Effects of hyper- and hypothyroidism on thyroid hormone concentrations in regions of the rat brain

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Am J Physiol Endocrinol Metab 285: E470–E480, 2003. First published May 7, 2003; 10.1152/ajpendo.00043.2003.—The purpose of this study was to investigate the effects of hyper- and hypothyroidism on thyroid hormone concentrations and deiodinase activities in nine regions of the rat brain. Four weeks of treatment with 75 μg thyroxine (T4)/kg body wt induced a two- to threefold increase in T4 levels in all of these brain regions, whereas the 3,5,3'-triiodothyronine (T3) concentrations were reduced in five brain regions and remained unchanged in four. Even after 8 wk treatment with 300 μg T3/kg, the T3 concentrations remained normal in cortical areas, the hippocampus and amygdala, and were elevated only in areas in which inner-ring deiodinase activity was low or absent, and in the hypothalamus. At the subcellular level, nuclear concentrations of T3 were diminished in hypothyroidism but remained unaltered in hyperthyroidism in all areas except the hypothalamus, where they were enhanced. Cortical mitochondrial succinate dehydrogenase activity was reduced in both hypo- and hyperthyroidism in spite of normal T3 concentrations in hyperthyroid animals. The results show that nuclear T3 concentrations fall in hypothyroidism but do not change during severe hyperthyroidism in any brain region except the hypothalamus. Further research is thus needed to clarify the mechanisms mediating the numerous biochemical and psychological effects of hyperthyroidism.

hyperthyroidism; 3,5,3’-triiodothyronine; thyroxine; nuclei; deiodinase

IN THE LAST FIFTEEN YEARS, an increasing number of studies have indicated that thyroid hormones have important physiological functions, not only during brain maturation but also in the adult vertebrate brain. To begin with, nuclear 3,5,3’-triiodothyronine (T3) α and β receptors are expressed throughout the adult rat brain (e.g., Refs. 4, 23, and 24). Second, hyper- and hypothyroidism may affect the expression of distinct genes in the adult rat brain (e.g., Ref. 19).

Third, the iodothyronine metabolizing enzymes, in particular the selenoenzymes type II deiodinase (D2; see Ref. 7) and type III deiodinase (D3; see Ref. 36), constitute an autoregulatory mechanism that functions to keep brain T3 concentrations stable as long as possible under conditions of peripheral hypo- or hyperthyroidism (reviewed in Ref. 26). However, despite these autoregulatory mechanisms, effects of hyper- and hypothyroidism on numerous biochemical and behavioral parameters have been described in the literature for over a century. Severe forms of both hypo- and hyperthyroidism may cause mental illnesses such as depression and paranoid psychosis (reviewed in Refs. 43 and 44), and symptoms such as nervousness, fatigue, weakness, and hyperactivity are common, even when the overall clinical picture is mild (5). At a biochemical level, thyroid hormone dysfunction may affect not only gene expression (see above) but also the characteristics of various neurotransmitter systems (e.g., Refs. 29, 30, and 37).

Surprisingly, however, to date no attempt has been made to quantify thyroid hormone concentrations in the brain itself in hyperthyroidism, e.g., after its induction in euthyroid laboratory animals. Three study groups have substituted hypothyroid rats with supra-physiological doses of thyroxine (T4). They found either no increase in T3 concentrations in the cortex or cerebellum (41) or “brain” (10) or an only 15% increase in cortical levels of T3 but a doubling of cerebellar T3 concentrations after the highest dose of 80 μg T4/kg body wt (12). Thus, to clarify in more detail the effects of hyperthyroidism on brain concentrations of T4 and T3, we studied thyroid hormone concentrations in nine brain regions in groups of rats rendered hyperthyroid.

It is widely accepted that T3 influences gene expression by binding to its specific nuclear receptor sites (reviewed in Ref. 1). However, effects on the mitochondria (reviewed in Ref. 45) and synaptosomes (e.g., Ref. 30) have also been reported. Moreover, the T3-producing enzyme D2 is presumably located intracellularly in the endoplasmatic reticulum (2). We therefore developed a method of separating homogenates into relevant...
subcellular compartments and measuring thyroid hormones in these fractions, i.e., in nuclei, mitochondria, synaptosomes, and microsomes. Because our method of subcellular fractionation yields a myelin pellet, iodothyronines were quantified also in this subcellular compartment.

MATERIALS AND METHODS

Materials. The following compounds were purchased from the following suppliers: thyroid hormones from Henning (Berlin, Germany); sucrose from Merck (Darmstadt, Germany); HPLC columns (Eurosphere 100 C18) from Knauer (Berlin, Germany); 0.2-μm filter (Minisart SRP 15) from Sartorius (Gottingen, Germany). All other chemicals were of reagent grade and were bought from Sigma (Munich, Germany). 3-Bromo-[5-125I]T1 and inner ring-labeled [5-125I]T3 were purchased from Formula (Berlin, Germany). 5'-[125I]T3, 3' -[125I]T3, and also antibodies against rat T4, T3, and 3,5-diiodothyronine (T2) were produced as previously described (34, 35).

Sprague-Dawley rats were bought from Tierzucht Schönewalde (Schönewalde, Germany).

Animal treatment. All animal experiments were approved by the Senate of Berlin. Adult male Sprague-Dawley rats with an initial weight of ~300 g and housed four to a cage were employed throughout. They had a 12:12-h light-dark cycle (6:00 AM to 6:00 PM) and received water and food pellets ad libitum. Before initiating any experiments and decapitation without treatment, all rats were allowed to remain undisturbed in their new environment for at least 3 wk. The rats in all groups were always decapitated between noon and 2:00 PM without anesthesia, and their brains were dissected immediately as described by Glowinski and Iversen (17).

Experiment 1 ("low-dose T4", hypothyroidism). To induce hypothyroidism, 0.25% methimazole and 1% KClO4 were added to the tap water of eight rats for 4 wk. Hyperthyroidism was induced in a further eight rats by adding 24 μg T3/30 ml drinking water, which is the approximate daily fluid intake of a rat. As the initial mean weight of this group of rats was 318 ± 15 g, this dose of T3 was approximately equal to 75 μg·kg body wt⁻¹·day⁻¹. One percent BSA was added to all bottles. The contents of all bottles were replaced daily, and the amount of fluid consumed was recorded. Eight control rats received tap water containing 1% BSA.

Experiment 2 ("high-dose T4"). An additional group of eight rats was treated with 96 μg T4/30 ml drinking water for 4 wk (approximately equal to 300 μg T4·kg body wt⁻¹·day⁻¹). All other conditions were the same as described for group 1. Eight control rats received tap water containing 1% BSA.

Experiment 3. For quantification of thyroid hormones in subcellular fractions and the determination of deiodinase activities and other functional parameters, groups of 12 rats each were treated for 8 wk as follows. 1) One group was administered low-dose T4 as described for group 1; 2) one group was treated with high-dose T4, as given for group 2; 3) one group was treated with 96 μg T3/30 ml tap water, equal to 300 μg T3·kg body wt⁻¹·day⁻¹; 4) one group was rendered hypothyroid as described under group 1; and 5) one control group received tap water containing 1% BSA.

To establish the methods for both subcellular fractionation and evaluation of subcellular fractions by biochemical "markers" and by electron microscopy, 36 control rats were decapitated. Quantification of T3 in subcellular fractions revealed that T3 was detectable in all fractions of the large brain regions of a single brain. However, to be able to detect T3 in all subcellular fractions of the smaller brain areas, the brains of two rats (for the hippocampus and limbic forebrain) and even three rats (for the hypothalamus, amygdala, and septum) had to be pooled. Thus 12 rats were needed for quantification of iodothyronines in subcellular fractions of experiment 3.

Subcellular fractionation. The method used to isolate subcellular fractions is a modification of the methods described by Lovtrup-Rein and McEwen (27), Whitaker (42), and Dodd et al. (9). All procedures were carried out at a room temperature of 4 °C. Briefly, frozen tissue was placed in ice-cold 0.32 M sucrose at a final dilution of 1:10 (wt/vol). The tissue was homogenized with a motor-driven glass-Teflon homogenizer (clearance 0.2 mm). Homogenate (100 μl) was immediately frozen for later quantification of thyroid hormone concentrations. The remaining homogenate was centrifuged in an L8-55 ultracentrifuge (Beckman Instruments) for 15 min at 850 g, yielding the supernatant (S1) and the crude nuclear pellet (P1). P1 was suspended in 2 ml 0.32 M sucrose and centrifuged for 10 min at 600 g. The resulting supernatant was unified with S1. The pellet (P2) was resuspended in 1.3 M sucrose containing 1 mM magnesium chloride and 1 mM potassium phosphate buffer, pH 6.5, and centrifuged for 45 min at 53,000 g, yielding the nuclear pellet. S1 was diluted with 0.32 M sucrose, layered on 1.2 M sucrose, and centrifuged at 187,000 g for 30 min. The resulting pellet contained the mitochondria. The interface (I1) was diluted with 0.32 M sucrose, layered on 0.85 M sucrose, and centrifuged at 187,000 g for 15 min. The resulting pellet was composed of synaptosomes, whereas the myelin was retained at the 0.85/ 0.32 interface (I2). The 0.32 M sucrose supernatant contained the microsomes. The interface containing the myelin was treated hypoosmotically to minimize cytotoxic inclusions.

Details on the development of the method of subcellular fractionation will be presented elsewhere (33a).

Characterization of the subcellular fractions by electron microscopy. Subcellular fractions were fixed in cacodylate-buffered 2% glutaraldehyde (pH 7.2) at 4 °C overnight. Post-fixation was performed in buffered 1% osmium tetroxide for 4 h at room temperature. Samples were then dehydrated in a graded series of acetone (30, 50, 70, 80, 96, and 2 × 100%, each for 15 min). After being embedded in araldite, the samples were cut in ultrathin sections (70 nm) on a Leica Ultracut. Staining was performed with uranyl acetate and tungstophosphoric acid during the dehydration procedure and with lead citrate after sectioning. Samples were examined with a Zeiss EM 902 transmission electron microscope.

The results of the study of the purity and integrity of the subcellular fractions by electron microscopy are presented in Fig. 1. As can be seen, the nuclei, mitochondria, myelin, and microsomes were clearly identifiable. Contamination with other cellular components was negligible. However, because of prior freezing at −80°C, many of the synaptosomes appeared empty, without the characteristic accumulations of synaptic vesicles and mitochondria (Fig. 1D). Brain tissue subfractionated immediately after dissection of the rat brains yielded far more characteristic synaptosomes (Fig. 1E). However, it was not feasible to carry out subcellular fractionation of the rat brain tissues immediately after death without a freezing step, since nine brain regions were dissected from each rat brain. Immediate subcellular fractionation is only possible with a single brain region. All experiments were therefore performed with synaptosomal preparations obtained after freezing in which the iodothyronines of interest were clearly measurable (see DISCUSSION).
Characterization of the subcellular fractions by “biochemical markers.” The quantification of DNA was performed with the “Wizard Genomic DNA Puriﬁcation Kit” (Promega, Madison, WI). Succinate dehydrogenase (SDH) activity was quantiﬁed as described by Pennington (33). Quantiﬁcation of 2’,3’-cyclic nucleotide 3’-phosphohydrolase (CNPase) was performed by the method reported by Kurihara and Takahasi (22). Measurement of NADPH-cytochrome c reductase activity was performed as described by Sottocasa et al. (40). Lactate dehydrogenase activity was quantiﬁed as reported by Johnson and Whittaker (20). Na+/K+-ATPase activity was quantiﬁed as reported by Esmann (14).

Figure 2 shows the results of the quantiﬁcation of subcellular markers. It reveals that the DNA concentrations and SDH, CNPase, and NADPH cytochrome c reductase activities were between 4- and 10-fold higher in their respective fractions than in the homogenate. The only relevant contamination was an approximately twofold increase in the SDH activity in the synaptosomal fraction, reﬂecting presynaptic mitochondria. Cytosolic contamination of the fractions was negligible, since lactate dehydrogenase activity was enhanced only in the combined supernatant.

Extraction, separation, puriﬁcation, and quantiﬁcation of iodothyronines. The extraction, separation, and puriﬁcation of the thyroid hormones from the whole homogenates were performed as recently described in detail (34). The procedure employed for the subcellular fractions was basically the same, with the following modiﬁcations.

After extraction with methanol, the samples were mixed in a vortex and treated in an ultrasonic bath until the pellet had dissolved. After the samples were ﬁltered and dried, HPLC processing was performed as follows. The gradient was programmed to increase from 32% acetonitrile to 40% between minutes 1 and 24 at a ﬂow rate of 1 ml/min, followed by 10 min of washing with 100% acetonitrile and reestablishment of the equilibrium (32% acetonitrile-67% H2O-1% acetic acid).

The samples were always run together with ~10% of the total number of samples that had been handled in exactly the same manner as the tissue samples. However, these “blanks” did not contain any tissue. The RIAs were performed as previously described in detail (34, 35). The sensitivities of all RIAs were in the low picogram range (ED80 between 1 and 2 pg). All samples of one experiment were processed in duplicate and assayed together within the same run.

To investigate whether molecules of T3 originally present in the cytosol adhered to subcellular fractions during the different centrifugation procedures, the midbrains of 12 control rats were processed as follows: 150 pg T3 were added to each of six midbrains at the start of the homogenization process. The levels of T3 in the homogenates and subcellular fractions of these six midbrains and six additional midbrains to which no T3 had been added were then quantiﬁed by RIA.

Serum concentrations. Serum concentrations of T3 and T4 were determined by a slightly modiﬁed double-antibody RIA as previously described for human serum (31). For assaying total T4 and T3 in the rat sera, standards were set up in iodothyronine-free rat serum.

Iodothyronine deiodinase assays. The deiodinase activities were measured as previously described (11). Tissue samples were homogenized individually on ice in 5–6 vol of 0.25 M sucrose and 10 mM HEPES (pH 7.0) containing 10 mM DTT and immediately frozen in a dry ice-acetone bath and stored at −80°C until assay.

Fig. 1. Electron microscopic images of the nuclear (A; magniﬁcation 1:3,000), mitochondrial (B; 1:12,000), and myelin (C; 1:7,000) fractions, the synaptosomal fraction after storage at −80°C (D; 1:20,000), the synaptosomal fraction obtained without prior freezing (E; 1:20,000), and the microsomal fraction (F; 1:30,000).
D2 assay. D2 activity was determined using \([5^\prime-\text{H}11032-\text{T}4]\) as substrate in the presence of 6 nM T4, 30 mM DTT, 1 mM G-n-propyl-2-thiouracil (PTU), and 1 \(\mu\)M MT3 to inhibit the inner-ring deiodination of T4 in those tissues containing significant D3 activity. The measurement was conducted after 45–90 min (usually 60 min) incubation at 37° C with 50–100 \(\mu\)g protein from the crude homogenate in 100 \(\mu\)l of 0.1 M potassium phosphate buffer (pH 7.0) and 1 mM EDTA. The reaction was started by the addition of the tissue homogenate and stopped by adding 50 \(\mu\)l ice-cold 5% BSA and 10 mM PTU, followed by 400 \(\mu\)l of 10% ice-cold TCA. After centrifugation at 4,000 \(g\) for 30 min, the supernatant containing the \(^{125}\text{I}\) was further purified by cation exchange chromatography. The iodide was then eluted with two 1-ml aliquots of 10% acetic acid, and the radioactivity was counted in a \(\gamma\)-counter.

D3 assay. For determination of D3 (inner-ring deiodinase), 20–70 \(\mu\)g protein were incubated in a final volume of 100 \(\mu\)l of 0.1 M potassium phosphate buffer (pH 7.4) and 1 mM EDTA with \(~1.2\) kBq (\(~50,000\) counts/min) inner-ring-labeled \([5^\prime-\text{H}11032]\)T3, at 50 nM T3, 20 nM DTT, and 1 mM PTU for 60 min at 37° C. Radioiodide release was measured as described above.

Protein contents. The protein contents were determined by the method employed by Bradford (3).

Statistics. Data are given as means \(\pm\) SE. Comparisons between the control group and each of the treatment groups were performed by the Mann-Whitney U-test. P values smaller than 0.05 were considered significant.

Table 1. Serum concentrations of T4 and T3 in different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>T4, ng/ml</th>
<th>T3, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.3 ± 1.2</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>ND</td>
<td>0.01 ± 0.003*</td>
</tr>
<tr>
<td>Low-dose T4</td>
<td>62.3 ± 2.6*</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.3 ± 2.2</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>High-dose T4</td>
<td>84.6 ± 3.4*</td>
<td>1.56 ± 0.20*</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40.0 ± 2.0</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>ND</td>
<td>0.03 ± 0.005*</td>
</tr>
<tr>
<td>T3</td>
<td>ND</td>
<td>3.20 ± 0.21*</td>
</tr>
<tr>
<td>Low-dose T4</td>
<td>64.3 ± 1.8*</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>High-dose T4</td>
<td>92.6 ± 1.9*</td>
<td>1.70 ± 0.18*</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE. T4, thyroxine; T3, 3,5,3’-triiodothyronine. \*P < 0.01; ND, not detectable.
thyroidism induced by adding 24 μg T4/30 ml to the rat’s drinking water for 4 wk. In hypothyroidism, the tissue concentrations of T4 were undetectable in all regions except the frontal cortex, where they were diminished significantly. T3 was undetectable in five brain areas and significantly reduced in four other areas. In hyperthyroid rats, the tissue concentrations of T4 were elevated significantly in all brain regions. However, the T3 levels were normal in four regions and significantly diminished in the other five areas.

Figure 4 shows the effects of 4 wk of treatment with 96 μg T4/30 ml tap water on the concentrations of T3 and T4 in homogenates of 9 brain regions. fCor, frontal cortex; pCor, parietooccipital cortex; Hip, hippocampus; Amy, amygdala; Lf, limbic forebrain; Hyp, hypothalamus; Mid, midbrain; Cbl, cerebellum; Med, medulla. *P < 0.05 and **P < 0.01 compared with controls. #Not detectable.

Figure 3. Effects of hypothyroidism and 4 wk of treatment with 24 μg T4/30 ml tap water−1day−1 on concentrations of 3,5,3’-triiodothyronine (T3; A) and thyroxine (T4; B) in homogenates of 9 brain regions. fCor, frontal cortex; pCor, parietooccipital cortex; Hip, hippocampus; Amy, amygdala; Lf, limbic forebrain; Hyp, hypothalamus; Mid, midbrain; Cbl, cerebellum; Med, medulla. *P < 0.05 and **P < 0.01 compared with controls. #Not detectable.
concentrations of T3 in aliquots of the homogenates and subcellular fractions of the midbrain with and without the addition of 150 pg T3 before homogenization (Fig. 7). The T3 levels were almost doubled in the homogenates but remained unchanged in the subcellular fractions.

Figure 8 shows the T3 concentrations in aliquots of the homogenates and subcellular fractions of the parietooccipital cortex, the hypothalamus, and cerebellum. The parietooccipital cortex is typical of the four regions whose homogenates showed no increase in T3 concentrations, even after high-dose T4 treatment (Fig. 4). The cerebellum is typical of those areas in which the T3 concentrations in the homogenates were elevated after high-dose T4 treatment. For space reasons Fig. 8 only shows the results for these two “representative” brain regions as well as those for the hypothalamus, which was the only brain area where cortical T3 concentrations were elevated in hyperthyroidism. In hypothyroid rats, the nuclear concentrations of T3 were dramatically reduced in the parietooccipital cortex and no longer detectable in the hypothalamus and cerebellum. Similar results were seen in all other brain regions (data not shown). Hyperthyroidism did not lead to increases in nuclear T3 concentrations in the parietooccipital cortex. The nuclear T3 levels were not enhanced, even in the cerebellum, where the T3 concentrations in the homogenates and most of the other subcellular fractions were elevated significantly after high-dose T4 treatment. Likewise, T3-induced hyperthyroidism resulted in elevated T3 concentrations in the homogenates and all subcellular fractions of the cerebellum. However, nuclear T3 concentrations remained normal. The only exception was the hypothalamus, where nuclear T3 concentrations were elevated significantly in both of the two groups treated with T3 and high-dose T4, respectively. It also seems worthy of note that, after treatment with low-dose T4, the T3 levels were reduced significantly in the homogenates of the parietooccipital cortex but remained unchanged in the nuclear fraction. Similar results were obtained in the remaining four brain regions, in which low-dose T4 treatment led to reduced T3 concentrations in the homogenates (Fig. 3).

Table 2 shows that the SDH activities were reduced significantly in the mitochondrial fractions of the parietooccipital cortex in the hypothyroid rats and also in the hyperthyroid rats, both after administration of T3 and after the high dose of T4. No significant effects on the activities of the other two enzymes were observed.

To establish whether the iodothyronine metabolite 3,5-T2 is enhanced in hyperthyroid rats, this hormone
was also quantified in aliquots of the homogenates and subcellular fractions of the frontal cortex. 3,5-T₂ was not detectable in the homogenates or any subcellular fraction of the control rats (data not shown). In the groups treated with T₃ and high-dose T₄, however, 3,5-T₂ concentrations were measurable in the mitochondria, albeit at very low levels. The mean values were 2.1±0.3 pg/mg protein in the rats treated with T₃ and 1.1±0.2 pg/mg protein after treatment with high-dose T₄.

DISCUSSION

The most surprising result of this study concerned the T₃ concentrations in the brains of the hyperthyroid rats. After 4 wk of treatment with a dose of T₄ that induced a significant rise in the serum and tissue T₄ levels, the tissue levels of T₃ were reduced in five brain regions and normal in four. Even after 8 wk of treatment with doses of T₃ and T₄ that led to two- to fivefold increases in the serum concentrations of T₃, the tissue levels of T₃ remained unchanged in four functionally important areas (two cortical areas, hippocampus, and amygdala). These results show that, in particular, those areas in which D₃ activity is very low or undetectable, such as the medulla, cerebellum, and midbrain (34), are not well protected against hyperthyroidism. However, the hypothalamus, where D₃ activity is no lower than in cortical areas (34), is a notable exception, as hypothalamic concentrations of T₃ were elevated drastically after administration of T₃ and high-dose T₄.

It is particularly intriguing that the nuclear T₃ concentrations remained normal, even in those areas in which the homogenates and also most of the other subcellular fractions of the respective brain areas showed significantly elevated T₃ levels (e.g., in the cerebellum, medulla, and midbrain).

The mechanism responsible for keeping T₃ levels within the normal range in cortical areas, the hippocampus and amygdala, is obviously the decrease in D₂ activity (Fig. 5). D₃ activity, on the other hand, was not elevated in cortical areas after the high dose of T₄ and even showed a paradoxical fall after administration of the lower dose of T₄. The mechanisms underlying the unexpected decreases in T₃ concentrations observed in five brain regions after administration of the low dose of T₄ are unclear. The most likely explanation is an "overreaction" of D₂, which becomes inhibited to an extent that results in diminished rather than normal T₃ concentrations.

Our results show that T₄ is a much "stronger" regulator of the D₂ activity compared with T₃, since D₂ activity is elevated in rats treated with T₃. This increase in D₂ activity is most likely because of the fall in serum and tissue concentrations of T₄ in these animals. Both this result and the finding that D₃ is far more sensitive to T₃ than to T₄ have previously been reported by others (13, 39). These findings are consistent with reports showing that the regulation of the D₂ activity may occur independently of nuclear T₃ receptor binding (16).

T₃ tissue concentrations were normal in rats treated with high-dose T₃, although T₄ serum concentrations were undetectable in these rats. Therefore, in the absence of T₄, T₃ may well enter the brain and ensure physiological T₃ concentrations at the cellular level.

The results obtained in hyperthyroid rats raise the question as to what mechanisms underlie the numerous biochemical and psychological abnormalities observed in hyperthyroidism. In light of our results, it would seem surprising that several well-designed and -performed studies in which far lower dosages of T₄ were administered than in our own have reported biochemical effects on the adult rat brain. One research group, for example, observed decreases in the number of β-adrenergic receptors and noradrenaline-stimulated cAMP formation in rat cerebral cortex after 9 days of treatment with 50 μg T₄·kg⁻¹·day⁻¹ (37). Other authors have found an increase in cortical β-adrenergic receptor density after 7 days of treatment with 250 μg T₄·kg⁻¹ (29). This and numerous other contradictory results of studies on the effects of hyperthyroidism on brain parameters may well be because of the fact that a dose of 50 μg T₄·kg may even decrease brain T₃ levels.
On the other hand, even discrete forms of hyperthyroidism cause mild to moderate psychiatric symptoms, such as nervousness, anxiety, fatigue, weakness, and hyperactivity in a substantial number of patients (5, 44). Our results militate against the assumption that these mental symptoms in the milder forms of hyperthyroidism are mediated by an increase in T3 concentrations at specific nuclear T3 receptor sites. Likewise, the numerous biochemical effects observed after administration of doses of thyroid hormones not exceeding those employed in the present study were most likely not mediated by increases in nuclear T3 receptor occupancy. However, it cannot be entirely excluded that increases in nuclear T3 concentrations in very small brain structures during hyperthyroidism could not be detected, since in our study relatively large brain regions were homogenized.

Hypothetically, these mental and biochemical effects could be because of effects of T4, which have now been well established (e.g., Ref. 15). Interestingly, Davis et al. (8) reported that T4 activates the MAPK and induces serine phosphorylation of the thyroid hormone receptor (TR)/β1. The effects of hyperthyroidism may theoretically also be mediated by elevated levels of T3 in other subcellular structures, such as the mitochondria or synaptosomes, and/or by other iodothyronine metabolites such as 3,5-T2 (18); or by “peripheral” influences, such as changes in heart rate, oxygen sup-

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**Fig. 6.** Concentrations of T3 in aliquots of the homogenate and subcellular fractions of 11 brain regions.

**Fig. 7.** T3 concentrations in aliquots of the homogenate and 5 subcellular fractions of the midbrain after addition of 150 pg T3 before homogenization and in control tissue. **P < 0.01 compared with controls.**
ply, glucose availability, etc.; or finally by effects of the underlying disease, for example immunological effects on specific brain parameters in Graves’ disease.

It is particularly intriguing that those mental and cognitive functions such as cognitive performance, mood, and anxiety, which are most often affected in hyperthyroidism, are mainly regulated in brain regions in which even high doses of T4 failed to induce increases in T3 concentrations (cortical areas, the hippocampus and amygdala) in the present study. In light of these data, it does not seem surprising any more that the adult vertebrate brain has long been considered to be unresponsive to thyroid hormones (e.g., Ref. 26) and that effects of thyroid hormone dysfunction on gene expression have been reported almost exclusively in brains of hypothyroid animals (e.g., Ref. 19).

It is also interesting that cortical SDH activity was reduced in both hypo- and hyperthyroidism in spite of normal mitochondrial T3 levels in hyperthyroid animals. Such common effects of hypo- and hyperthyroidism may be involved in the as yet unexplained fact that both types of thyroid dysfunction may also induce very similar psychopathological symptoms (43, 44). Another explanation for this phenomenon may be our finding that brain concentrations of T3 can also be reduced in hyperthyroidism.

With regard to the reliability of the quantifications of thyroid hormones in subcellular fractions, one major concern we had before performing these experiments was that we might measure some artifacts resulting from a “redistribution” of the lipophilic thyroid hormones during the different steps of the centrifugation procedure. However, T3 added during homogenization was recovered from the homogenates only, and not from the subcellular fractions. The possibility of artifacts resulting from another “redistribution effect” cannot be excluded: molecules of T3 not covalently bound to structures such as myelin or synaptosomes may separate from them during centrifugation and then be recovered in the homogenates or supernatants. However, such redistribution effects involving synaptosomes or nuclei are not likely to occur. In regard to the synaptosomes, a previous study (28) found a maximum binding capacity of high-affinity synaptosomal binding sites of 4.2 pg T3/mg protein in broken synaptosomes compared with 3.0 pg T3/mg protein in intact synaptosomes in adult rat cortex and 1.7 pg T3/mg protein in the cerebellum. These values are almost identical to

Table 2. Activities of succinate dehydrogenase in mitochondria, of Na+K+-ATPase in synaptosomes and of CNPase in the myelin of the parietooccipital cortex of groups of thyroid dysfunc. 

<table>
<thead>
<tr>
<th></th>
<th>Succinate Dehydrogenase Activity, ( \mu \text{mol} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1} )</th>
<th>Na+K+-ATPase Activity, ( \text{nmol} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1} )</th>
<th>CNPase Activity, ( \text{mol} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.6 ± 0.39</td>
<td>377.3 ± 11.1</td>
<td>1,729 ± 29</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>10.7 ± 0.85**</td>
<td>364.9 ± 14.9</td>
<td>1,771 ± 21</td>
</tr>
<tr>
<td>( T_3 )</td>
<td>11.1 ± 0.08**</td>
<td>367.8 ± 17.1</td>
<td>1,723 ± 29</td>
</tr>
<tr>
<td>( T_4 ) (24 \mu g)</td>
<td>13.1 ± 1.40</td>
<td>377.5 ± 10.9</td>
<td>1,738 ± 15</td>
</tr>
<tr>
<td>( T_4 ) (96 \mu g)</td>
<td>11.8 ± 0.70**</td>
<td>381.7 ± 26.9</td>
<td>1,737 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE. CNPase, 2',3'-cyclic nucleotide 3'-phosphohydrolase. **P < 0.01.
our own results and indicate that the specific T₃ binding sites in brain synaptosomes are localized mainly on synaptic membranes. Quantification of T₃ on broken synaptosomes isolated after freezing therefore poses no problem.

With regard to the nuclear concentrations of T₃, Schwartz and Oppenheimer (38) have reported high concentrations of T₃ binding sites in the cortex (0.73 ng T₃/mg DNA), a smaller binding capacity for the hypothalamus (0.1 ng T₃/mg DNA), and the lowest capacity for the cerebellum (0.01 ng T₃/mg DNA; see Ref. 36). Others have reported similar results in vivo (6) and in primary cultures of rat brain (21). These data are entirely consistent with our own results, i.e., no enrichment in nuclear T₃ in the hypothalamus, cerebellum, medulla, or midbrain.

In conclusion, the quantification of T₃ in subcellular fractions seems to be reliable and enabled us to detect changes in hormone concentrations that were not evident from measurements in the homogenate. In particular, nuclear T₃ concentrations remained unchanged in the cerebellum of hyperthyroid rats in spite of elevated hormone levels in the homogenates of the same tissues. The effects of pharmacological and nonpharmacological treatments on thyroid hormone metabolism and concentrations in rat brain. Endocrinology 141: 1027–1040, 2000.

REFERENCES


