Developmental Changes in Rat Brain 5'-Deiodinase and Thyroid Hormones during the Fetal Period: The Effects of Fetal Hypothyroidism and Maternal Thyroid Hormones[1]

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ABSTRACT. We have studied the ontogenesis of 5'-deiodinase (5'D) activity in rat brain during fetal life, its capacity to respond to maternal or fetal hypothyroidism, and its regulation by maternal thyroid hormones. Type II 5'D (5' D-II) activity increases 4-fold during the period studied (17 to 22 days of gestation), mainly between days 19 and 21. Fetal brain T4 concentrations increase in parallel with fetal plasma T4, whereas fetal brain T3 concentrations increase 18 times (days 17–21), six times more than would have been expected from the small increase in fetal plasma T3 levels. Maternal thyroidectomy did not affect 5'D-II activity or thyroid hormone concentrations in fetal brain (except brain T4 at 18 days of gestation). Fetal hypothyroidism, induced by giving a goitrogen (methimazole) to the mothers, depleted all fetal tissues studied, including the fetal thyroid, from thyroid hormones. By 19 days of gestation, the fetal brain was able to respond to hypothyroidism with a 3- to 5-fold increase in 5'D-II activity. Earlier onset of treatment with methimazole led to 2- to 3-fold increases in 5'D already at 17 and 18 days of gestation, showing that when fetal thyroid secretion starts the fetal brain 5'D-II is able to respond to hypothyroidism. Replacement of methimazole-treated mothers with physiological doses of T4, given by constant infusion, increased T4 and T3 concentrations in fetal brain, and inhibited fetal, as well as maternal, brain 5'D-II activity. But treatment of the mothers with T3 did not change T3 concentrations in the fetal brain, despite the increase in fetal plasma T3, and actually increased 5'D-II in fetal brain. Maternal cerebral 5'D-II was not inhibited by T3 treatment. Inverse relationships were found between the 5'D-II and thyroid hormone concentrations in the fetal brain. These correlations were not identical for fetuses from thyroidectomized and control mothers. In fetuses from thyroidectomized dams, brain 5'D-II is more sensitive to a decrease in brain T4 than in the progeny of control

dams. The present results describe the developmental changes in rat cerebral 5'D-II activity and its regulation by thyroid hormones. Although fetal plasma T3 is 10% of adult levels, T3 concentrations in fetal brain increase almost to adult levels, suggesting an important role of local T3 production from T4, and thus, of 5'D-II in fetal brain. In addition, brain 5'D-II responds to thyroid hormone deficiency and can be modulated by maternal thyroid hormones when the fetus is hypothyroid. (*Pediatr Res* 24: 588–594, 1988)

Abbreviations

5'D, 5'-deiodinase
5'D-II, type II-5'deiodinase
MMI, methimazol
PTU, propylthiouracil
C, control
T, Thyroidectomy, thyroidectomized
T4, thyroxine, tetraiodo-thyronine
T3, 3', 3, 5 -triiodo-thyronine
rT3, reverse T3
3', 5', 3, triiodo-thyronine
RIA, radioimmunoassay
DTT, dithiothreitol
BW, body weight
dg, days of gestation
P, protein
TSH, thyroid-stimulating hormone

Thyroid hormones are known to play an important role in brain maturation. Their absence during development leads to irreversible brain damage (1–3). The defects resulting from thyroid hormone deficiency in the developing brain are well described both for congenital hypothyroidism and endemic cretinism (4–6), but the biochemical mechanisms involved are not yet fully defined (7, 8), nor the timing at which the developing brain is more sensitive to the absence of thyroid hormones. Nuclear receptors for T3 have been described in the adult and fetal brain (9–12) but the postreceptor events are also still poorly understood.

Most nuclear and intracellular T3 of the adult and neonatal brain is locally produced from T4, by 5'D (13–15). The deiodination process, different from the one described in liver and kidney, has been identified in its kinetics characteristics: low Km

(2 nM), with preference for T4 as substrate and insensitive to inhibition by PTU. Its physiologic regulation has extensively studied (16–19): it increases in hypothyroidism and is regulated by thyroid hormones (20–22). Thyroid hormone concentrations in brain are maintained by several mechanisms, which include selective uptake and deiodinative processes (23, 24).

In previous studies (25, 26) we have reported that thyroid hormones are present in the fetal brain (days 20 and 21 of gestation), in greater amounts (about 50% of adult levels) than was previously thought, despite low plasma T3 levels. In contrast with other fetal tissues, maternal thyroidectomy had little effect on fetal brain T4 and T3 concentrations (25), suggesting that mechanisms for the maintainance of cerebral T3 homeostasis are already present at this stage of development.

The aims of the present work were to study: *1*) the development of 5'D-II in the fetal brain, *2*) the possible contribution of this enzyme to the changes in fetal brain T3 concentrations, *3*) the capacity of 5'D-II to respond to fetal or maternal hypothyroidism, and *4*) its possible modulation by maternal thyroid hormones when the fetus is hypothyroid.

MATERIALS AND METHODS

Experimental design. Female Wistar rats were used. They were fed, housed, and T as previously described (25). Two months after T, or later, rats with undetectable plasma T4 were mated (T dams) with normal males. Intact age-paired females were also mated (C dams). The day of appearance of the vaginal plug was considered as day 0 of fetal age (25).

Some of the C and T dams were given 0.02% MMI as drinking water, starting at 14 dg (CMMI or TMMI), except for a single experiment, when pregnant rats were given MMI from 12 dg. Some of the MMI-treated dams were implanted at 15 dg with osmotic minipumps delivering T4 (1.8 μg T4/100 g BW/day) or T3 (0.45 μg T3/100 g BW/day) at a constant rate, as detailed elsewhere (26).

C, CMMI, T and TMMI dams were killed, bled, and perfused as described (25), except that 6-propyl-2-thiouracil (PTU) was excluded from the perfusion media to avoid interference with the measurement of 5'D. Fetuses were obtained at different ages between 17 and 22 dg. Fetuses from MMI-treated C or T dams infused with T4 or T3, were only studied at 21 dg.

Plasma, liver, and brain were obtained from the mothers. Fetuses were dissected out from the uterus, bled (from 18 dg onward), separated from the placenta, placed on ice, and weighed. The brain, thyroid, as well as other fetal tissues were dissected out, weighed, and quickly frozen on dry ice. Brain samples were used for the determination of 5'D activity and thyroid hormone concentrations.

Synthesis of radioactive rT3 or T4. High specific activity ^{125}I-rT3 or ^{125}I-T4 were synthesized as previously described (27), using 3',3-T2 or 3',3,5-T3 as substrates, respectively, and used for the deiodinase assay. ^{125}I-T3 and ^{131}I-T4 were obtained with the same method and used as tracers for individual recovery calculations during the preparation of plasma or tissue samples for the determination of T3 and T4 by RIA (25, 26).

Tracers used for the deiodinase assay were purified by descending chromatography (28), eluted with methanol-NH$_4$OH (9:1), evaporated to dryness, and kept in ethanol.

Determination of 5'D activity. Iodothyronine 5'D activity was assayed in fetal brain homogenates as previously described (17), measuring the release of [125]I$^-$ from ^{125}I-T4 or ^{125}I-rT3 (100,000 cpm/vial) during 1 h, in the presence of 2 nM T4 or rT3, respectively, 20 mM DTT and in the presence (5'D-II) or absence (5'D-total) of 1 mM PTU. When using T4 as substrate, 1 μM T3 was included in the reaction media, to prevent inner ring deiodination (5D). The Iodide was separated by ion-exchange chromatography on Dowex-50W-X2 columns equilibrated in 10% acetic acid (29). The protein content was determined using the method of Lowry *et al.* (30). The amount of protein used

was 100–150 μg P/vial. Results are expressed as fmols Iodide/h/mg P.

Production of Iodide was linear from 50 to 250 μg of protein/vial, in control and hypothyroid fetal brain, and up to 2 h of incubation at 37° C. Occasionally, the amounts of [125]Iodide and 3',3 T$_2$ (or T3, when using T4 as substrate) produced during the incubation were checked by descending chromatography using the system described by Bellabarba et al. (28), to assess the production of equal amounts of iodide and metabolites.

All the values shown are the mean of at least five to six determinations using duplicates, and the fetal samples of a given group were obtained from at least two different dams. Most experiments were repeated more than once to check for reproducibility.

Several series of homogenates were assayed using T4 as well as rT3 as substrates. As already described (see "Results" and "Discussion"), when using 2 nM rT3, 5'D-II constituted a lower percentage of total 5'D, than when using T4. Differences observed in 5'D-II (as % of total 5'D) when using rT3 or T4 were minimal in MMI-treated fetuses (5–10%), and somewhat higher in C fetuses (20–30% higher when using T4 as substrate).

Other determinations. Thyroid hormone concentrations were determined by RIA, as previously described (31). In brief, plasma and brain samples were extracted with methanol and purified using chloroform-methanol extraction, and back-extraction into an aqueous phase, followed by purification of this phase on BIO-RAD AG 1×2 resin columns, as described in detail elsewhere (25, 26). Thyroid hormone concentrations were determined in the purified extracts.

Statistical analysis. Mean values ± SEM are given. These values and the significance of difference between group means (Student's *t* test) were calculated as described by Snedecor and Cochran (32). For comparisons of mean values in experiments involving several experimental groups, one- or two-way analysis of variance was used after square root or logarithmic transformations to ensure homogeneity of variances (Bartlett's test), if not found with the raw data. When the variance ratio indicated the presence of statistically significant differences between mean values, these were identified by the protected least significant difference test (32).

RESULTS

Ontogenesis of 5'D activity in fetal brain. The development of 5'D activity was examined in fetal brain homogenates, using samples from 17 to 22 dg. This comprises a developmental period starting before onset of fetal thyroid secretion, at 17.5–18 dg, up to a few hours before birth.

In some assays, both rT3 and T4 were used as substrates of the deiodinative reaction. As already described (17, 24), the % of inhibition by PTU is higher using rT3 than T4, but the conclusions are the same using either substrate. Due to very low activities found on days 17 and 18 when using rT3, T4 was used as the preferred substrate for exploring 5'D-II activities.

When T4 was used as substrate, 5'D-II activities were around 80% of total 5'D activities, indicating that under our conditions most T4 5'-D activity is PTU insensitive (type II). Figure 1 (*upper panel*) shows a 6-fold increase in brain T4 5'D-II during the developmental period studied, the main increase taking place between 19 and 21 dg. This means that, in 2 days, the potential capacity of fetal brain to produce T3 increases three times. As will be explained below (Fig. 2), conclusions are similar using rT3 as substrate.

The changes in thyroid hormone concentrations occurring in the fetal brain and plasma are illustrated in the *lower* and *middle* panels of Figure 1 (note different scales for plasma and brain T4 and T3). As may be seen, brain and plasma T4 concentrations increased markedly between 18 and 21 dg, about 9- and 8-fold, respectively, in only 3 days. T4 concentrations in fetal brain doubled each day of gestation (18–21 dg). Brain T3 concentra-
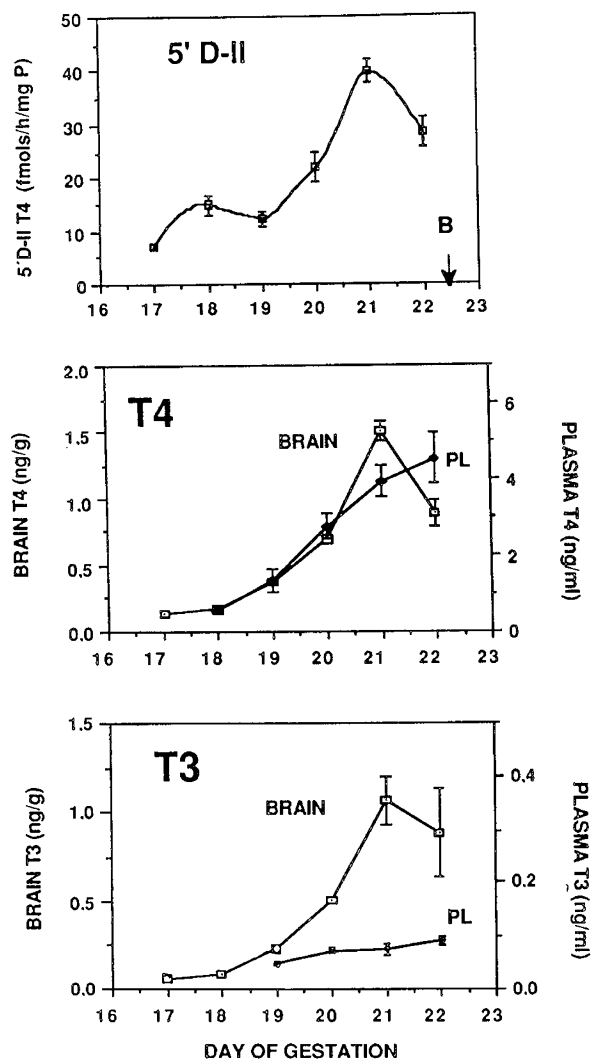
Fig. 1. The *upper panel* shows T4 5'D-II activity in fetal brain (17–22 dg). A total of 2 nM T4 was used as substrate. Results are expressed in fmols of Iodide/h/mg P (six fetal samples/group, with fetuses from two different dams in each group). Increases were statistically significant ($p < 0.05$) between days 17 and 18 and from 19 to 21 dg, with a decrease between 21 and 22 dg. B, indicates birth. The *middle panel* shows T4 concentrations in brain and plasma of fetuses from C mothers during the same period as specified above. No fetal plasma was available on day 17. Results are expressed as ng T4/g or ml. Increases are statistically significant from 18 to 21 dg, both for brain and plasma, with a decrease between 21 and 22 dg for brain T4. The *lower panel* shows T3 concentrations in brain and plasma of fetuses from C mothers. No plasma values were obtained at 17 and 18 dg. Results are expressed as ng T3/g or ml. Increases are statistically significant from day 18 for brain T3, and only between 19 and 22 dg for plasma T3.

tions increased even more: 18-fold increase from days 17 to 21, and about 12-fold between days 18 to 21, but there was only a relatively small increase (2-fold) in the plasma T3 levels during the same period.

Effects of maternal thyroidectomy on ontogenesis of 5'D. Fetal brain T4 and T3 concentrations are shown in Table 1, for fetuses from T and C dams. They were the same for both groups, except for lower T4 levels at 18 dg, and lower T3 levels at 21 dg in the brain of fetuses from T dams as compared with those from C dams. Determinations in fetal brains from T dams at 17 dg were not performed because of insufficient amount of tissue.

Maternal T affected the development of the fetuses as previously reported (25). It decreased the number of fetuses per dam
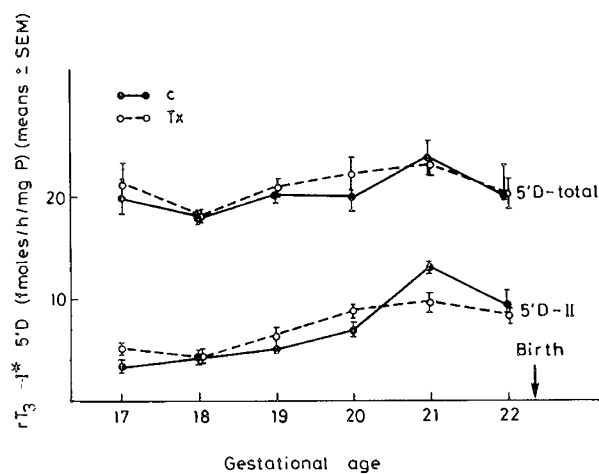

Fig. 2. Total and 5'D-II activity in fetal brain during gestation (days 17 to 22). Fetal brain from C and T mothers were used for comparison (*closed* and *open circles*, respectively). A total of 2 nM rT3 was used as substrate. No statistically significant difference was found between fetal brain from C and T dams, except in 5'D-II activities at 21 dg.

Table 1. *Thyroid hormone concentrations in fetal brain in fetuses from C and T dams (mean ± SEM)*

Day of gestation	T4 (pg/g)		T3 (pg/g)	
	C dams	T dams	C dams	T dams
17	124 ± 20		60 ± 14	
18	170 ± 25	94 ± 21*	85 ± 4	77 ± 7
19	364 ± 19	407 ± 135	231 ± 6	206 ± 10
20	688 ± 20	690 ± 10	505 ± 15	560 ± 10
21	1503 ± 75	1541 ± 61	1071 ± 134	813 ± 45*
22	880 ± 100	1232 ± 273	886 ± 251	821 ± 119

*$p < 0.05$ or less, with respect to their C group.

from $11 ± 0.6$ for C dams to $6.6 ± 0.2$ for T dams ($p < 0.005$). The BW and organ weights of fetuses from T mothers were lower, although in the case of the brain, the relative weight increased (25).

Figure 2 shows the developmental changes in total and type II 5'D in the brain of 17- to 22-day-old fetuses from C and T mothers (using rT3 as substrate). Total 5'D did not change during the period studied. On the contrary, and in agreement with the data shown in Figure 1 (using T4 as substrate), 5'D-II increased steadily from 17 dg, reaching peak values at 21 dg, with an overall 4-fold increase in the C group. The increase was statistically significant from day 19 onward in fetuses from C dams, and from day 20 in those from T dams. The relative proportion of 5'D-II over the total 5'-D activity also increased from 17% on day 17, to 55% on day 21.

No differences were found in total 5'D and 5' D-II activities between fetal brain from C and T dams, although in some assays small, but statistically significant differences were found, as the ones seen in Figure 2 at 21 dg. In one experiment, using 17- and 18-day-old fetuses, we have found that fetal brain 5'D-II was increased at 18 dg [$9.4 ± 0.82$ ($n = 9$) *versus* $3.9 ± 0.56$ fmol/h/mg P ($n = 12$) in fetuses from T and C mothers, respectively, $p < 0.001$]. T4 concentrations were lower in fetal brain at 18 dg (Table 1).

Effect of fetal hypothyroidism on fetal brain 5'D activity. A combination of fetal and maternal hypothyroidism was induced by treating C or T dams with MMI from 14 dg. Treatment of the mother with MMI resulted in undetectable concentrations of T4 and T3 in the fetal thyroids, and markedly reduced their concentration in all fetal tissues (26, 33). In the fetal brain, T4 and T3 were also reduced (25–40% of C values): approximately 0.1 ng/g on 18–19 dg, and less than 0.4 ng/g on 20–21 dg.

The effects of MMI treatment on fetal brain 5'D activity are shown in Figure 3. The *bars* represent total 5'D activity, whereas the hatched area represents the PTU-insensitive fraction, namely 5'D-II activity. No difference was observed in brain 5'D activity between fetuses from C or T mothers, either in the basal 5'D levels or in its response to MMI treatment. Treatment with MMI produced a 3- to 5-fold increase in 5'D-II activity between 19 and 21 dg. MMI caused a small, but statistically significant, increase at 17 dg, both in fetuses from C and from T dams, but not at 18 dg.

This observation raised the question if 5'D-II in fetal brain is able to respond to hypothyroidism at the early age of 17–18 dg, or if the lack of a clear response in 5'D-II was due to the fact that the degree of hypothyroidism achieved was not severe enough to give a clear increase. To further clarify this point, pregnant rats were given MMI at 12 dg. This was done because we had observed (Ruiz de Oña C, Obregón MJ, unpublished data) that a period of 5 days of maternal treatment with MMI is needed to get a clear-cut increase in fetal brain 5'D-II. Results are summarized in Figure 4. The enzymatic activity increased 2-fold at 17 dg, and 3-fold at 18 dg.

Effect of placental transfer of thyroid hormones. We have previously shown that infusion of physiologic doses of T4 to pregnant rats on MMI, results in placental transport of the hormone and increased levels of T4 and T3 in most fetal tissues studied, as compared to those found in fetuses from dams on MMI alone (26). We wanted to know if 5'D-II in fetal brain is sensitive to maternal transfer of T4 and we compared this transfer with that of T3. C and T dams, treated with MMI and infused with thyroid hormones were used.

The thyroidal status of the mothers were assessed by their plasma thyroid hormone and TSH concentrations. A complete study of the thyroidal state of the mothers is presented elsewhere (33). In brief, the doses of T4 and T3 infused into the MMI-treated C and T dams were not supraphysiologic, as their plasma T4, T3, and TSH were comparable to those found in C dams.

As already indicated, fetal T4 and T3 concentrations were markedly decreased by treatment of the dams with MMI. Maternal infusion of T4 increased T4 concentrations in fetal plasma and brain to 33–50% of C levels, as well as brain T3 (40–60% of C levels), but did not increase fetal plasma T3 (26, 33). Maternal infusion with T3 increased fetal plasma T3 to C levels, as well as T3 concentrations in many fetal tissues (33), but not in the brain. It actually decreased fetal brain T4 concentrations.

Figure 5 shows the responses of 5'D-II in the 21-day-old fetal brain from the eight groups of treated dams. The increase in enzymatic activity in response to MMI is partially reversed by treatment of the mother with T4, but not by maternal infusion with T3. In the T3-treated groups, 5'D-II activity in the fetal brains actually increased, in agreement with a decrease in brain T4. Similar responses were obtained using rT3 as substrate (see legend to Fig. 5). These results show that the hypothyroid fetal brain is able to respond to maternal thyroid hormone.

We have analyzed the relationship between brain thyroid hormone concentrations and 5'D-II activity in fetal brain, and results are presented in Figure 6. We found that the inverse relationship between brain T3 and 5'D-II activity (*lower panel*) is identical in fetuses from C and T dams, but the one found between fetal brain T4 and 5'D-II (*upper panel*) is different for fetuses from C and T mothers. Brain 5'D-II activity is more sensitive to small variations in brain T4 concentration in the fetuses from T mothers, and 5'D-II activity is already increased at T4 concentrations that are close to the normal range, *e.g.* 900 pg T4/g, whereas lower T4 concentrations have to be reached in the brain of fetuses from C dams to observe a 5'D-II response of comparable intensity. These results suggest different set-points
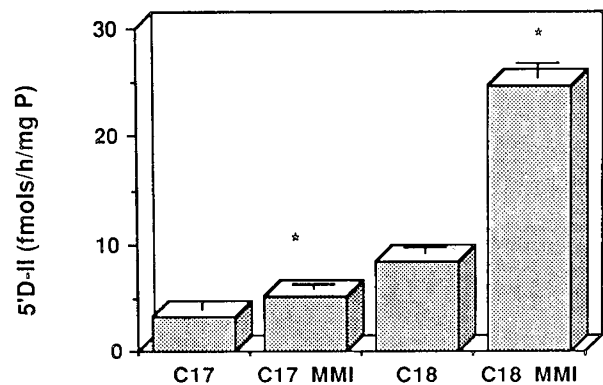

Fig. 4. Effect of MMI on 5'D-II activity from 17- and 18 days-old fetal brain. T4 was used as substrate. In this experiment C mothers were treated with MMI from day 12 of gestation. *Asterisks* represent statistically significant differences between C- and CMMI-treated fetuses at the level of $p < 0.05$ or less. Results are the means obtained in two different experiments ($n = 12$/group).
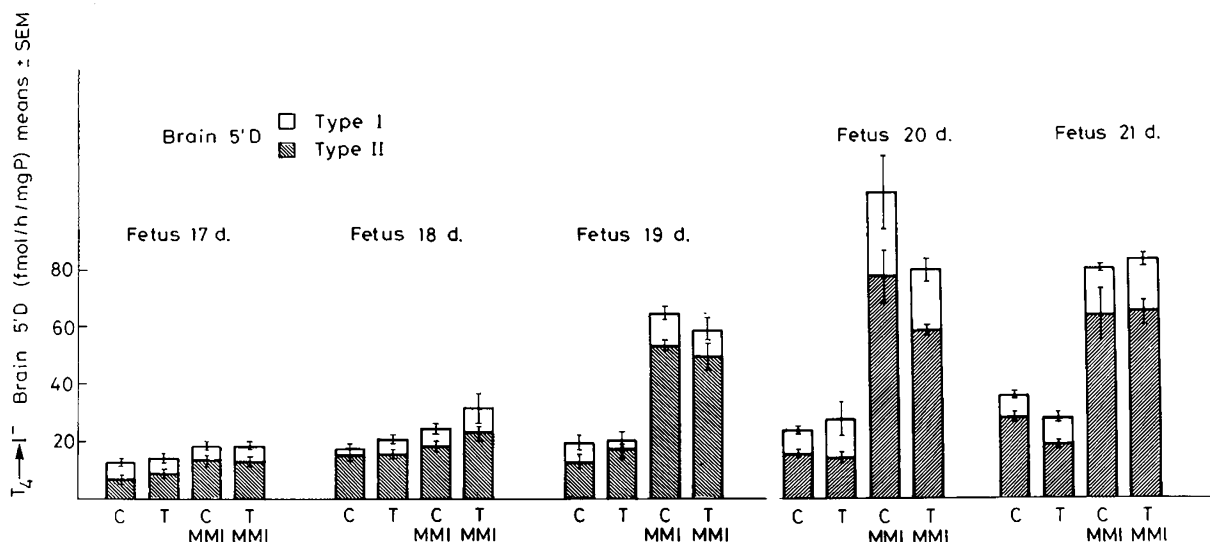

Fig. 3. Effects of MMI treatment on T4 5'D-11 activity in fetal brain from C and T mothers, from days 17 to 21 of gestation, using T4 as substrate. Total *bars* represent total 5'D activity, and the *hatched bars* represent 5'D-II activity. The increases in brain 5'D-II activity due to MMI treatment were always statistically significant for C and T groups, except in 18-day-old fetuses. No differences were found between C and T groups, either in basal or stimulated 5'D-II.
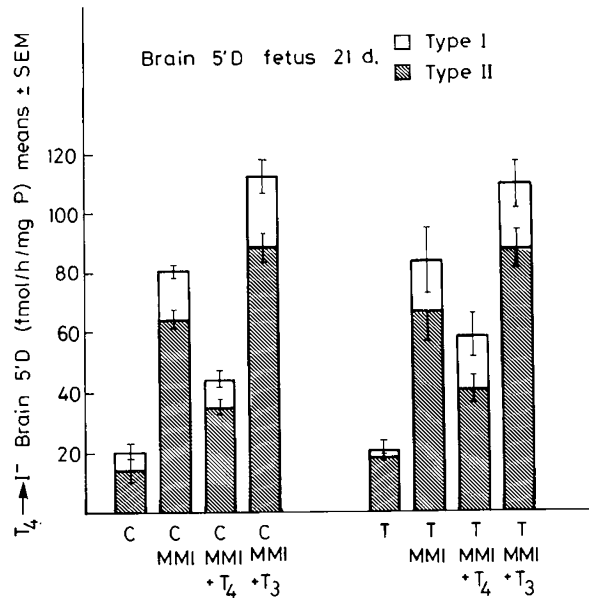
Fig. 5. Effect of maternal transfer of T4 or T3 on 5'D-II activity in hypothyroid fetal brains. Brains from 21-day-old fetuses were used. *Bars* have the same meaning as in Figure 3. All differences between the C (or T) groups were statistically significant. No statistically significant difference was found between the C and T groups, *e.g.* CMMI *versus* TMMI. Results using 2 nM rT3 as substrate were the same, although the inhibition by PTU was 10–20% higher.

Table 2. *T4 5'D-II in maternal brain from C or T pregnant rats, under MMI treatment, infused with T4 or T3 (mean ± SEM)**

Treatment of the dams	*n*	5'D-II
C	4	14.5 ± 5.1
CMMI	5	29.4 ± 7.4†
CMMI + T4	3	3.9 ± 0.9†‡
CMMI + T3	4	41.9 ± 5.7†§
T	5	96.5 ± 4.3
TMMI	3	64.9 ± 1.6†
TMMI + T4	5	5.2 ± 1.5†‡
TMMI + T3	4	57.6 ± 6.2†§

* C and T dams were given 0.02% MMI as drinking water, starting at 14 dg (CMMI or TMMI). Some of the MMI-treated dams were implanted at 15 dg with osmotic minipumps delivering T4 (1.8 μg T4/100 g BW/day) or T3 (0.45 μg T3/100 g BW/day).

† Difference with respect to data from the respective C or T group was significant, at the 5% level or less.

‡ Difference with respect to data from the respective CMMI or TMMI group was significant, at the 5% level or less.

§ The same as † and ‡ but with respect to MMI + T4 groups.

of regulation of 5'D-II deiodinase activity in the brain of fetuses from C and T mothers.

5'D-II was also analyzed in cerebral cortex from C and T mothers treated with MMI, and infused with T4 or T3, and the activities found are presented in Table 2. Treatment with T4 inhibited maternal cerebral 5'D-II, whereas treatment with T3 did not affect or suppress 5'D-II activities in the maternal brain.

DISCUSSION

The present study tries to identify mechanisms that regulate the availability of thyroid hormones to the fetal brain during a period in which it is undergoing active development, with an especially rapid neuroblast multiplication. We have focused our attention on the 5'D mechanisms that contribute to T3 homeostasis in the fetal brain, as studies on T4 or T3 uptake by the brain during the fetal period are technically difficult, or might involve T4 metabolization during placental transfer.

Ontogenic development of 5'D. We describe the ontogenic pattern of fetal brain 5'D-II activity. A previous report using rT3 as substrate (34) found very low 5'D-II activity in 17-day-old fetal brain, with a 3-fold increase on the day of birth. Intermediate ages were not reported. Present results are in agreement with such findings.

The different profiles found for total 5'D activity when using T4 or rT3 as substrates confirms (17) that different potentialities are explored with each iodothyronine. They also show the specificity and preference of the enzyme for T4 as substrate, and that only the PTU-insensitive fraction of 5'D (type II) activity increases. Using rT3 as substrate, greater inhibition by PTU was observed than using T4, as described for adult brain (17, 24). We have some preliminary kinetic data (C. Ruiz de Oña, M. J. Obregon, F. E. del Ray, and G. Morreale de Escobar, unpublished results) comparing adult and fetal brain, using rT3 and T4 as substrates. For total 5'D, T4 V_{max} and T4 V_{max}/Km are higher in fetal than in adult brain, whereas when using rT3, the opposite occurs as both parameters are lower in fetal than in adult brain. When exploring 5'D-II, V_{max}/Km are similar when using either rT3 or T4 as substrate.

We have found a continuous increase in fetal brain 5'D-II activity during the period of fetal development comprising onset of active fetal thyroid secretion. The increase was found using
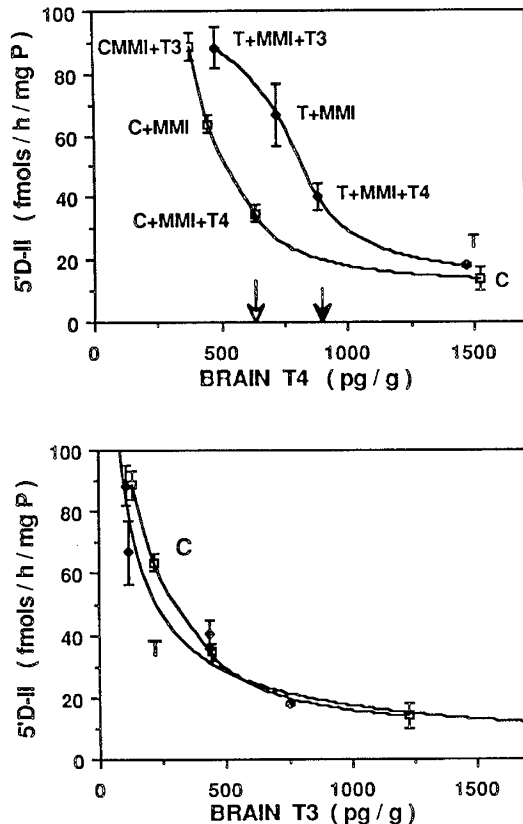

Fig. 6. Inverse relationships between thyroid hormone concentrations and 5'D-II activity in fetal brain, from C and T dams. The *arrows* show brain T4 concentrations at which a significant increase in 5'D-II activity is found in fetuses from C and T dams.

either rT3 or T4 as substrate. This increase suggests an increasing demand for local T3 production. This conclusion is supported by several findings reported here. The concentrations of T4 in the brain increase between 17 and 21 dg at a somewhat greater rate than plasma T4 levels. This points to plasma T4 as the main source of cerebral T4. In contrast, cerebral T3 increases 6 times more than plasma T3, clearly indicating that plasma T3 is not the main source of cerebral T3. Moreover, infusion of T3 into MMI-treated dams increased fetal plasma T3, and the T3 concentration in many fetal tissues, but did not increase cerebral T3 (33). This also clearly supports the conclusion that 5'D-II is essential for T3 homeostasis in the fetal brain, and that the fetal brain depends on local generation of T3, not on the uptake of plasma T3.

It is likely that during the period studied, the uptake of T4 by the brain might be increasing, and that this change also plays a role in determining the cerebral availability for T4, and ultimately, the generation of cerebral T3. As already indicated, brain T4 levels increased at a somewhat greater rate than plasma T4. But, considering the increasing activity of 5'D-II, leading to increasing concentrations of T3 in the brain, it is possible that brain to plasma T4 ratios would lead to underestimation of the actual increase of T4 uptake by the brain. Actually, brain/plasma T4 ratios in the fetal brain (0.24) are four times higher than the ratio found in the adult brain (0.06) (31). There is no evidence suggesting changes in the cerebral uptake of T3, which does not appear to contribute much to cerebral T3, at least as far as may be assessed from the data obtained at 21 dg. At present there is little information regarding a possible role of decreased cerebral degradation of T3, or decreased exit time, in the maintainance of fetal brain T3 concentrations.

The profile of 5D activity has been described to be high during the fetal and neonatal period, as compared with adult brain (35-37). The 5D activity is inversely correlated with 5'D activities. Studies done using fetal brain cells in primary culture (38) show that inner ring deiodination is 25 times more active than the 5'D pathway. Studies done in glial cells in primary culture (39) show that 5D activity is found predominantly in astrocytes. How both pathways influence each other during fetal life remains a matter of discussion, but it is possible that rT3 production regulates 5'D-II activities in a similar way as in the adult brain (22).

Responses to hypothyroidism. We have not found any major, or consistent, effect due to maternal thyroidectomy either in the 5'D-II activities (Figs. 2 and 3), nor in the developmental pattern of T4 or T3 concentrations in the fetal brain, except at 18 dg (Table 1). This findings were expected (25), as no major differences in thyroid hormone concentrations in the brain were observed between fetuses from C and T dams (20 and 21 dg), despite the fact that T4 and T3 concentrations were lower in the thyroid and other tissues of fetuses from T dams. A role of deiodinative mechanisms to maintain cerebral T3 concentrations could not be excluded at that time.

Some of the present data suggest that maternal hypothyroidism does affect 5'D-II in the fetal brain (18 dg), before the fetal thyroid is able to compensate for the decrease in cerebral T4. Thus, although 5'D-II levels were still very low, this activity increased in 17- and 18-day old fetal brains, when obtained from T, or MMI-treated C, dams. We believe this finding of interest, as it would indirectly show that the mechanisms to maintain cerebral T3 are becoming operative in parallel with the onset of fetal thyroid secretion.

When fetal thyroid hormone deficiency is induced by the administration of MMI to the mother, clear-cut responses of 5'D-II are apparent. Present results extend to fetal development the observation that neonatal hypothyroidism results in clear increases in brain T4 5'D activities from the first day of extrauterine life (19). Brain T4 and T3 in fetuses from MMI-treated dams were always very low at all times studied, and support the

conclusion that the 5'D-II response was caused by thyroid hormone deficiency of the fetal brain.

T4 concentrations in 17-day-old fetuses are very low (0.2 nM). This fact raises the question about a different set-point for the regulation of the enzyme during these early stages of brain development.

Effect of maternal thyroid hormones. The adequacy of the dose of T4 used to infuse the pregnant dams, has been extensively discussed in our previous report (26, 33). Infusion of T4 into the MMI-treated mothers increased the concentration of T4 and T3 in the fetal brain, mitigating the effects of MMI. The infusion of T3 to the mother increased fetal plasma T3, and the T3 concentration in many fetal tissues (33), but did not mitigate the cerebral T3 deficiency caused by treatment with MMI. It is shown here that 5'D-II activities clearly responded to the different T4 concentrations in the fetal brain.

Despite the small amount of T4 transferred to the fetus (at least as is reflected in brain T4 concentrations), it is enough to inhibit 5'D-II in the fetal brain, and to produce a clear increase in brain T3, whereas treatment of the mother with T3 actually increased brain 5'D-II activity, possibly as a consequence of the further decrease in cerebral T4 level. Despite these increases in 5'D-II activity in MMI and MMI + T3 treated groups, the high activities were unable to maintain brain T3 concentrations within the normal range (Figs. 5 and 6), possibly due to the lack of substrate: T4.

The inverse relationships found between 5'D-II activities and brain thyroid hormones show that the fetal brain from T dams is better prepared to respond to a situation of fetal hypothyroidism, as the set-point at which 5'D-II starts to respond to T4 deficiency is more sensitive in the fetus from T dams. It could be argued that the different responses could be caused by differences in T4 inner-ring deiodinative pathways (5-D) between the fetal brain from C and T dams. In fact, it has been reported that the amount of fetal plasma rT3 is reduced under the situation of maternal thyroidectomy (40), but, unfortunately, brain rT3 production was not studied in this situation, and it is known that the inhibition of brain 5'D-II, if any, is accounted for by rT3 locally produced from T4 (22). There is also evidence that, in the adult brain, rT3 production is lower in hypothyroid than in C rats (22, 41). In summary, it is possible that the amount of rT3, as a product of T4 metabolism, is changed in the fetal brain under the situation of maternal thyroidectomy. However, the differences between the curves obtained for C and T rats (Fig. 6) do not result from the differences in 5'D-II activities, but from the higher concentrations of T4 in the brain from TMMI and TMMI + T4 fetuses, as compared to their respective C groups. This suggests that maternal thyroidectomy, combined with fetal hypothyroidism might increase T4 uptake by the brain, or decrease T4 degradation or exit time. However, neither the possible changes in cerebral rT3 levels nor in T4 concentrations would totally explain the different set-point of the 5'D-II response to cerebral T4 concentrations between fetuses from C and T dams. The mechanisms that could induce a different sensitivity in the fetal brain from T dams remain to be elucidated.

In summary, present results report for the first time the ontogenic development of T4 5'D-II in the rat fetal brain. During this period, 5'D-II and cerebral concentrations of T4 and T3 are rapidly increasing. The present study also demonstrates that in the rat fetal brain, T3 is derived entirely from T4 by local deiodination, and at the end of gestation reaches values near the adult thyroid hormone concentrations, despite very low plasma T3 levels. Although during fetal development 5'D-II and thyroid hormone concentrations are all increasing, 5'D-II is able to respond to cerebral thyroid hormone deficiency. It does so as early in gestation as 17 to 18 dg. These results suggest a role for thyroid hormones in fetal brain development, considering that mechanisms are already present to preserve cerebral T3 concentrations. Such a role, however, remains to be defined.

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