5′ Deiodinase Activity in Brain Regions of Adult Rats: Modifications in Different Situations of Experimental Hypothyroidism

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Received 27 February 1992; Accepted 12 August 1992

SERRANO-LOZANO, A., M. MONTIEL, M. MORELL AND P. MORATA. 5′ Deiodinase activity in brain regions of adult rats: Modifications in different situations of experimental hypothyroidism. BRAIN RES BULL 30(5/6) 611-616, 1993.—In the central nervous system, type II 5′ deiodinase (SD-II) is highly regulated, as judged by the dramatic changes in enzyme levels observed after abrupt alterations in thyroid status. In this work, the 5′-DII activity has been studied in different situations of experimental hypothyroidism (propylthiouracil, methimazole, thyroidectomy, and low iodine diet), in various brain regions (pituitary, cerebellum, brain stem, hypothalamus, cortex, and whole brain) in adult rats. Propylthiouracil and methimazole significantly increase the activity in all brain regions. These increases are higher in rats treated with methimazole. Thyroidectomy significantly increases the activity in cortex and pituitary. A low iodine diet significantly increases in all brain regions except in the hypothalamus. The concentration of triiodothyronine (T3) studied in the major brain regions remained unchanged. The results obtained show a compensatory mechanism in pituitary and other brain regions in order to maintain the T3 levels in brain tissue.

Type II 5′-deiodinase Hypothyroidism Brain regions Adults rats

PREVIOUS studies show that approximately 80% of T3 in brain cortex and pituitary of euthyroid rats is produced from thyroxine (T4) by 5′ deiodination, a reaction catalyzed by type II 5′ deiodinase (5D-II) (1,16).

Enzymatic 5′ deiodination of T4 can be demonstrated in homogenates of different regions of the brain (10). 5D-II has been described in the central nervous system, pituitary gland, and also in brown adipose tissue (BAT) and placenta (9,12,28); nevertheless, there is a possibility that other tissues may also contain 5′-DII (28). It is interesting to observe that the enzyme activity has a maturational pattern, which suggests that it may be of physiological relevance for the development of the brain (11).

5′-DII enzyme has been distinguished from the 5′-DI in being insensitive to the thioureylene drug propylthiouracil (PTU), which is a strong inhibitor of the 5′-DI (8,16). Goswami et al. (6) found that administration of PTU in vivo provokes inhibition of 5′-D in pituitary and BAT, but no inhibition was observed in the brain. PTU content is found in the pituitary and BAT, but no PTU was detected in the brain, suggesting that PTU may be excluded by the blood–brain barrier. On the other hand, these authors (6) found that at the low dithiothreitol concentrations (<5 mM), PTU inhibits 5′D in the brain, pituitary, and BAT homogenates of hypothyroid rats that predominantly contain the 5′ DI activity. Thus, in in vitro studies the 5′ DI and 5′ DII are affected by PTU in a similar manner but in different degrees when added in vitro (15).

The iodothyronine are the most recognized regulators of cerebro-cortical SD-II activity. T4, 3,3',5'-triiodothyronine (reverse T3, rT3) and T3 caused rapid suppression of 5′D-II activity in hypothyroid rats (24,25). When serum levels progressively decrease by administration of methimazole (MMI), 5′-D-II activity increases markedly, the highest values being found in hypothyroid tissues (23).

However, the 5′D-II activity in other brain regions is less known: hypothalamus or brain stem. On the other hand, although it is known that the hypothyroidism provokes an increase in 5′-D-II activity in the cortex brain and pituitary (23), most studies have been done in congenital hypothyroidism or neonatal hypothyroidism, but the 5′D-II modification in adult hypothyroidism is less known. Ruiz-Marcos et al. (20) have demonstrated thyroid hormone-dependent alterations in the morphology of the cortical neurons in adult rats. Iodine deficient diets provoke different changes in T3 serum than other hypothyroid situations.
so it is interesting to determine the 3'D-II activity in these situations.

The aim of this paper has been to study the 3'D-II activity in different situations of experimental hypothyroidism in several brain regions of adult rats.

METHOD

Reagents

Leverotary iodothyronines used in all studies, and 6 n pro
pyl-2-thiouracil (PTU), 2-mercapto-1-methylimidazole (MMI),
dithiothreitol (DTT), Triton X-100 were obtained from Sigma
Chemical Co. (St. Louis, MO). 131I, 125I-T3 were purchased from
Amersham Corp. England. Other reagents were obtained from
Boehringer Mannheim and Merck (Germany). Standard labora-
tory diet and low iodine diet were from Panlab Lab. (Spain).

Animals and Treatments

Male Wistar rats, maintained at 20 ± 2°C, and at 14h:10h
light:dark schedule, were used in this study. All the animals were
weaned on day 21, the treatments beginning on day 28, and
1ight:dark schedule, were used in this study. All the animals were
they were sacrificed by decapitation on day 120 after birth. Seven
experimental groups were used:

1. Control (C) Euthyroid rats, fed with standard laboratory diet
(containing 0.49 mg iodine/kg diet) and tap water ad lib.

2. PTU rats fed with standard laboratory diet and tap water ad
lib containing 20 mg/100 ml water.

3. MMI rats fed with standard laboratory diet and tap water ad
lib containing 20 mg/100 ml water.

4. Thyroidectomized rats (Tx). A group of animals selected after
birth were fed with a laboratory diet from weaning onwards
and thyroidectomized surgically under anaesthesia with ether
on day 28 after birth, followed by intraperitoneal injection
of 100 𝜇Ci 131I to each animal after 7 days of thyroidectomy
in order to ensure complete thyroid removal (17).

5. Thyroidectomized control rats (C.Tx). This group of rats un-
derwent a sham operation, using the same surgical manipu-
lations without removing the thyroid gland. After 7 days,
each animal was IP injected with 100 𝜇l of isotonic phosphate-
buffered saline solution pH 7.4 (PBS), containing 1 mg/ml
of sodium thiosulphate (dilution buffer of 131I). This buffer
consisted of (Na2HPO4 8 mM, K2HPO4 1 mM, 0.15 M NaCl).

Low Iodine Diet

Rats were fed with a low iodine diet (containing 25 𝜇g iodine/
kg diet) from 28 days of life onwards. During the days 28, 29,
and 30 of life, they received 1% KClO4 as drinking water in
order to accelerate thyroidal depletion of total iodine, no KClO4
was given afterwards. The animals were divided into two groups.

6. low iodine diet (LID) rats. This group received LID, distilled
water until completion of the experiment.

7. LID Control (LID+I). Rats were fed the same bath of diet
and distilled water containing KI at one concentration of
0.65 𝜇g/ml.

Preparation of Samples

Serum was obtained by centrifugation at 1000 × g for 20
min and stored at −40°C for T3 and T4 determinations. T3 and
T4 concentrations were measured by RIA, after an extraction
step with ethanol. The method used is described by Takaishi et
al. (26) and Obregón et al. (18). In order to avoid interferences,
standard curves (from commercials standard) to each sample
type were prepared, which were subjected to the same extraction
process.

Isolation of Liver Cytosolic and Mitochondrial Fraction

Liver homogenates were made 1:10 with 0.25 M of sucrose,
1 mM DTT, 1 mM EDTA and 15 mM Tris CI pH 7.4. The
homogenates were filtered through two layers of gauze and cen-
trifuged at 1000 × g for 10 min. The supernatant was centrifuged
at 12000 × g for 10 min. The pellet was washed in the homogenation medium and centrifuged at 12000
× g for 10 min. The pellet was resuspended and homogenized
in a glass potter homogenator with 0.5 ml of 0.25 M of sucrose,
1 mM DTT, 5% of Triton X-100 and 15 mM Tris CIH. The
homogenates were centrifuged at 30000 × g for 20 min. The
supernatant was used as the source of hepatic mitochondrial
-3-23-glycerophosphate dehydrogenase (α-GPD) (EC 1.1.99.5).

Enzymatic Assay

ME was assayed as described by Hsu and Lardy (7). Mitochon-
drial α-GPD was determined following the method of
Dawson and Thorne (2), using 2,6-dichlorophenol as the electron
acceptor.

Monodeiodination of T4 to T3

Whole brain and brain regions (pituitary, hypothalamus,
brain stem, cerebellum, and cerebral cortex) were dissected with
fine scissors, weighed, and homogenized with a motor glass-teflon
buffered saline solution pH 7.4. The resulting homogenates were used for
the deiodination studies.

Assay of 5'D-II in Brain Regions

The brain regions homogenates were incubated with non-
radioactive T4, and the amount of T3 generated was measured by
radioimmunoassay (RIA) in an ethanol extract of tissue. As-
says were carried out by the method developed by El-Zaheri et
al. (4) with some modifications; for example, the time of incu-
bation and the final concentration of T4 and DTT. In our ex-
periments the final concentrations were: 100 mM DTT, 5 𝜇g/
ml T3, and incubation time was 120 min.

The percentage the recovery of T3 by ethanol extraction was
determined in all experiments by adding 125I T3 to aliquots of
homogenates just before the addition of ethanol. The percent
recovery average was 94%. Protein concentration in the ho-
mogenates was determined by the method of Lowry et al. (14).

The T3 generated from T4 after 120 min of incubation was
expressed as ng of T3 per mg of protein.

Statistical Analysis

The results are expressed as mean ± SEM (standard error
mean). All data were analyzed for statistically significant differ-
ces by analysis of variance (ANOVA): design completely alea-
tory.

RESULTS

Hypothyroid State of Animals

Body weight, serum levels T3 and T4, and liver enzymatic
activity α-GPD and ME were used as parameters for screening
hypothyroid state. Figure 1 shows body weight evolution, from 28 days of life until completion of experiment. Both pharmacologically induced hypothyroidism and surgical hypothyroidism produced a significant decrease in body weight throughout the experiment; however, light differences were observed with a low iodine diet. Table 1 shows serum thyroid hormones and liver $\alpha$-GPD and ME in the different groups studied. $T_1$ and $T_4$ were significantly decreased in MMI- and PTU-treated animals and
in surgical hypothyroidism. Nevertheless, in LID only T4 decreases significantly, although T3 levels were higher than control. The liver α-GPD and ME activities were significantly decreased with respect to the controls in the different experimental states of hypothyroidism.

**Concentration of T3 in Different Brain Regions**

Table 2 shows the T3 content in brain regions of the rat in normal and different experimental states of hypothyroidism. It is noteworthy that T3 content is high in pituitary and hypothalamus, although values in cortex, cerebellum, brain stem, and whole brain were lower and very similar.

In hypothyroidism induced by MMI, the tissue levels of T3 unchanged significantly with respect to the controls in all brain regions; however, in PTU-treated rats there is a decrease in pituitary (p < 0.05) although in other regions these unchanged significantly against control. In surgical and LID-induced hypothyroidism, the concentrations of T3 in brain regions show no significant differences in groups studied according to the respective controls.

**Conversion of T4 to T3 by Different Brain Regions of Adult Rats**

T4 to T3 converting activity data in brain regions of adult rats in different states of hypothyroidism are summarized in Table 3. In all experimental conditions the activity is highest in hypothalamus and pituitary regions, whereas in cortex, brain stem, cerebellum, and whole brain, similar values were found.

A significant increase (p < 0.05) in the T4 to T3 converting activity was found in pharmacologically induced hypothyroidism (MMI and PTU) in all brain regions, against the control. It is interesting to detach that MMI provokes a higher stimulation in the conversion of T4 to T3 than PTU. Surgical hypothyroidism (Tx) provokes an increase in the activity of 5′ deiodinase type II significantly in cortex and pituitary. Low iodine diet (LID) significantly increases the 5′ D-II activity in cortex, cerebellum, brain stem, pituitary, and whole brain, although it remains significantly unchanged in hypothalamus.

**DISCUSSION**

In brain and posterior pituitary gland, more than half of the intracellular T3 is produced locally by the enzyme-catalized phenolic ring deiodination of T4 (13). The T4 has a very low biological activity: in this way, 5′ deiodination may be considered as an active pathway for generating active thyroid hormone.

Cerebrocortical and pituitary type III′ deiodinase are highly regulated as judged by the deiodinated changes in enzyme levels observed after abrupt alterations in thyroid status (24,25).

In this work the 5′D-II deiodinase activity variations on several brain regions during different hypothyroid states induced by drugs, thyroidectomy, or low iodine diet has been studied.

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**TABLE 1**

SERUM LEVELS OF TRIIODOTHYRONINE, THYROXINE, α-GLYCEROPHOSPHATE DEHYDROGENASE, MALIC ENZYME ACTIVITIES IN THE LIVER OF CONTROLS AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th></th>
<th>T4 (ng/dl)</th>
<th>T3 (ng/dl)</th>
<th>α-GPD (nmol/min/mg protein)</th>
<th>ME (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>24.85 ± 1.18 (12)</td>
<td>3.01 ± 0.22 (12)</td>
<td>11.82 ± 0.99 (8)</td>
<td>22.17 ± 0.85 (8)</td>
</tr>
<tr>
<td>PTU</td>
<td>1.91 ± 0.35 (12)*</td>
<td>0.90 ± 0.08 (12)*</td>
<td>5.56 ± 0.36 (11)*</td>
<td>11.76 ± 1.08 (11)*</td>
</tr>
<tr>
<td>MMI</td>
<td>9.83 ± 1.35 (12)*</td>
<td>0.73 ± 0.10 (12)*</td>
<td>3.65 ± 0.18 (7)*</td>
<td>12.94 ± 0.85 (7)*</td>
</tr>
<tr>
<td>CTx</td>
<td>26.75 ± 1.47 (12)</td>
<td>4.69 ± 0.24 (12)</td>
<td>14.54 ± 0.68 (8)</td>
<td>22.59 ± 1.87 (8)</td>
</tr>
<tr>
<td>Tx</td>
<td>4.91 ± 1.49 (12)*</td>
<td>1.09 ± 0.10 (12)*</td>
<td>7.75 ± 0.90 (8)*</td>
<td>16.86 ± 1.64 (8)*</td>
</tr>
<tr>
<td>LID + I</td>
<td>31.58 ± 2.54 (12)</td>
<td>3.17 ± 0.12 (12)</td>
<td>9.56 ± 0.59 (8)</td>
<td>22.36 ± 2.53 (8)</td>
</tr>
<tr>
<td>LID</td>
<td>48.08 ± 6.35 (12)*</td>
<td>1.24 ± 0.05 (12)*</td>
<td>3.47 ± 0.15 (8)*</td>
<td>10.31 ± 1.31 (8)*</td>
</tr>
</tbody>
</table>

The results are means ± SEM (standard error means). The number of animals is indicated in parentheses.

In comparison with respective control animal in each brain region, p < 0.05.

C: Control; PTU: propylthiouracil; MMI: methimazole; CTx: thyroidectomized control; Tx: thyroidec-tomized; LID: low iodine diet; LID + I: LID control.

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**TABLE 2**

CONCENTRATIONS OF TRIIODOTHYRONINE (pg/mg PROTEIN) IN BRAIN REGIONS OF CONTROL AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Brain Stem</th>
<th>Hypothalamus</th>
<th>Pituitary</th>
<th>Whole Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>50.75 ± 5.14 (6)</td>
<td>41.38 ± 3.53 (6)</td>
<td>49.12 ± 3.26 (6)</td>
<td>184.66 ± 47.64 (6)</td>
<td>662.25 ± 267.47 (6)</td>
<td>49.86 ± 3.73 (6)</td>
</tr>
<tr>
<td>PTU</td>
<td>28.54 ± 8.37 (6)</td>
<td>32.86 ± 8.29 (6)</td>
<td>29.51 ± 7.35 (6)</td>
<td>170.34 ± 42.79 (6)</td>
<td>201.98 ± 25.01 (6)*</td>
<td>33.70 ± 14.54 (6)</td>
</tr>
<tr>
<td>MMI</td>
<td>40.63 ± 4.13 (6)</td>
<td>29.71 ± 7.01 (6)</td>
<td>29.69 ± 6.19 (6)</td>
<td>153.34 ± 68.06 (6)</td>
<td>451.56 ± 116.56 (6)</td>
<td>35.30 ± 6.90 (6)</td>
</tr>
<tr>
<td>CTx</td>
<td>30.28 ± 7.32 (6)</td>
<td>26.03 ± 4.98 (6)</td>
<td>29.01 ± 10.32 (6)</td>
<td>131.44 ± 27.29 (6)</td>
<td>185.48 ± 27.97 (6)</td>
<td>24.57 ± 2.75 (6)</td>
</tr>
<tr>
<td>Tx</td>
<td>30.69 ± 4.46 (6)</td>
<td>20.87 ± 2.60 (6)</td>
<td>21.04 ± 2.28 (6)</td>
<td>124.59 ± 17.54 (6)</td>
<td>389.26 ± 67.70 (6)</td>
<td>20.69 ± 2.27 (6)</td>
</tr>
<tr>
<td>LID + I</td>
<td>30.22 ± 2.59 (6)</td>
<td>27.20 ± 2.22 (6)</td>
<td>20.57 ± 2.85 (6)</td>
<td>88.64 ± 12.16 (6)</td>
<td>237.70 ± 24.20 (6)</td>
<td>33.42 ± 2.58 (6)</td>
</tr>
<tr>
<td>LID</td>
<td>40.11 ± 14.56 (6)</td>
<td>29.96 ± 5.93 (6)</td>
<td>25.83 ± 2.18 (6)</td>
<td>101.66 ± 14.69 (6)</td>
<td>94.12 ± 15.03 (6)</td>
<td>20.61 ± 2.59 (6)</td>
</tr>
</tbody>
</table>

The results are means ± SEM (standard error means). The number of animals is indicated in parentheses.

In comparison with respective control animal in each brain region, p < 0.05.

C: Control; PTU: propylthiouracil; MMI: methimazole; CTx: thyroidectomized control; Tx: thyroidec-tomized; LID: low iodine diet; LID + I: LID control.
The animal hypothyroid states were demonstrated by their body weight decrease, low serum thyroid hormones concentration, and the reduced malic and α-GPD enzyme activities, which independently or together have been extensively used as index thyroid state. Malic enzyme and α-GPD decrease significantly in these groups according to Obregon et al. (19) and Santisteban et al. (21). These enzymes have been measured in liver, because enzyme brain levels are not an adequate index for thyroid state screening (8,22), although some authors found decreases in malic enzyme in brain development of hypothyroid animals (3). The serum T₃ and T₄ concentration are decreased in PTU-, TX-, and MMI-treated animals, although in PTU animals there is less decrease in T₄ levels because PTU inhibits peripheral T₄ to T₃ conversion, by 5'-I inhibition.

In LID rats, T₄ decreases, although there is an increase in T₃ levels. This is explained by different authors as an adaptation mechanism to prolonged LID diet, with an increase of T₃ thyroid synthesis and TSH stimulation (5,19).

The SD-II activity is increased in all experimental groups in comparison with respective control animal in each brain region, p < 0.05. Only in PTU-treated animals the T₃ concentrations were decreased significantly in pituitary, possibly due to an inhibitory effect of PTU on 5'-deiodination. Thus, it is possible that the ultimate impact thyroid hormone deficiency may have on brain 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 other brain regions.

**REFERENCES**

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