was abolished by IOP. Because of these differences, the T_3:T_4 ratio in hypothyroid cortex was 10-fold that in euthyroid rats. This difference in T_3:T_4 ratio was also abolished by IOP. Using the specific activity of the injected T_4, the concentrations of T_4 and T_3 derived from the T_4 dose were calculated. The tissue T_4 concentration was threefold greater in the euthyroid controls than in the 20-mg-MMI group ($P < 0.001$), and in both groups IOP treatment increased the concentration of T_4 severalfold ($P < 0.001$). Despite the reduced concentration of T_4 in the cortex of the hypothyroid pups, the concentration of T_3 derived from T_4 [T_3 (T_4)] was 3.3-fold higher in the 20-mg-MMI rats than in the controls ($P < 0.001$). This concentration of T_3 is about three times the reported T_3 concentration of 1.2 ng/g in adult euthyroid rat cerebral cortex (35). At the time of death, the serum concentration of T_4 derived from the dose was 21.4±1.2 ng/ml in the control group and 17±1.9 ng/ml in the MMI-treated rats ($P < 0.001$). However, these figures may be ~25% overestimated since they are based on serum TCA-precipitable-^{131}I (see below).

In a second experiment we evaluated whether the T_3 acutely generated from T_4 would have a biological effect. IOP was used for the same purpose as in the previous experiment. Because in that experiment, the

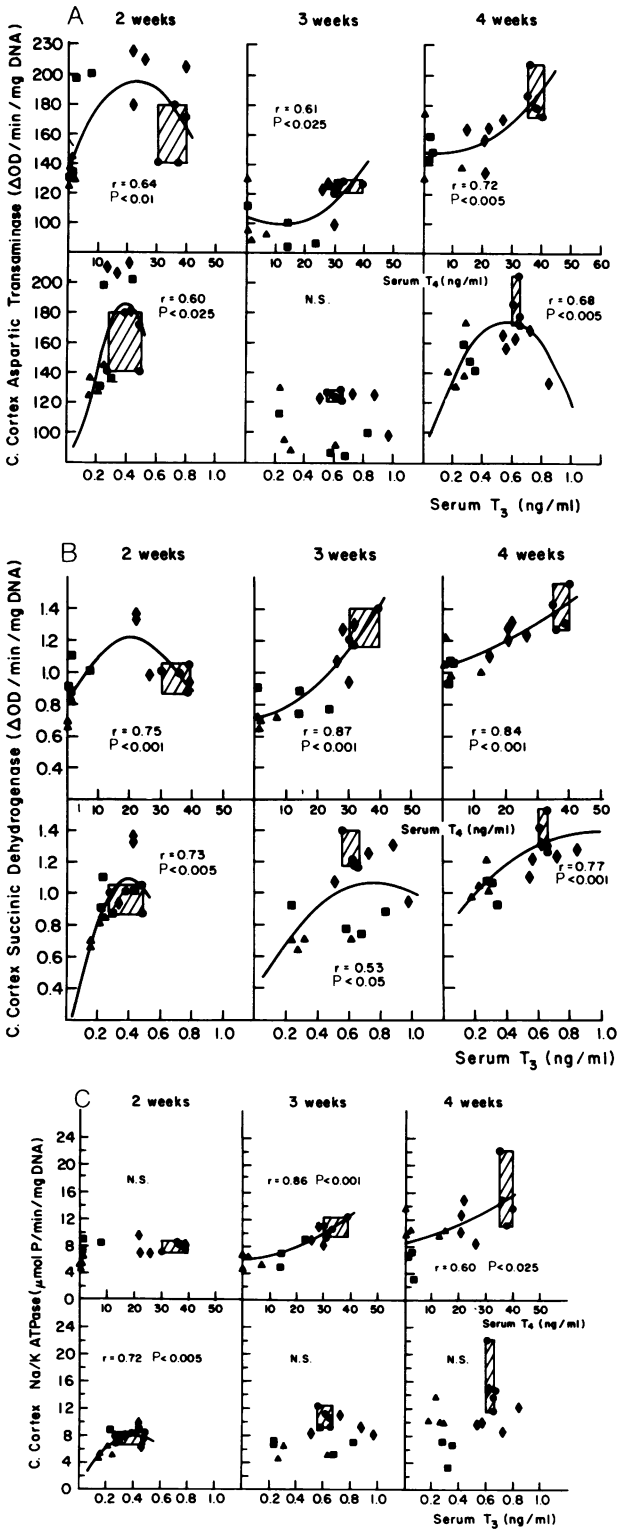

FIGURE 4 A–C. Relationship between Cx AT (A), S.D. (B), Na/K dependent ATPase (C), and serum T_4 and T_3 concentrations in neonatal rats with MMI-induced congenital hypothyroidism. All three enzyme activities were measured in crude homogenates as described. Symbols and explanations are as for Fig. 1. Correlation coefficients and regression lines correspond to a parabolic model, as for Fig. 3. C. cortex, cerebral cortex.

amount of T_3 generated was severalfold the estimated adult concentration and in the IOP-injected pups a significant increase in cortex T_4 concentration occurred, we reduced the T_4 dose to 60 ng/pup, (150–300 ng/100 g bw), one-twelfth to one-sixth of the normal daily T_4 production rate for this age. The time interval after the T_4 injection was also increased to 24 h to allow expression of biological effects. Two 10-mg/100 g bw doses of IOP were injected 16 h before and just preceding the [^{125}I]T_4. Approximately 16 h before killing the pups, [^{131}I]T_3 (5 ng) was injected subcutaneously to estimate the plasma [^{125}I]T_3 contribution to tissue [^{125}I]T_3 and the influence that differences in T_3 distribution and degradation might have had on the observed levels of [^{125}I]T_3.

The results of this experiment are shown in Tables IV and V. At 24 h only a minimal increase in serum T_4 of 2.6–2.7 ng/ml was observed in both euthyroid and MMI-treated rats. The increment in T_4 in the Cx of euthyroid rats was 0.34 ng/g, ~30% of the normal endogenous T_4 content (35, 36), but in the hypothyroid pups this increment was only 0.06 ng/g. This, and the large increments in cortex T_4 induced by IOP, indicate that the lower cortex:serum T_4 ratio in hypothyroid pups is due mainly to accelerated T_4 degradation in these animals. A similar phenomenon of less magnitude was observed in the liver. The T_3 derived from the T_4 in serum, cortex, and liver was significantly higher in hypothyroid animals and the difference exceeded that expected from the differences in the dose per unit weight. Due to differences in weight, the hypothyroid rats received a mean of 1.6 times more T_4 than the euthyroid pups, but the [^{125}I]T_3 was 3.3, 2.5, and 4.6 times higher in the serum, liver, and cortex of the MMI-treated rats than in the euthyroid controls.

The serum [^{131}I]T_3 in hypothyroid rats given T_4 was 0.23±0.03% of the dose per milliliter, whereas in the euthyroid controls it was 0.13±0.01% of the dose per milliliter ($P < 0.001$), suggesting a significant, albeit modest, decrease in T_3 clearance in the hypothyroid pups. If the 1.6-fold greater body weight of the control pups is taken into consideration, and assuming proportionality between body weight and volume of distribution, differences in fraction removal rate of T_3 do not turn out to be a major factor in the observed values of serum [^{131}I]T_3 in the T_4-treated hypothyroid pups. Furthermore, the cortex:serum ratio of [^{131}I]T_3 was not different in any of these groups, indicating that there was no "sequestration" of T_3 in the nervous

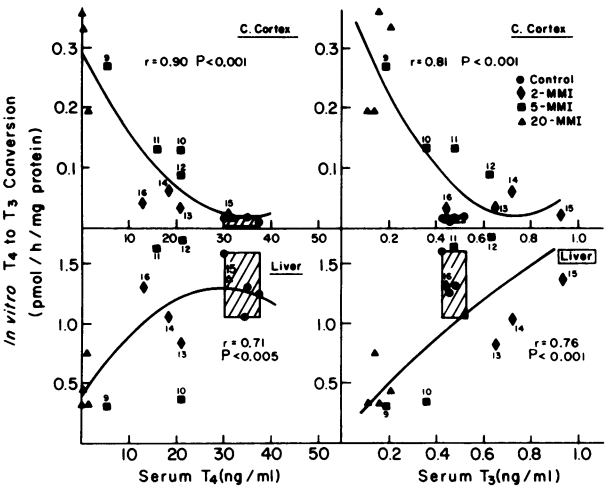

FIGURE 5 Relationship between Cx and liver I 5'D and the corresponding serum T_4 and T_3 concentrations in 2½-wk-old neonatal rats with MMI-induced congenital hypothyroidism. In both tissues enzyme activity was measured in crude homogenates as described in Methods. In both tissues, activity is expressed on a protein basis. Symbols and explanations are as for Fig. 1. Correlation coefficients and regression lines correspond to a parabolic model as for Fig. 3. The numbers by the symbols are used to identify each individual animal.

tissue. Using the injected [^{131}I]T_3, we estimated that, whereas 75% of the newly formed [^{125}I]T_3 in both euthyroid and hypothyroid cortex was generated locally, in the liver virtually 100% was accounted for by the plasma [^{125}I]T_3. IOP administration markedly reduced T_4 to T_3 conversion and also decreased the degradation rate of T_3 as judged by both tissue and serum concentrations of [^{131}I]T_3. In IOP-treated rats, all the [^{125}I]T_3 found in both cortex and liver was accounted for by the plasma T_3, indicating that cortex T_4 to T_3 conversion was virtually abolished by this drug.

The enzyme activities (Table V) were reduced in all hypothyroid animals compared with the controls ($P < 0.001$). AT was significantly higher in the 20-ng-MMI group treated with 60 ng of T_4 than in any of the other groups of hypothyroid controls. This was the only enzyme that showed a significant increase after T_4. This response has been reproduced in several other experiments. IOP blocked this effect of T_4 despite the marked increase in serum and tissue T_4 that occurred in these animals (Table IV). This was not a nonspecific effect of IOP because it did not affect the level of the enzyme in hypothyroid pups receiving only tracer [^{125}I]T_4 (3 ng). Liver α-GPD also failed to increase with this small dose of T_4, which may be accounted for by the minimal elevation of serum T_3 and the long latency of this enzyme to respond to thyroid hormone (37).

DISCUSSION

We have recently described the mechanism of the pituitary response to a failing thyroid gland (reviewed in 13). In essence, the decreased serum T_4 leads to pituitary hypothyroidism and the ensuing increase in TSH stimulates the thyroid to secrete T_3, keeping serum T_3 within normal limits until well advanced thyroid failure. The results of the present experiments show that this mechanism is indeed operative in the neonatal rat, because T_3 was maintained within normal limits in the face of markedly decreased (<10% of

TABLE III
Effect of Thyroid Status on Cx T_3 Concentration Generated 16 h after Injecting 600 ng T_4/100 g bw to 2-Wk-Old Hypothyroid Rats

			Treatment		Cx:Serum*			Iodothyronine in cortex§	
	Condition	n	T_4	IOP	T_4	$T_3(T_4)$	Cx T_3/T_4‡	T_4	T_3
					×10³			ng/mg DNA	
A	Eu	4	+	—	39±3	1.5±0.6	1.2±0.1	0.8±0.0	1.0±0.1
B	Eu	4	+	+	58±8	1.4±1.0	0.2±0.0	1.9±0.1	0.3±0.0
C	Hypo	4	+	—	17±5‖	4.3±0.3‖	13.0±3.8‖	0.3±0.1‖	3.3±0.1‖
D	Hypo	4	+	+	51±7	1.1±0.5	0.2±0.0	1.8±0.1	0.3±0.0

Data represent mean±SD.
Eu, euthyroid controls; Hypo, hypothyroidism induced by 20 mg/100 ml MMI in drinking water as described in Methods. IOP (10 mg/100 g bw) was injected intraperitoneally ~18 h before death, and [^{131}I]T_4 (600 ng/100 g bw) was injected intravenously 2 h later.
* Cx:Serum, cortex to serum ratio of [^{131}I]T_4 or [^{131}I]T_3 (T_4) expressed as percentage dose per milligram DNA divided by percentage dose per milliliter serum.
‡ Cx T_3/T_4, [^{131}I]T_3 counts per minute [^{131}I]T_4 counts per minute in paper chromatography of butanol extracts of Cx.
§ Calculated from the specific activity of the injected [^{131}I]T_4. To calculate the mass of T_3, the tissue radioactivity was multiplied as well by (2 × [651/777]) because of changes in specific activity and in molecular weight in the process of [^{131}I]T_4 to [^{131}I]T_3 conversion.
‖ $P < 0.001$ with respect to A. For IOP, all P values < 0.001 with respect to A (Eu) or C (Hypo).

TABLE IV

Effect of Thyroid Status on Serum, Cx, and Liver Thyroid Hormone Concentrations 24 h after an Intravenous Injection of 60 ng T_4 to 2-Wk-Old Rats

Treatment	Group		
	Euthyroid	Hypothyroid	
	A	B	C
	mean±SD of n = 4/group		
[^{125}I]T_4 (*ng/100 g bw*)*	159±7	263±27	286±11
[^{131}I]T_3 (*ng/100 g bw*)	13±1	22±2	24±1
IOP (*mg/100 g bw*)	—	—	10 × 2
Serum T_4 (*ng/ml*)	2.6±0.5	2.7±0.3	13.0±2.5‖
Serum $T_3(T_4)$ (*ng/ml*)	0.03±0.00	0.10±0.01‡	0.02±0.01‖
Serum [^{131}I]T_3 (*% dose/ml*)	0.13±0.01	0.23±0.03‡	1.80±0.47‖
Cx increment (*ng/g*)			
T_4	0.34±0.06	0.06±0.01‡	1.02±0.21‡‖
Total $T_3(T_4)$	0.38±0.06	1.76±0.28‡	0.02±0.01‡‖
Local $T_3(T_4)$	0.28±0.06	1.29±0.21‡	0.00±0.00‡‖
Liver increment (*ng/g*)			
T_4	1.47±0.19	0.95±0.05¶	4.81±1.21‖
Total $T_3(T_4)$	0.14±0.01	0.35±0.04‡	0.10±0.06‖
Total $T_3(T_4)$	0.00±0.00	0.00±0.03	0.04±0.06
Tissue/serum [^{131}I]T_3 (*ng/g÷ng/ml*)			
Cortex	3.73±0.72	4.56±0.95	2.37±1.05
Liver	11.33±1.41	7.96±0.97§	4.76±0.96‡

* The weight of the animals is given in Table V. IOP, 10 mg/100 g bw, was injected intraperitoneally 40 and 24 h before death. [^{125}I]T_4 was injected intravenously 24 h and [^{131}I]T_3 subcutaneously 16 h before killing the animals. [^{131}I]T_3 was used to estimate the local production of [^{125}I]T_3 by subtracting ([tissue/serum [^{131}I]T_3] × serum [^{125}I]T_3) from the total observed tissue [^{125}I]T_3. The gravimetric increments of T_4 and T_3 derived from the T_4 dose were calculated as described in Table III.

§ $P < 0.01$ with respect to A.

¶ $P < 0.005$ with respect to A.

‡ $P < 0.001$ with respect to A.

‖ $P < 0.001$ with respect to B.

normals) serum T_4 (Tables I and II, Fig. 1). Because of the elevation of TSH and because the Cx, as the anterior pituitary, derives most of the T_3 from local T_4 to T_3 conversion, one would expect that the Cx of pups with low serum T_4—in spite of the normal serum T_3—would show evidence of hypothyroidism. However, the present findings suggest that there are other mechanisms operative at cellular levels that can compensate for significant reductions in T_4 supply in the neonatal rat with partial thyroid impairment.

MMI-induced congenital hypothyroidism. The addition of graded concentrations of MMI to the drinking water provides a model that induces graded hypothyroidism with a wide spectrum of T_4 concentrations suitable for our purpose. In addition, this model better reflects the situation most commonly seen in humans in that athyreosis is a less common cause of congenital hypothyroidism than is dysgenesis or ectopically located and poorly functioning thyroid tissue (38, 39). Because of the residual thyroid tissue in these infants, serum T_4 is variably reduced and often serum T_3 is normal. In another common cause of congenital thyroid impairment, iodine deficiency, normal cord serum T_3, and low T_4 are common findings (40, 41). Therefore, the experimental model used in the present studies has distinct parallels to human disease.

The possibility that MMI, by a mechanism other than the well known antithyroid effect, may account for our findings is remote. In Methods we have presented evidence that the drug does not affect the enzyme assays nor can it reduce the enzyme levels in surgically thyroidectomized rats. The better correlation of our results with serum thyroid hormone levels than with the dose of MMI and the effect of T_4 treat-

TABLE V

Effect of 60 ng of T_4 on Various Cerebral Cortex Enzymes and Hepatic α-GPD in 2-Wk-Old Hypothyroid Rats

		Group				
		Euthyroid	Hypothyroid			
	Treatment	A	B	C	D	E
		mean±SD; n = 4/group				
	[^{125}I]T_4 (*ng*)	60	60	60	3	3
	[^{131}I]T_3 (*ng*)	5	5	5	5	5
	IOP	—	—	+	—	+
Cx						
AT		130±10	88±6*	75±6	74±7	77±7
S.D.		13.6±1.0	8.9±1.7	9.4±0.5	8.7±0.7	8.7±0.8
Na/K ATPase		6.3±0.4	3.9±0.3	3.9±0.4	3.4±0.2	3.6±0.4
Liver						
α-GPD (×10^3)		33±4	14±4	16±1	12±2	14±2
Body weight (*g*)						
Basal		33±2	23±2	21±1	19±2	22±1
After		41±2	26±2	21±2‡	21±4	20±2‡
	P (paired t)	<0.025	<0.001	NS	NS	<0.025

Groups A, B, and C are the same as shown in Table IV. All groups belong to the same experiment. The timing of T_3, T_4, and IOP are as described for Table IV.

When a *P* value is not indicated, it is either because it is NS or because it is not relevant. All hypothyroid *P* values were <0.001 with respect to A.

* $P < 0.05$ with respect to C, D and E.

‡ $P < 0.025$ with respect to B.

AT, S.D., and α-GPD are expressed as optical density units per minute per milligram DNA (cortex), or per milligram of mitochondrial protein (liver). Na/K-dependent ATPase is expressed as micromoles P per minute per milligram DNA.

ment on the enzymes (Table V) in MMI-treated pups point in the same direction. In addition, the levels of all three enzymes have been shown to be reduced to an even greater extent in ^{131}I-induced neonatal hypothyroidism (4, 5). Although the thyroid hormone plasma levels were not given in those reports one would assume that ^{131}I can induce a more severe form of hypothyroidism. At the same time, those results (4, 5) indicate that qualitatively similar data are obtainable in hypothyroidism induced by other means.

Effect of the various MMI doses on cerebral enzymes. In spite of the reduced levels of serum T_4 in virtually all MMI-treated pups, at 2 wk only the 20-mg-MMI group showed biochemical evidence of hypothyroidism in the cortex with decreased AT, S.D., and ATPase (Fig. 2), whereas when the animals increased in age, and also in the duration of MMI exposure, modest tissue hypothyroidism was observed after exposure to lower quantities of MMI. In all these situations, the changes observed in these enzymes were modest, contrasting with the concomitant marked increases of the I 5'D in the MMI-treated animals. The 10-fold increase in I 5'D observed at 2 wk in the pups receiving the highest doses of MMI compared with the three- to fivefold increase in the pups on the same MMI dose later on (Figs. 2, 3), and the relationship between the levels of AT, S.D., and ATPase and serum T_4 and T_3 at different ages, all suggest that the increased I 5'D activity in response to hypothyroidism is of high adaptive value. Furthermore, one can speculate that the success of such a mechanism in maintaining cerebral cortical euthyroidism will presumably depend on at least two, though perhaps more, factors. The first is the quantity of tissue substrate (T_4) available and the second, the magnitude of the increase in I 5'D activity achievable. Thus, in the 2-wk-old rats, normal AT, S.D., and ATPase activities are seen in the 5-mg-MMI but the 20-mg-MMI animals show hypothyroid responses. Because the I 5'D is increased 10-fold in both, it would appear that inadequate quantities of T_4 are available in the latter. Although, on average, the serum T_4 was not statistically lower in 20-mg-MMI rats than in 5-mg-MMI at 2 wk (Table I), it tended to be higher in the latter (Fig. 1), and the experiments injecting low doses of T_4 indicate that minimal increments in serum T_4, within the experimental

measurement error, could give rise to significant amounts of T_3 in the cortex (see below). On the other hand, despite similar serum T_4 concentrations at 3 and 4 wk, the 5- and 20-mg-MMI groups have subnormal cortical enzyme levels. It would appear that the three- to fivefold increases in I 5'D characteristic of these ages are insufficient to maintain the euthyroid state. The capacity for I 5'D to increase during hypothyroidism may be a function of age, as shown in Figs. 2 and 3, and is suggested by the fact that there is a spontaneous two- to threefold increase in cerebral I 5'D beginning at 10 d in euthyroid pups (8).

Capacity of the Cx to generate significant amounts of T_3 from comparatively small doses of T_4. To support the hypothesis that the above mechanism could explain these results, it should be possible to demonstrate in vivo that the quantities of T_3 provided to the cortex via 5'-deiodination of small amounts of T_4 are quantitatively and physiologically significant. The results shown in Tables III–V demonstrate that this is the case. Thus, Table III shows that after injection of a small dose of [^{131}I]T_4, the cortex:serum [^{131}I]T_3 ratio is threefold higher in hypothyroid rats than in euthyroid animals. The increased [^{131}I]T_3 in the cortex is not explained by increased concentrations of T_4 in the cortex of the hypothyroid rats, because both serum T_4 concentration and the cortex:serum ratio of T_4 are actually lower in hypothyroid rats than in euthyroid animals. These data suggest rapid T_4 to T_3 conversion in the hypothyroid animal. Furthermore, the elevated T_3, both in absolute terms and relative to the T_4 concentration seen in the hypothyroid neonate, is abolished by IOP. The results shown in Table IV add support to and expand the information provided by the above findings. In this experiment, the injected T_4 dose was only 150–300 ng/100 g bw, and at 24 h this was associated with an insignificant increase in the serum T_4. The concomitant injection in these pups of [^{131}I]T_3 allowed estimates of the fraction of the tissue T_3 increments that could be attributed to local T_4 to T_3 conversion using the approach that we have previously described (6, 12). Also, we could determine how much of the observed differences in tissue [^{125}I]T_3 (T_4) can be accounted for by changes in T_3 distribution and degradation. As would be expected from our studies in adult rats (17, 42), most of the [^{125}I]T_3 in the Cx could be attributed to T_4 and T_3 conversion occurring within the Cx in euthyroid pups. In both euthyroid and hypothyroid pups, 75% of the newly formed T_3 (T_4) found at 24 h in the Cx was produced locally. This may be disturbing at the first glance because the 10-fold increase in I 5'D would allow one to expect a greater relative contribution of the local source. We interpret the finding to be purely coincidental. Thus, these figures have been obtained 24 h after an intravenous pulse of T_4, and in Table IV it is apparent that the concentration of T_4 has decreased markedly at that time in the cortex of these hypothyroid pups, i.e., 0.06±0.01 ng/g compared with 0.34±0.06 ng/g in the controls. On the other hand, because the serum T_3 (T_4) was higher in hypothyroid pups by roughly the same factor as the total Cx T_3 (T_4) was, and because the tissue:plasma ratio of [^{131}I]T_3 was not different, the serum contribution was higher in hypothyroid pups than in the controls by the same factor as the tissue T_3 (T_4) was elevated. The relative contribution of local as compared with the serum source to cortex T_3 found under these conditions does not represent the steady-state conditions where endogenous concentrations of T_4 and T_3 must be taken into consideration.

The higher serum T_3 (T_4) in hypothyroid pups may be due to two factors. One is decreased T_3 degradation, as suggested by the higher concentration of [^{131}I]T_3, 0.23±0.01% of the dose per milliliter in hypothyroid pups as opposed to 0.13±0.01% in controls. However, because the hypothyroid animals were smaller by approximately the same factor, the difference in serum concentration is not readily accounted for by decreased fractional T_3 degradation. Furthermore, assuming that the difference is totally due to reduced volume of distribution, the overall rate of serum T_4 to T_3 conversion is at least the same in hypothyroid neonatal rats as in euthyroid pups. At any rate, regardless of the factor(s) responsible for the higher serum T_3 (T_4) in the hypothyroid neonates, the cortex:serum ratio of [^{131}I]T_3 was not affected by the thyroid status, indicating that differences in T_3 clearance are equally reflected in both compartments.

Comparison between liver and cortex I 5'D and T_3 generation. In this context, it is interesting that the liver I 5'D showed a change in opposite direction to the cortex with hypothyroidism (Fig. 5). In agreement with this in vitro finding, the increased concentration of newly formed T_3 (T_4) found in this tissue in MMI-treated pups is accounted for by the observed higher serum T_3 (T_4). Two important implications can be derived from these findings: (*a*) the liver is not as important a source of T_3 in neonatal hypothyroid rats as it is in the euthyroid adult rat or even the euthyroid neonate, and (*b*) the increased I 5'D observed in the cortex of the hypothyroid pups is physiologically relevant. Thus, in Table IV, had the cortex not undergone the observed increment in I 5'D, the elevation in serum T_3 (T_4) observed in the MMI-treated neonates would have resulted in an increment of only 0.75 ng T_3/g in the cortex, the sum of the serum contribution, 0.47 ng T_3/g, plus the local contribution of 0.28 ng T_3/g found in euthyroid rats. Instead, the actual increment, 1.76 ng T_3/g, was 2.3-fold greater.

In summary, in hypothyroid neonatal rats, mecha-

nisms other than the increase in TSH secretion and the ensuing augmented thyroidal T_3 secretion contribute to the maintenance of normal tissue T_3 concentrations. One of the mechanisms studied here in the cerebral cortex is a marked increase in fractional T_4 to T_3 conversion. This mechanism may be present in other tissues and even reflected in the serum because, in spite of the reduced liver I 5'D, there was at least an equal fraction of the T_4 converted to serum T_3 in hypothyroid pups when compared with their euthyroid counterpart. Lastly, this mechanism is more effective in some tissues such as Cx, where local T_4 to T_3 conversion is an important source of tissue T_3, than in liver, where it is not (6, 12, 13, 17).

An important question raised by these findings is the signal that stimulates the increase in I 5'D, because based on the above hypothesis, one woud assume that the cerebral cortical T_3 concentration is normal or near normal when this compensation has occurred. Fig. 3 demonstrates how closely related the activity of the I 5'D is to the concentration of T_4 in the serum and how poorly it relates to the level of serum T_3 despite the fact that there is an obvious relationship between concentrations of serum T_3 and T_4 in these MMI-treated pups (Fig. 1). The activity of the enzyme thus seems to be governed by the level of serum T_4 rather than by the T_3 generated from it. At present, we do not know how the enzyme is regulated by thyroid hormones, though other data from our laboratory indicate that this increase in activity is due to an increase in the maximum velocity (V_{max}) as opposed to the Michaelis constant (K_m) of the enzyme (10, 24). It is also not clear whether this V_{max} increase represents an activation of latent enzyme units or changes in the synthetic rate of the enzyme. It is possible, given the marked heterogeneity of cerebral cortical tissue, that cells responding to the change in T_4 with an increase in I 5'D are different from those that are reflected by the activities of AT, S.D., and ATPase. The possibility must also be raised that the enzyme is regulated by the level of T_4 directly, without the necessity for the generation of T_3 and the subsequent interaction of this T_3 with the nuclear receptor. Whatever the answer to the questions posed by these data, the findings suggest that the intracellular T_3 concentration is carefully regulated in the developing brain. It is most interesting that in the Cx the capacity to respond to hypothyroidism appears maximal during the time when the maturation rate is highest and the normal adaptive feedback regulation of the pituitary-thyroid axis is not completely developed (2, 3, 15).

In terms of the clinical implications of these findings, it would appear important to evaluate the neurological and psychological outcome of the congenitally hypothyroid infant in terms of the serum concentrations of T_4 rather than of T_3. That the laudable outcome of treatment of infants with congenital hypothyroidism discovered by screening is so much better than has been reported in the literature before the time of screening was begun (43) may be due to the fact that clinically detected congenital hypothyroidism is likely to be more severe than that discovered by the sensitive screening process. The data at 3 and 4 wk in these rats suggest that this compensatory mechanism cannot protect the Cx indefinitely. This is consistent with the fact that earlier intervention with thyroid hormone is consistently more successful in preventing irreversible mental retardation in the congenitally hypothyroid infant (44).

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