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Effects of Pharmacological and Nonpharmacological Treatments on Thyroid Hormone Metabolism and Concentrations in Rat Brain*

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ABSTRACT

The activities of the 5′I-deiodinase (5′D-I), 5′II deiodinase (5′D-II) and 5III-deiodinase (5D-III) isoenzymes and tissue concentrations of thyroxine (T_4) and triiodothyronine (T_3) were measured in up to 10 regions of the rat brain after acute and subchronic nonpharmacological (sleep deprivation, 12 h fasting, 14 days' calorie-reduced diet) and pharmacological (ethanol, haloperidol, clozapine, lithium, carbamazepine, desipramine, fluoxetine, tranylcypromine, and mianserin) treatments. All of these treatments induced significant and sometimes dramatic changes in 5′D-II activities and tissue concentrations of thyroid hormones and, to a lesser extent, in 5D-III activity. The activity of 5′D-I remained unaffected. The results revealed a surprising specificity for each type of treatment in terms of the isoenzyme and hormone affected, the direction of the change, the brain region affected and the time of day. The changes in thyroid hormone concentrations frequently failed to correspond in any way to those in deiodinase activities and unexpected effects such as inhibition of both 5′D-II and 5D-III were seen, indicating that there may be additional pathways of iodothyronine metabolism in the CNS. In conclusion, particularly 5′D-II activity and thyroid hormone concentrations in the CNS are highly sensitive to many different kinds of influence that may induce changes in neuronal activity. However, these changes in deiodinase activities do not ensure stable tissue concentrations of T_3, but were, on the contrary, in most cases accompanied by marked changes T_3 levels in the tissue. The implications of these findings for the physiological role of thyroid hormones in the CNS are discussed. (*Endocrinology* **141:** 1027–1040, 2000)

THYROID HORMONE metabolism in rat CNS is subject to a highly specific regulatory mechanism that differs substantially from that described in other tissues such as the liver or kidney. In these latter organs most of the active iodothyronine compound 3,3′,5′triiodothyronine (T_3) is taken up directly from the blood, whereas the T_3 supply of the brain depends mainly on cellular uptake and intracellular deiodination of thyroxine (T_4) (1). Furthermore, the mechanisms of deiodination in the CNS are also very different from those described in the liver or kidney. Whereas in peripheral tissues of the rat type I 5′iodothyronine deiodinase (5′D-I) catalyses both phenolic and tyrosyl ring deiodination of T_4 and T_3, in the CNS two other isoenzymes catalyze the production and metabolization of T_3. Type II 5′-iodothyronine deiodinase (5′D-II) catalyses 5′-deiodination of T_4 and rT_3 to T_3 and 3,3′-T_2, respectively. Type III 5-iodothyronine deiodinase (5D-III) catalyses tyrosyl ring deiodination of T_4 to rT_3 and that of T_3 to 3,3′-T_2, thereby inactivating T_3 (2–4; for a review see Ref. 5). The physiological significance of 5′D-I in the rat CNS is unclear and the levels of this isoenzyme in the human CNS are below the limit of detection (6). All three deiodinases have recently been cloned in rats and humans and proved to be selenoproteins (7–9). Subsequent investigations revealed that 5′D-II and 5D-III are also expressed in several tissues other than the CNS, *e.g.* in the placenta, skeletal muscle, ovaries, testes, and thyroid gland, etc. (10, 11).

As regards the regulation of the activities of 5′D-II and 5D-III in the CNS, there is good evidence that the activity of 5′D-II is inhibited and that of 5D-III stimulated by different iodothyronine compounds, whereas in hypothyroidism the reverse changes occur (12, 13). It is believed that the purpose of this "autoregulatory mechanism" is to protect the CNS against unphysiological changes in T_3 concentrations in the case of hypo or hyperthyroidism.

However, recent evidence suggests that the concentrations of T_3 in the CNS may vary substantially after pharmacological interventions and even under physiological conditions. There is, for example, a significant circadian rhythm in 5′D-II deiodinase activity in the rat CNS, with significant variations in tissue levels of T_3 (14). We recently reported the surprising finding that even mild stress such as handling a rat causes dramatic increases in 5′D-II activities and T_3 concentrations in specific regions of the rat brain (15).

As clinical studies support an effect of antidepressant drugs on thyroid hormone metabolism, in recent years we have investigated the effects of different antidepressant medications on thyroid hormone metabolism and concentrations in the rat CNS. We hypothesized that all of these drugs would enhance the tissue concentrations of T_3 in at least one relevant brain region. Stimulation of 5′D-II was indeed found in 8 out of 11 brain regions following treatment with the norepinephrine reuptake inhibitor desipramine (16). However, T_3 levels were elevated in only two of these regions (17). Further investigations

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revealed that many different drugs used in the treatment and prophylaxis of affective disorders do in fact affect deiodinase activities in rat brain. The results of these studies which are reported here showed effects that were specific for each type of treatment in terms of the isoenzyme affected, the direction of the change, the brain region involved and the time of day. No common effects on deiodinase activities of all the drugs investigated were evident.

These data are therefore disappointing from a psychopharmacological point of view, but raise the question as to whether thyroid hormone homeostasis in the CNS is more sensitive and its regulation more complex than generally thought. To learn more about this issue we also investigated the effects of a variety of nonpharmacological (sleep deprivation, 12 h fasting, and 14 days of calorie-reduced diet) and nonantidepressant, pharmacological (ethanol, neuroleptic drugs and one anticonvulsant) treatments on the activities of both 5′D-II and 5D-III and also on the tissue concentrations of T_4 and T_3. The results are reported together with those of investigations on four antidepressants and one prophylactic drug. The activities of 5′D-I in the CNS and liver and pituitary were determined in several selected subgroups only as the physiological relevance of this isoenzyme in rat CNS is questionable (5) and its activity has not been demonstrated in human brain (6).

The results obtained for Group 17 (treatment with DMI, Table 1) and the deiodinase activities (but not the tissue levels of T_3 and T_4) of the groups treated subchronically with fluoxetine, lithium and carbamazepine (Groups 15, 16, and 18) have been previously reported in a psychopharmacological context (16–19). All other results presented in this study have not been previously published.

TABLE 1. Serum concentrations of thyroxine (T_4) and triiodothyronine (T_3) in all experimental groups

Group	Design	Treatment duration	T_4 (nmol/liter)	T_3 (nmol/liter)	Mean drug serum concentration
1.	Sleep deprivation	24 h	90.6 ± 3.1[a]	2.3 ± 0.2[a]	
	Controls		65.3 ± 2.0	1.3 ± 0.1	
2.	12-h food deprivation	12 h	70.3 ± 4.0	0.7 ± 0.2[a]	
	Controls		60.2 ± 3.5	1.0 ± 0.03	
3.	14-day calorie-reduced diet (5 am)	14 days	54.4 ± 3.2	1.3 ± 0.06	
	Controls		59.2 ± 3.4	1.4 ± 0.1	
4.	14-day calorie-reduced diet (8 pm)	14 days	63.5 ± 3.2	1.6 ± 0.2	
	Controls		60.3 ± 4.2	1.2 ± 0.03	
5.	Ethanol 30 min	30 min	71.5 ± 4.6	1.0 ± 0.2	0.84 ± 0.05‰
	Ethanol 120 min	120 min	75.3 ± 4.5	1.1 ± 0.1	0.39 ± 0.03‰
	Controls		83.0 ± 2.2	1.3 ± 0.03	
6.	Ethanol 14 days (8 am)	14 days	77.8 ± 4.5	1.1 ± 0.08	n.m.
	Controls		85.7 ± 2.5	1.2 ± 0.04	
7.	Ethanol 14 days (8 pm)	14 days	66.0 ± 2.4	0.9 ± 0.02	n.m.
	Controls		66.1 ± 3.4	0.9 ± 0.06	
8.	Haloperidol 1 mg/kg	14 days	74.3 ± 2.5	1.2 ± 0.05	Not detectable
	Controls		79.3 ± 1.5	1.0 ± 0.1	
9.	Clozapine 20 mg/kg	14 days	90.7 ± 3.6[a]	1.7 ± 0.08[a]	n.m.
	Controls		79.0 ± 2.6	1.2 ± 0.02	
10.	Lithium 7.5 mmol/kg (0800 h)	12 h	17.4 ± 2.2[a]	0.3 ± 0.03[a]	2.6 ± 0.1 mmol/liter
	Controls		57.4 ± 2.5	0.8 ± 0.03	
11.	Lithium 7.5 mmol/kg (2000 h)	24 h	26.1 ± 0.7[a]	0.4 ± 0.01[a]	1.9 ± 0.1 mmol/liter
	Controls		58.6 ± 1.3	0.7 ± 0.03	
12.	Lithium 3 mmol/kg (0800 h)	12 h	63.5 ± 2.5	0.8 ± 0.1	0.44 ± 0.07 mmol/liter
	Controls		58.2 ± 3.2	1.0 ± 0.2	
13.	Carbamazepine 40 mg/kg (0800 h)	12 h	60.2 ± 0.2	0.8 ± 0.1	Not detectable
	Controls		61.3 ± 4.0	1.0 ± 0.1	
14.	Carbamazepine 40 mg/kg (2000 h)	24 h	64.3 ± 4.0	1.0 ± 0.1	Not detectable
	Controls		71.5 ± 2.2	1.1 ± 0.3	
15.	Lithium 0.15%	14 days	79.5 ± 4.5[a]	1.4 ± 0.03[a]	0.7 ± 0.08 mmol/liter
	Lithium 0.3%		45.8 ± 5.2[a]	0.9 ± 0.02[a]	1.3 ± 0.1 mmol/liter
	Controls		61.4 ± 3.3	1.2 ± 0.03	
16.	Carbamazepine 0.4%	14 days	40.7 ± 6.2[a]	1.0 ± 0.05[a]	6.0 ± 0.7 mg/liter
	Controls		60.8 ± 3.2	1.2 ± 0.05	
17.	Desipramine 5 mg/kg	14 days	70.6 ± 2.0	1.4 ± 0.1	
	Desipramine 20 mg/kg		57.5 ± 3.0[a]	1.1 ± 0.1	n.m.
	Desipramine 50 mg/kg		44.2 ± 6.5[a]	0.4 ± 0.2[a]	
	Controls		68.5 ± 2.5	1.2 ± 0.05	
18.	Fluoxetine 15 mg/kg	14 days	59.4 ± 1.8[a]	1.0 ± 0.2	n.m.
	Controls		74.3 ± 1.5	1.1 ± 0.1	
19.	Tranylcypromine 5 mg/kg	14 days	62.3 ± 7.0[a]	1.0 ± 0.2	n.m.
	Controls		81.5 ± 3.5	1.4 ± 0.1	
20.	Mianserine 20 mg/kg	14 days	60.3 ± 1.5[a]	1.2 ± 0.1	n.m.
	Controls		70.3 ± 2.3	1.2 ± 0.3	
21.	Young controls		68.4 ± 2.5	1.2 ± 0.1	
	Old controls		63.2 ± 2.6	1.0 ± 0.1	

[a] <0.05.
n.m., Not measured.

Materials and Methods

Materials

T_4, T_3, rT_3, 3,3′-T_2 and 3,5- T_2 of the highest available purity were obtained from Henning GmbH (Berlin, Germany). (5′-125I)-T_4, (5′-125I)-rT_3 and (3′-125I)-T_3 were prepared for iodothyronine deiodinase assays and RIAs by radioiodination of T_3, 3,3′-T_2 and 3,5-T_2 respectively, as previously described (20). The tracers with specific radioactivities of 50–75 MBq/nmol were repurified immediately before use with disposable Sep-Pak C18 cartridges (Waters Associates, Milford, MA) yielding a purity >99% with ^{125}I- as the only contaminant. Inner-ring labeled [5-^{125}I]-T_4 and [5-^{125}I]-T_3 (specific radioactivity 1.0–1.5 MBq/nmol) were purchased from R. Thoma, (Formula GmbH, Berlin). Dithiothreitol (DTT) was purchased from Roche Molecular Biochemicals (Mannheim, Germany); 6-n-propyl-2-thiouracil (PTU), iopanoic acid, (IOP) and aurothioglucose (ATG) were obtained from Sigma (Munich, Germany) All other chemicals were of reagent grade.

Carbamazepine, desipramine, and clozapine were gifts from Novartis Pharmaceuticals (Basel, Switzerland). Fluoxetine was donated by Eli Lilly & Co. (Indianapolis). Lithium, tranylcypromine, haloperidol and mianserin were bought from Sigma. Pellets containing carbamazepine and lithium as well as the control pellets were prepared by Altromin (Lage, Germany).

Animal treatments

All of the animal experiments described in this study were evaluated and approved by the Animal Protection Committee of the Berlin Senate. Adult male euthyroid Sprague Dawley rats weighing 250 to 300 g were employed throughout. They were housed in individual cages on a 12-h light, 12-h dark schedule (lights on at 0600 h) and had access to food and water *ad libitum*. Unless stated otherwise 24 rats were used for each experiment. Deiodinase activities were measured in 6 rats in each group, while thyroid hormone concentrations were determined in a further 6. The remaining 12 served as controls (6 for deiodinase activities and 6 for thyroid hormone concentrations). Experimental groups which received a drug for 14 days were always decapitated 24 h after the last dose unless otherwise stated. Drugs were usually administered and the rats decapitated at about noon, unless otherwise stated. The following groups were investigated (see Table 1).

Group 1 (sleep deprivation). Each of the 12 rats was placed in one of 6 drums which were rotated at a speed of one revolution per 45 sec. The rats were placed in the drums at 1000 h and remained there for 24 h. Food and water were available *ad libitum* throughout the whole procedure. At between 1000 h and 1200 h on the next day they were killed by decapitation without anesthesia together with 12 control rats, which received no treatment at all.

Group 2 (12 h fasting). This group was completely deprived of food at 2000 h and decapitated at between 0800 and 0900 h on the next morning. The 12 control rats received no specific treatment.

Groups 3 and 4 (14 days on a calorie-reduced diet). Twelve rats received a diet adjusted on a daily basis to achieve a weight reduction of approximately 50% within a 2-week period. As control rats of the same age usually undergo a weight increase of approx. 30% within 14 days, the diet was adjusted so as to induce a weight loss of approx. 20% of the initial weight during a 2-week period. On Day 14, Group 3 had lost 16.2 ± 4.5% of their initial body weights and Group 4 15.3 ± 3.9%, while the two control groups had gained 29.5 ± 3.5% and 31.1 ± 4.5%, respectively.

Group 5 (ethanol, acute). Twelve rats received 1 g ethanol/kg body weight and 12 control rats received the same volume of saline by gavage at approximately 1200 h. Twelve rats were decapitated 30 min later, and the other twelve 120 min later.

Groups 6 and 7 (ethanol, 14 days). Twelve rats received a 5% solution of ethanol as sole fluid during a 14-day period. Six of these rats were decapitated at 0800 h and the remaining 6 at 2000 h, each group of 6 together with the corresponding controls, which received pure water *ad libitum*. The ethanol was not withdrawn before decapitation. In this group only deiodinase activities were determined.

Groups 8 and 9 (neuroleptic drugs). Group 8 was treated for 14 days with 1 mg/kg haloperidol, which was suspended in a 2% Tween 80 solution and administered by gavage. Haloperidol is a "classical" and potent neuroleptic drug used in the treatment of schizophrenia. It is strong antagonist of dopamine 2 receptors and induces extrapyramidal side effects. Group 9 was given 20 mg/kg clozapine ip once daily for 14 days. The clozapine was also suspended in a 2% Tween 80 solution. Clozapine is an atypical and potent neuroleptic drug that does not have extrapyramidal side effects. The control rats of Group 8 received the same volume (7.5 ml) of a 2% Tween 80 solution (dissolved in saline) by gavage. The controls of Group 9 received the vehicle ip.

Groups 10 and 11 (lithium, acute, I). Twelve rats each received a single ip injection of 7.5 mmol/kg lithium chloride at approximately 2000 h and 12 control rats received an injection of saline ip. Twelve rats were decapitated after 12 h, the remaining twelve 24 h later, each group together with 12 control rats.

Group 12 (lithium, acute, II). As the dose of lithium employed for acute treatment in Groups 10 and 11 (7.5 mmol/kg) yielded highly toxic serum concentrations of lithium (2.6 ± 0.1 and 1.9 ± 0.1 mmol/liter, see Table 1), a further group was treated with one ip injection of 3 mmol lithium/kg at 2000 h and killed 12 h later. The control rats received an injection of saline ip.

Groups 13 and 14 (carbamazepine, acute). Twelve rats received 40 mg/kg carbamazepine ip at 2000 h, 12 control rats received saline ip. Twelve rats were killed after 12 h, the remaining twelve 24 h later.

Group 15 (lithium, subchronic). Twelve rats received a 0.15% lithium diet and the other 12 a 0.3% lithium diet for two weeks. Twelve control rats received the same pellets without lithium.

Group 16 (carbamazepine, subchronic). Twelve rats received a 0.4% carbamazepine diet for 2 weeks. Twelve controls received the pellets without carbamazepine.

Groups 17 to 20 (antidepressants, subchronic). Different groups of rats were treated with the drugs shown in Table 1 for 14 days. Desipramine is a norepinephrine reuptake inhibitor, fluoxetine a serotonin reuptake inhibitor, tranylcypromine an MAO inhibitor and mianserin an atypical antidepressant. All these drugs were dissolved in NaCl. Desipramine and fluoxetine were administered by gavage, tranylcypromine, and mianserin ip. The control rats received saline by the same route as the respective drug-treated group.

Group 21 (age effects). Six rats each weighing approximately 200 g were decapitated together with 6 rats aged 2 yr. In this group only deiodinase activities were determined.

All rats were decapitated without anesthesia. Their brains were dissected according to Glowinski and Iversen (21). The pituitaries and livers were also removed and all tissues stored immediately at −70 C. Blood was drawn from the decapitation wound, centrifuged and the serum stored at −20 C.

Iodothyronine deiodinase assays

The deiodinase activities were measured as previously described (16). Tissue samples were homogenized individually on ice in 5 to 6 vol of 0.25 M sucrose, 10 mM HEPES (pH 7.0) containing 10 mM dithiothreitol (DTT) and immediately frozen in a dry ice/acetone bath and stored at −80 C until assay. The measurement of 5′D-I, 5′D-II and 5D-III was based on the release of radioiodide from the ^{125}I-labeled substrates (22).

5′D-I and 5′D-II assay. The activity of 5′D-I was determined by measuring the release of radioiodide from 100,000 cpm (~ 2.5 kBq) (5′-125I)-rT_3 at 5 nM rT_3, 20 mM DTT, in the presence (for 5′D-II) and absence (5′D-I + 5′D-II) of 5′D-I inhibiting 6-*n*-porpyl-2-thiouracil (PTU) (4). 5′D-II activity was determined using (5′-125I)-T_4 as substrate in the presence of 6 nM T_4, 30 mM DTT, 1 mM PTU, and 1 μM T_3, to inhibit the inner ring deiodination of T_4 in those tissues containing significant 5D-III activity (4).

The measurement was conducted after 45- to 90-min (usually 60 min) incubation at 37 C with 50–100 μg of protein from the crude homogenate in 100 μl of 0.1 M potassium phosphate buffer (pH 7.0), 1 mM EDTA. The reaction was started by the addition of the tissue homogenate and

stopped by adding 50 μl ice-cold 5% BSA and 10 mM PTU, followed by 400 μl of 10% ice-cold trichloroacetic acid. After centrifugation at 4000 × *g* for 30 min, the supernatant containing the $^{125}I^-$ was further purified by cation exchange chromatography on 1.6-ml Dowex 50 WX 8 columns (100–200 mesh) (Serva GmbH and Co., Heidelberg, Germany). The iodide was then eluted with 2 1-ml aliquots of 10% acetic acid and the radioactivity was counted in a γ-counter.

5D-III assay. For determination of 5D-III (inner-ring deiodinase) 20–70 μg protein were incubated in a final volume of 100 μl 0.1 M potassium phosphate buffer (pH 7.4), 1 mM EDTA with approximately 1.2 kBq (~50,000 cpm) inner-ring labeled [5-^{125}I]-T_3, at 50 nM T_3, 20 nM DTT, and 1 mM PTU for 60 min at 37 C. Radioiodide release was measured as described above.

Preliminary experiments established that for each tissue (a) the reaction rates were constant over time for up to 120 min in the presence and absence of PTU; (b) the reaction rates were proportional to protein concentrations in the ranges used (50–100 μg/tube in the 5′D-I and 5′D-II assays; 20–70 μg/tube in the 5D-III assay) and (c) after incubation, equal amounts of 3,3′-T_2 and I^- were produced from rT_3 (5′D I+II assay) or from T_3 (5D-III assay) in homogenates from each of the different tissues, as determined by reversed-phase HPLC of the incubation extracts. Likewise, it was established that equal amounts of T_3 and I^- were produced from T_4 in the T_4 5′D-II assay.

In all assays, control incubations substituted homogenization buffer for tissue homogenates and the amount of $^{125}I^-$ produced in the tissue-free controls (usually 0.3–0.5% of the total radioactivity added) was then subtracted from the sample results.

Because the substrates were randomly labeled with ^{125}I at the equivalent 3′ or 5′ positions of the phenolic ring (for rT_3 and T_4) or at the equivalent 3 or 5 positions of the tyrosyl ring (for inner-ring labeled T_3), the labeled iodide release was half that of the degraded iodothyronines. This was accounted for in the analysis of the data. The reaction conditions selected were such that <10–15% of the substrate was consumed by enzymatic deiodination. Each experimental point was determined in triplicate with coefficients of variation of less than 5%.

Determination of tissue concentrations of T_4 and T_3

The tissue concentrations of T_4 and T_3 were determined by RIA as previously described (17, 23). Briefly, tissue samples were homogenized in 100% methanol-containing 1 mM PTU and 100 μM phloretin, extracted into chloroform-methanol and back extracted into an aqueous phase, which was then purified through Bio-Rad Laboratories, Inc. AG 1 x 2 resin columns (Bio-Rad Laboratories, Inc. Richmond, CA). The iodothyronines were eluted with 70% acetic acid, evaporated to dryness and taken up in the RIA buffer. The limits of sensitivity were 3.0 and 2.5 pg for T_4 and T_3, respectively. All the samples of a particular tissue were processed individually and extracted and assayed together at the same time. Each sample was determined in triplicate. Intraassay variations were 5.1 and 7.6% for the T_4 and T_3 RIAs, respectively; the corresponding interassay variations were 5.9 and 8.2%. Molar cross-reactivities of the anti-T_4 and anti-T_3 sera have been reported previously (20). The results were corrected on the basis of individual recovery data obtained after addition of maximal specific activity [^{125}I]T_3 and [^{131}I]T_4 to every sample during the initial extraction process. The quantities of radioactive T_3 and T_4 added (~1,000 cpm per sample) represented approximately 0.02–0.5% of the amount of endogenous hormone. Recovery ranged between 80 and 90% for extracted T_3 and between 70 and 80% for T_4. Tissue concentrations are given in nanograms of T_4 or T_3 per gram wet weight.

Determinations of the serum concentrations of T_4, T_3, and TSH

The serum levels of T_4 and T_3 were determined by a slightly modified double-antibody RIA as previously described for human serum (20). For assaying total T_4 and T_3 in the rat sera, standards were set up in iodothyronine-free rat serum. The serum levels of TSH were measured by a specific RIA developed for the rat, using immunoreactants kindly supplied by the National Institute of Arthritis, Diabetes & Digestive and Kidney Diseases of the National Institutes of Health (Bethesda, MD).

Statistical analysis

The data are given as means ± SEM. *P* values of less than 0.05 were considered significant. Individual comparisons were performed using the Mann-Whitney *U* test. Altogether, 267 statistical comparisons have been calculated in Table 1 and Figs. 1–8. When a significance level of $P < 0.05$ was applied, approx. 13 "significant" results would be expected to occur by chance. Strict application of Bonferroni's correction of the *P* value would result in a level of significance of $P > 0.00025$, in which case the results of only 12 statistical calculations would remain significant. However, in our study we found 98 significant results. Thus, if Bonferroni's correction were strictly applied, it is likely that several relevant findings would be lost. We therefore discuss critically whether all results yielding *P* values of less than 0.05 seem plausible or may reflect statistical artefacts.

Results

Group 1

Twenty-four hour sleep deprivation significantly enhanced 5′D-II activity in the cortex, hippocampus, limbic forebrain and midbrain, but not in the hypothalamus or cerebellum (Fig. 1). The tissue levels of T_3 were elevated in all brain regions except the hypothalamus. 5D-III activity and the tissue levels of T_4 were not significantly influenced. 5′D-I activity was measured in the cortex, hippocampus, and liver and found to be unchanged (data not shown). The serum concentrations of T_4 and T_3 were also significantly higher than in the controls (Table 1). However, the TSH levels remained unchanged (2.12 ± 1.02 ng/ml and 2.18 ± 0.99 ng/ml for the controls and experimental rats, respectively).

Group 2

The effects of 12-h food deprivation on thyroid hormone parameters are presented in Fig. 2. As in Group 1, significant increases in 5′D-II activity were noted in four brain regions; however, in contrast to the results for Group 1, the levels of T_4 in the groups having fasted were dramatically lower than in the controls in three out of four brain regions, whereas the tissue levels of T_3 remained unchanged. The serum levels of T_4 remained unchanged, but those of T_3 were significantly lower in the fasting group than in the controls (Table 1). The serum levels of TSH were not significantly affected in this or any of the remaining groups (data not shown).

Groups 3 and 4

In contrast to the results after 12 h fasting, 14 days on a calorie-reduced diet induced a pronounced reduction in 5′D-II activity in the frontal cortex in the rats killed at 0500 h, but not in those killed at 2000 h (Fig. 2). In addition, the group decapitated in the early morning also had significantly lower 5D-III activities and tissue concentrations of T_3 and T_4 than the controls. In the group decapitated in the evening, a nonsignificant trend in the same direction was noted for the tissue levels of T_4 and T_3. No significant effects were noted for the serum concentrations of T_4, T_3, or TSH.

Group 5

Administration of ethanol significantly reduced the activity of 5D-III in the frontal cortex after 120 min and in the amygdala after both 30 and 120 min (Fig. 3, *left*). All other tissue and serum parameters were unaffected. The mean

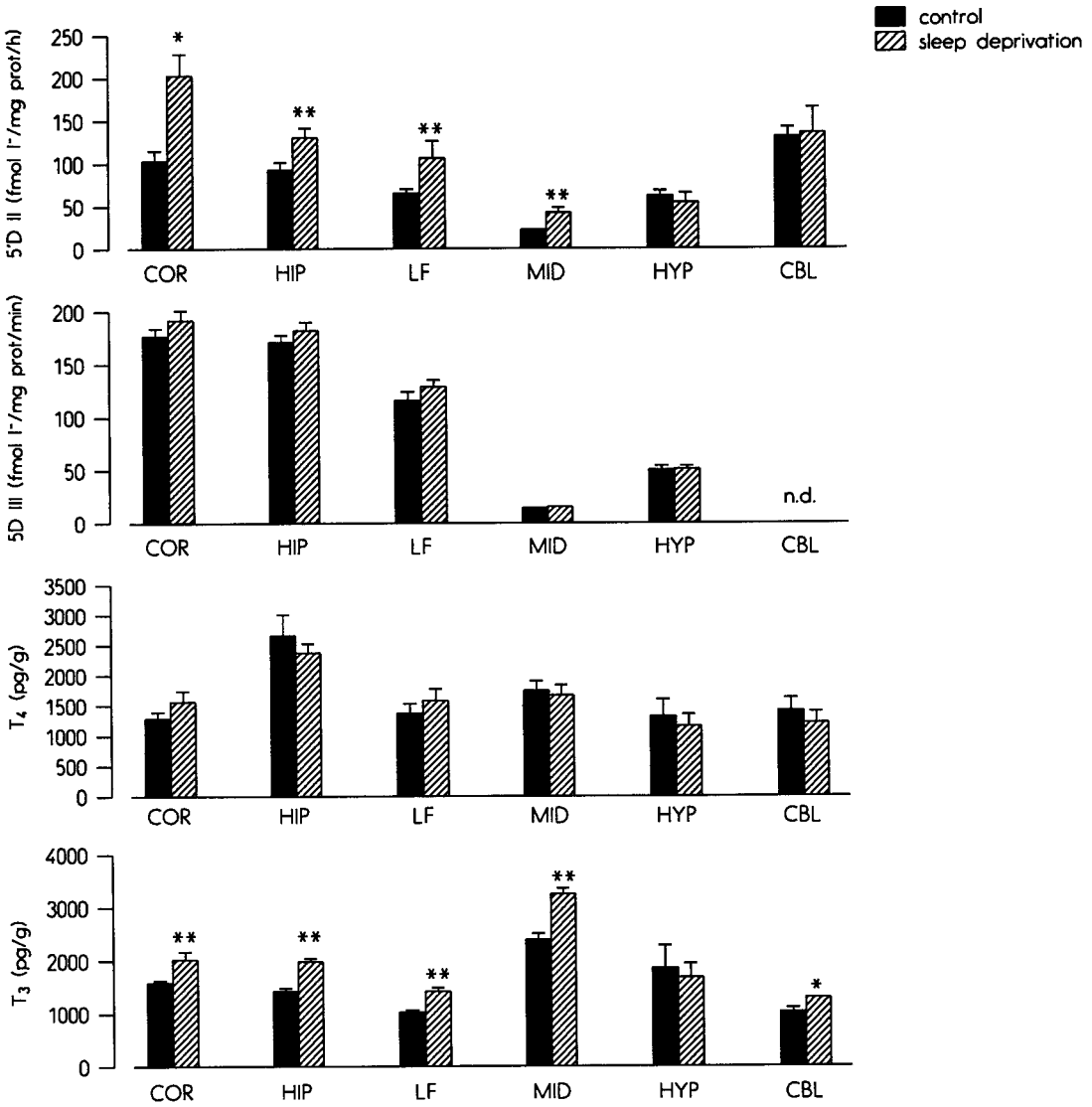

FIG. 1. Effects of 24-h sleep deprivation on the activities of the 5′II deiodinase (5′D-II) and the 5III deiodinase (5D-III) and on concentrations of T_4 and T_3 in 6 regions of the rat brain. COR, Cortex; HIP, hippocampus; LF, limbic forebrain; MID, midbrain; HYP, hypothalamus; CBL, cerebellum.

blood concentrations of ethanol were 0.84 ± 0.05‰ and 0.39 ± 0.03‰ after 30 and 120 min, respectively.

Groups 6 and 7

After 14 days of ethanol treatment, 5′D-II activity was enhanced in the cortex at 2000 h only and in the amygdala at both measuring times (Fig. 3, *right*). 5D-III activity was reduced in the cortex at 0800 h and in the amygdala at 2000 h only. The tissue levels of T_4 and T_3 were not measured in this group and the serum levels were not significantly affected (Table 1).

Group 8

Fourteen days of haloperidol treatment induced an increase in 5′D-II activity in the cortex. All other parameters shown in Fig. 7 remained unchanged. Also, 5′D-I activity was not affected in the cortex, pituitary or liver (data not shown).

Group 9

Fourteen days of treatment with the atypical neuroleptic drug clozapine inhibited 5′D-II activity in 5 out of 10 brain regions and 5D-III activity in 4 out of 8 brain regions (Fig. 4). 5′D-I activity was unchanged in all these brain regions and also in the pituitary, liver, and kidney. The tissue concentrations of T_4 and T_3 were measured in the cortex. They were not significantly affected by clozapine (data not shown). The serum concentrations of T_4 and T_3 rose significantly (Table 1), whereas the TSH levels remained unchanged (data not shown).

Groups 10 and 11

Twelve hours after a single administration of the highly toxic dose of 7.5 mmol/kg lithium (*i.e.* at 0800 h) a dramatic, almost 6-fold increase in 5′D-II activity was found in the frontal cortex. The increase was also significant in the group

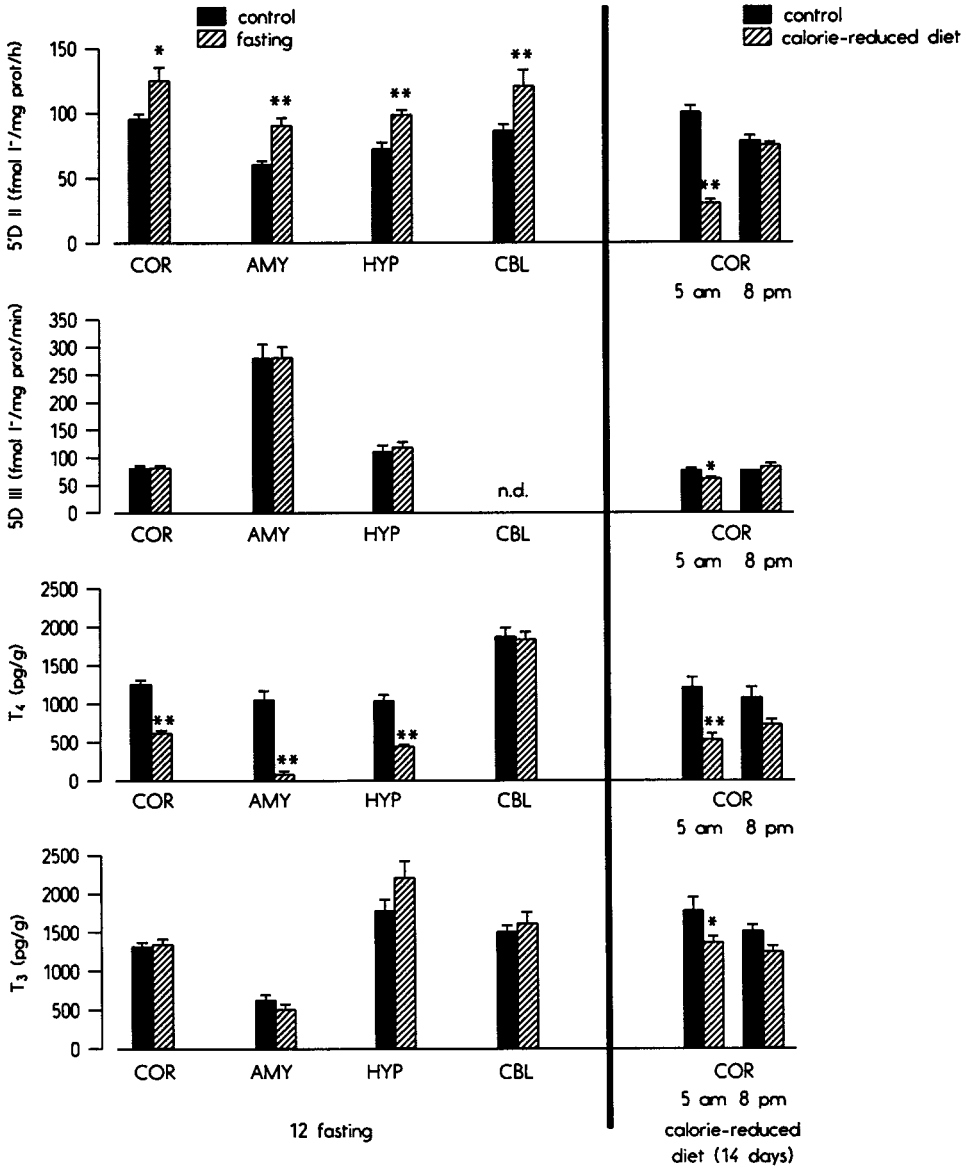

FIG. 2. *Left side*, Effects of 12-h fasting on deiodinase activities and on thyroid hormone concentrations in four regions of the rat brain. *Right side*, Effect of 14 days of calorie-reduced diet on deiodinase activities and thyroid hormone concentrations in the cortex in two groups of rats decapitated at 0500 h and 2000 h, respectively. AMY, Amygdala. For an explanation of all other abbreviations, see Fig. 1.

killed 24 h after administration of lithium (2000 h, Fig. 5). This increase was specific for the cortex because no effects were seen on the 5′D-II activities in the hypothalamus and pituitary (data not shown). The 5D-III activities remained unchanged, but cortical levels of T_4 fell sharply to about 25% of the original values at both measuring times. The tissue concentrations of T_3 were also significantly reduced, but to a lesser extent. The serum concentrations of T_4 and T_3 were drastically reduced at 0800 h and still significantly lower than in the controls at 2000 h (Table 1). The TSH levels remained unaffected (data not shown). The mean serum concentrations of lithium were in the toxic range at both 0800 h (2.6 ± 0.1 mmol/liter) and 2000 h (1.9 ± 0.1 mmol/liter).

Group 12

The effects of a single dose of the low dose of 3 mmol/kg lithium on thyroid hormone parameters after 12 h are shown in Fig. 6. 5′D-II activity was significantly elevated in all four brain regions. The tissue levels of T_4 were distinctly lower than in controls, whereas cortical concentrations of T_3 were significantly enhanced in three out of four areas, respectively. The serum levels of thyroid hormones were not affected. The mean serum concentration of lithium was 0.44 ± 0.02 mmol/liter, *i.e.* below the range considered to be clinically effective in patients with affective disorders.

Groups 13 and 14

The only effect of an acute dose of carbamazepine was a significant increase in 5D-III activity at 2000 h in the frontal cortex (Fig. 5). The serum levels of carbamazepine were not detectable at either 0800 h or 2000 h.

Group 15

Fourteen days of administration of two different dosages of lithium had opposing effects on 5′D-II activities (Fig. 5,

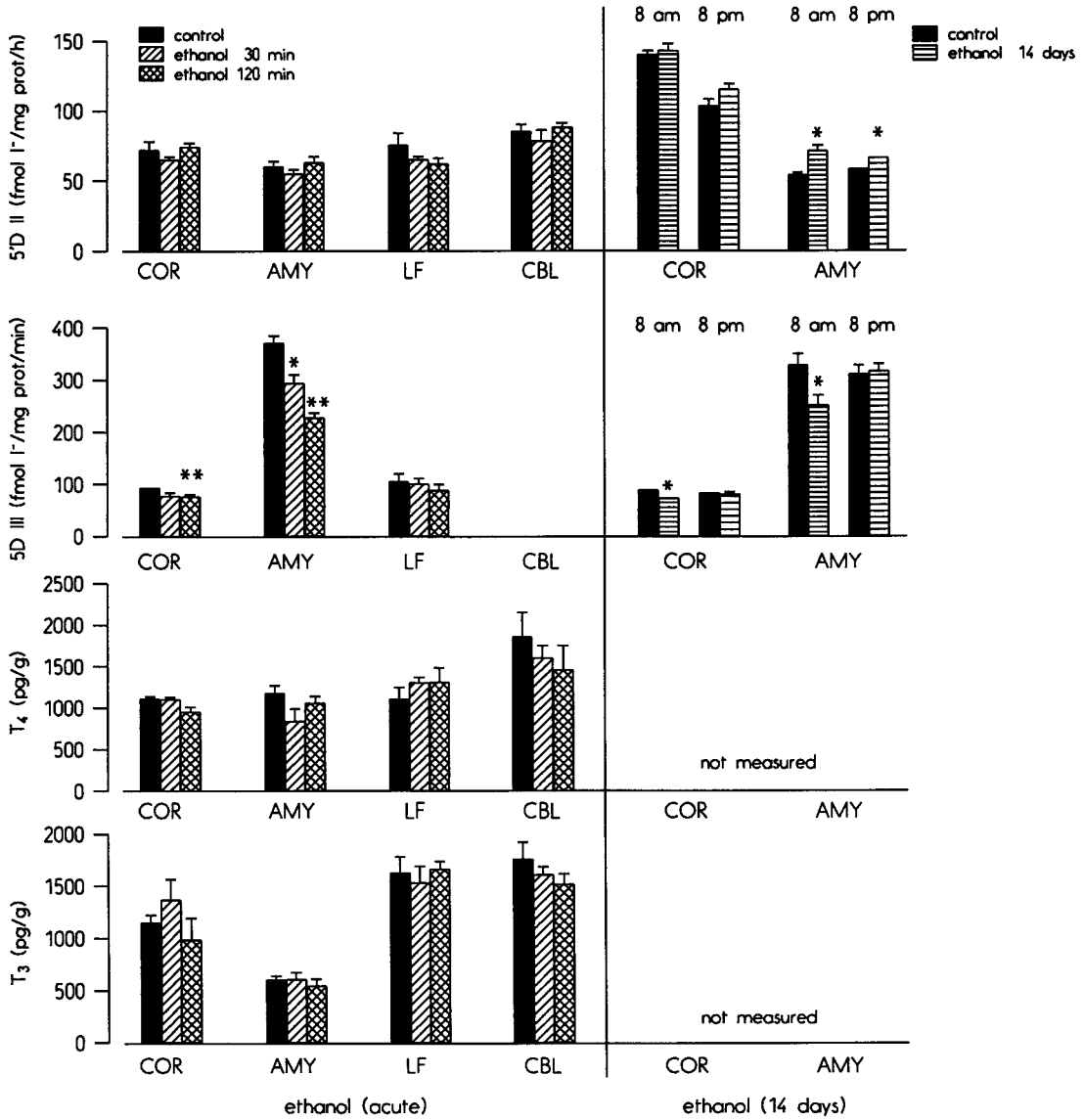

FIG. 3. *Left side*, Deiodinase activities and thyroid hormone concentrations in four regions of the rat brain 30 min and 120 min after intragastral administration of 1 g ethanol/kg. Right side, Effects of 14 day's treatment with a 5% ethanol solution as sole liquid on deiodinase activities in cortex and amygdala in groups of rats decapitated at 0800 h and 2000 h, respectively. For an explanation of the abbreviations, see Fig. 1.

right). The 0.15% lithium diet reduced 5′D-II activity in the cortex, whereas the 0.3% lithium diet enhanced it. T_4 levels were significantly elevated after the low dose and significantly reduced after the high dose. Likewise, the serum concentrations of T_4 and T_3 were enhanced after the 0.15% diet and lowered after the 0.3% diet (Table 1). The activities of 5D-III were, however, significantly reduced after administration of both dosages.

Group 16

Fourteen days of consumption of a 0.4% carbamazepine diet raised the activity of 5′D-II and reduced the tissue levels of T_4 and T_3 in the cortex (Fig. 5, *right*). The serum concentrations of T_4 and T_3 were also significantly lowered (Table 1).

Groups 17–20 (antidepressant drugs)

Fourteen days of treatment with the low dose of desipramine (5 mg/kg) raised the levels of T_3 in cortical tissue. However, the activities of both deiodinases remained unchanged (Fig. 7). The higher dose of 20 mg/kg induced a significant increase in 5′D-II activity and also in the tissue concentrations of T_3. After the toxic dose of 50 mg/kg, there was a pronounced increase in 5′D-II activity and the tissue concentrations of both T_4 and T_3 were significantly lower than in the controls. Fluoxetine induced an increase in 5′D-II activity and a reduction in 5D-III activity. Tranylcypromine significantly enhanced 5D-III activity without inducing any changes in thyroid hormone concentrations. None of these treatments significantly affected 5′D-I activity (data not shown). Fourteen days of treatment with the atypical anti-

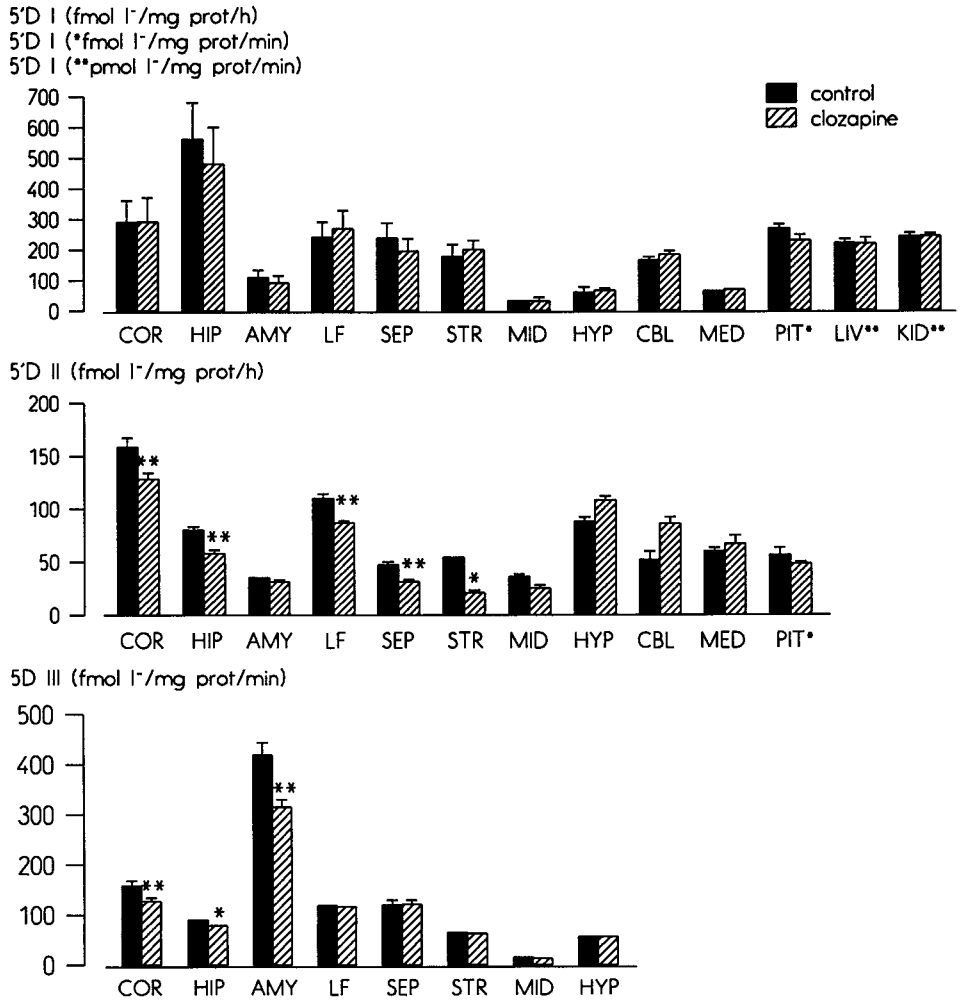

FIG. 4. Effects of 14 days of treatment with 20 mg/kg clozapine (ip.) on deiodinase activities in 10 regions of the brain and the pituitary, liver, and kidney in the rat. 5′D-I, 5′I deiodinase; SEP, septum; STR, striatum; MED, medulla; PIT, pituitary; LIV, liver; KID, kidney. For an explanation of all other abbreviations, see Fig. 1.

depressant drug mianserin led to a rather irregular pattern of changes in deiodinase activities and thyroid hormone concentrations (Fig. 8). Lowered concentrations of 5′D-II were seen in the cortex, hippocampus, and limbic forebrain and decreases in 5D-III concentrations in the hippocampus and amygdala. However, the T_4 levels were elevated in the hippocampus and reduced in the limbic forebrain, while those of T_3 remained unaffected. No effects were seen on 5′D-I activity in any brain region. Scrutiny of Table 1 reveals that several T_4 concentrations were significantly lowered after administration of all four antidepressant drugs. The serum levels of T_3 were significantly reduced only after the toxic dose of desipramine.

Group 21

The activities of 5′D-II and 5D-III were measured in the cortex and amygdala in young and old rats. 5′D-II activity was significantly higher in the young rats than in the old rats, in both regions (111.8 ± 6.6 *vs.* 54.0 ± 7.6 fmol I^-/mg protein/h, $P = 0.001$ and 52.4 ± 5.6 *vs.* 32.7 ± 1.8 fmol I^-/mg protein/h, $P = 0.01$ for the cortex and amygdala, respectively). There was no significant difference between the activities of 5D-III in cortex in the young and old rats (78.6 ± 4.4 *vs.* 86.2 ± 5 fmol I^-/mg protein/min). In the amygdala, however, 5D-III activity was again significantly higher in the younger rats than in the old rats (568 ± 36 *vs.* 301 ± 25 fmol I^-/mg protein/min, $P = 0.01$). A nonsignificant trend toward lower serum levels of T_4 and T_3 was found in the old rats (Table 1).

Discussion

The main conclusion of this study is that the activities of 5′D-II and, to a lesser degree, those of 5D-III in the rat CNS seem to be highly sensitive to many different kinds of physiological influences and pharmacological treatments. In some of the experiments the changes in deiodinase activities did not maintain physiological tissue levels of T_3 but were accompanied by changes in these levels.

As regards the validity of our significant results, as we have already commented in *Materials and Methods*, owing to the large number of statistical calculations (~267) about 13 significant results will have occurred by chance. We cannot therefore rule out the possibility that some "isolated" significant results may indeed have occurred by chance. However, almost all of our significant findings were obtained either in different brain regions, after different dosages or at two different measuring times, or they were so pronounced that they even fulfilled the $P =$

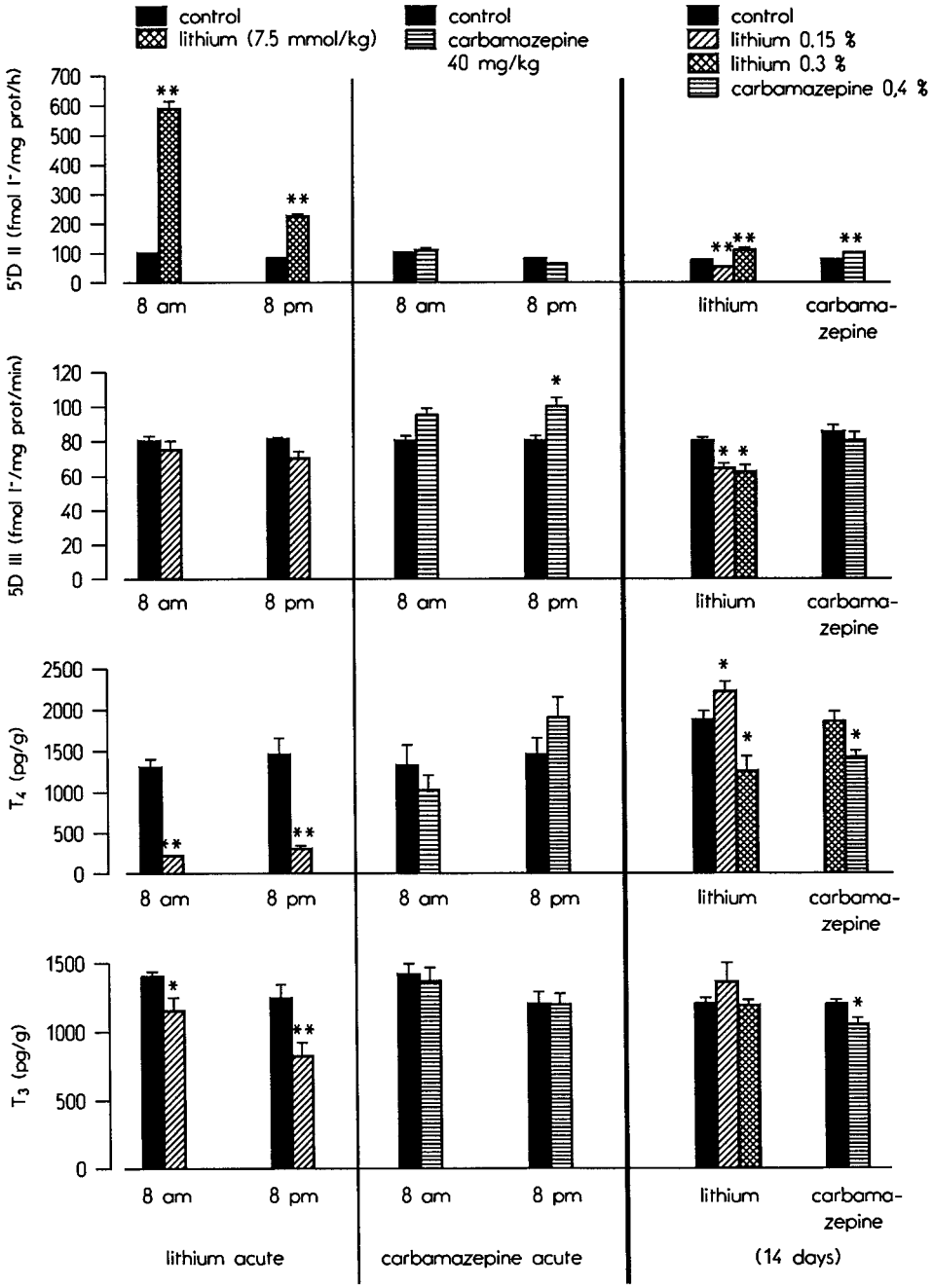

FIG. 5. *Left side*, Deiodinase activities and thyroid hormone concentrations in the cortex in different groups of rats having received a single administration of 7.5 mmol/kg lithium or 40 mg/kg carbamazepine and killed 12 and 24 h later, respectively. *Right side*, Deiodinase activities and thyroid hormone concentrations in the cortex in groups of rats having received a 0.15% and a 0.3% lithium diet or a 0.4% carbamazepine diet for 14 days. For an explanation of the abbreviations, see Fig. 1.

0.00025 criterion. The great majority of our significant findings can therefore be regarded as valid. One potential methodological shortcoming should, however, be mentioned. It was recently shown that studies using broken cell deiodinase assays may not adequately predict the rate of deiodination *in vivo* in intact cells (24). The same may well apply to the activities of 5′D-II and 5D-III. In light of these new findings our results on deiodinase activities must be confirmed by in-vivo experiments before they can be regarded as valid. Furthermore, several of the changes found to be significant in our study were quantitatively small and therefore of unknown physiological significance.

Only a few of our significant findings are easy to interpret. Sleep deprivation, for example, induced an enhancement in 5′D-II activity in four brain regions and an increase in the tissue concentrations of T_3 in the same areas. As the serum concentrations of T_4 were enhanced, the brain levels of T_4 probably did not fall, despite a simultaneous increase in 5′D-II activity. It is, however, unclear why the T_3 levels were elevated in the cerebellum, although the activity of 5′D-II was not affected. The possibility that the increase in serum levels of T_3 leads to direct uptake of T_3 in this and also in the other brain regions cannot be excluded. Why the serum levels of T_4 and T_3 were significantly elevated, despite the fact that the TSH concentrations remained unchanged, is also

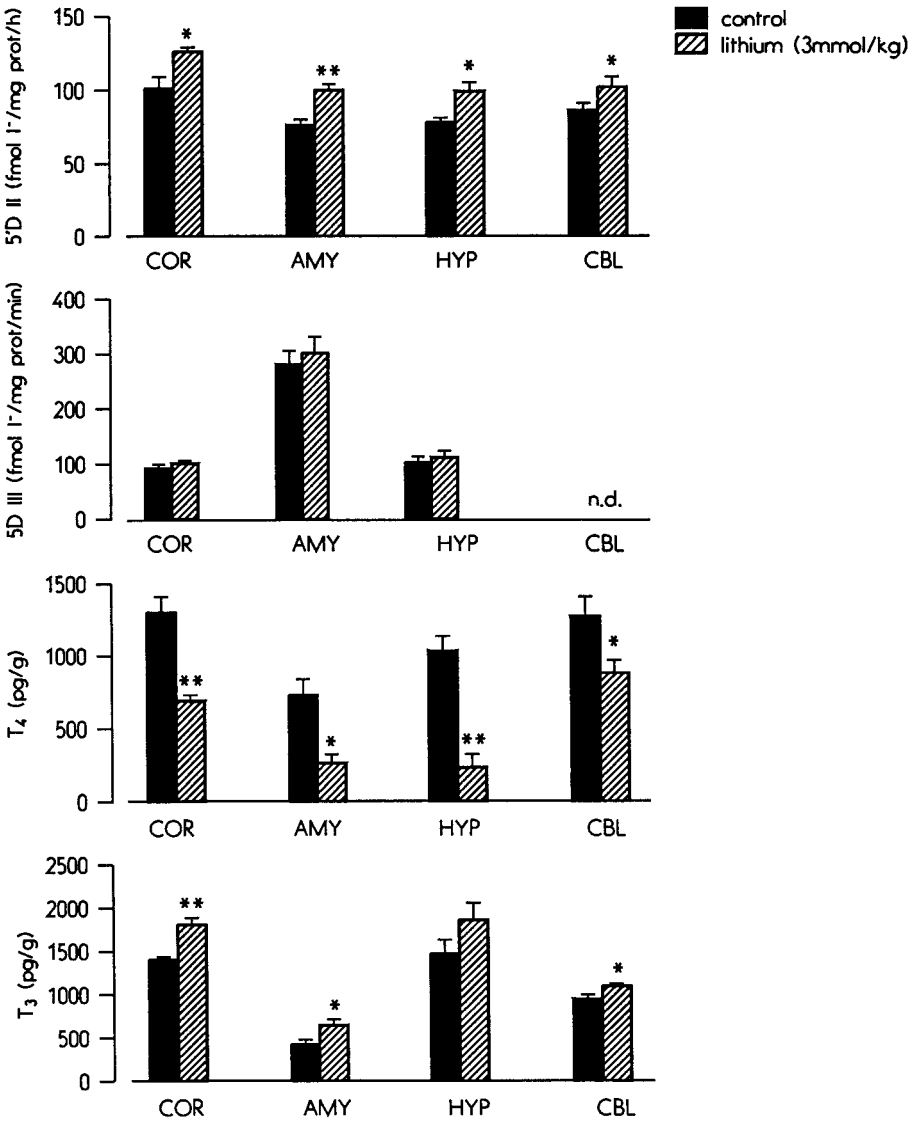

FIG. 6. Deiodinase activities and thyroid hormone concentrations 12 h after a single dose of 3 mmol/kg lithium in regions of the rat brain. For an explanation of the abbreviations, see Fig. 1.

unexplained. A direct stimulatory effect on the thyroid, such as stimulation of the sympathetic nervous system (25) should be considered.

One of the most unexpected findings of this study was the dramatic decrease in tissue levels of T_4 after different kinds of intervention such as 12 h fasting, a calorie-reduced diet for 14 days, and a single, relatively low dose and a single toxic dose of lithium (Groups 2, 3, and 10–12). After 12 h fasting and acute administration of lithium, 5′D-II activities were enhanced, while after 14 days on a calorie-reduced diet they were lowered (Figs. 2, 4, and 6). It also seems doubtful whether the relatively small increases of approx. 30–50% in 5′D-II activity after 12 h fasting and the low dosage of lithium (Fig. 6) were responsible for the sharp decline in tissue concentrations of T_4 to between 10 and 50% of the initial levels, particularly as these were evident after only 12 h. As the tissue concentrations of T_3 remained unchanged after a 12-h fast (Fig. 2) and were only slightly enhanced after injection of lithium (Fig. 6), it is unclear to which iodothyronine metabolite T_4 was metabolized during fasting. As the activity of 5D-III remained unchanged, enhanced degradation to reverse T_3 seems unlikely. Other candidates are T_3 sulfate and 3,3′-T_2 sulfate, which have been detected in rat brain and astrocytes in culture, respectively (26, 27). Deiodination of T_3 to 3,5-T_2 would also seem conceivable. 3,5-T_2 has recently been detected in human (28) and rat brain (29), but the enzyme catalyzing this reaction has not yet been characterized.

Another open question is the mechanism underlying the enhanced 5′D-II activities seen in many of our experimental groups (Groups 1, 2, 8, 10–12, 15, 16, 17, and 18). The increases in 5′D-II activity in most of our experimental groups might be explained on the basis of a fall in tissue concentrations of T_4 [*e.g.* Group 2 (except for the cerebellum) and Groups 10, 11, 12, and 15–18]. However, such an interpretation would not explain the mechanism underlying the fall in tissue levels of T_4, particularly in the groups in which the serum levels of T_4 remained unchanged (*e.g.* Groups 2 and 12). Furthermore, after sleep deprivation, the activity of 5′D-II was enhanced despite the fact that the tissue levels of

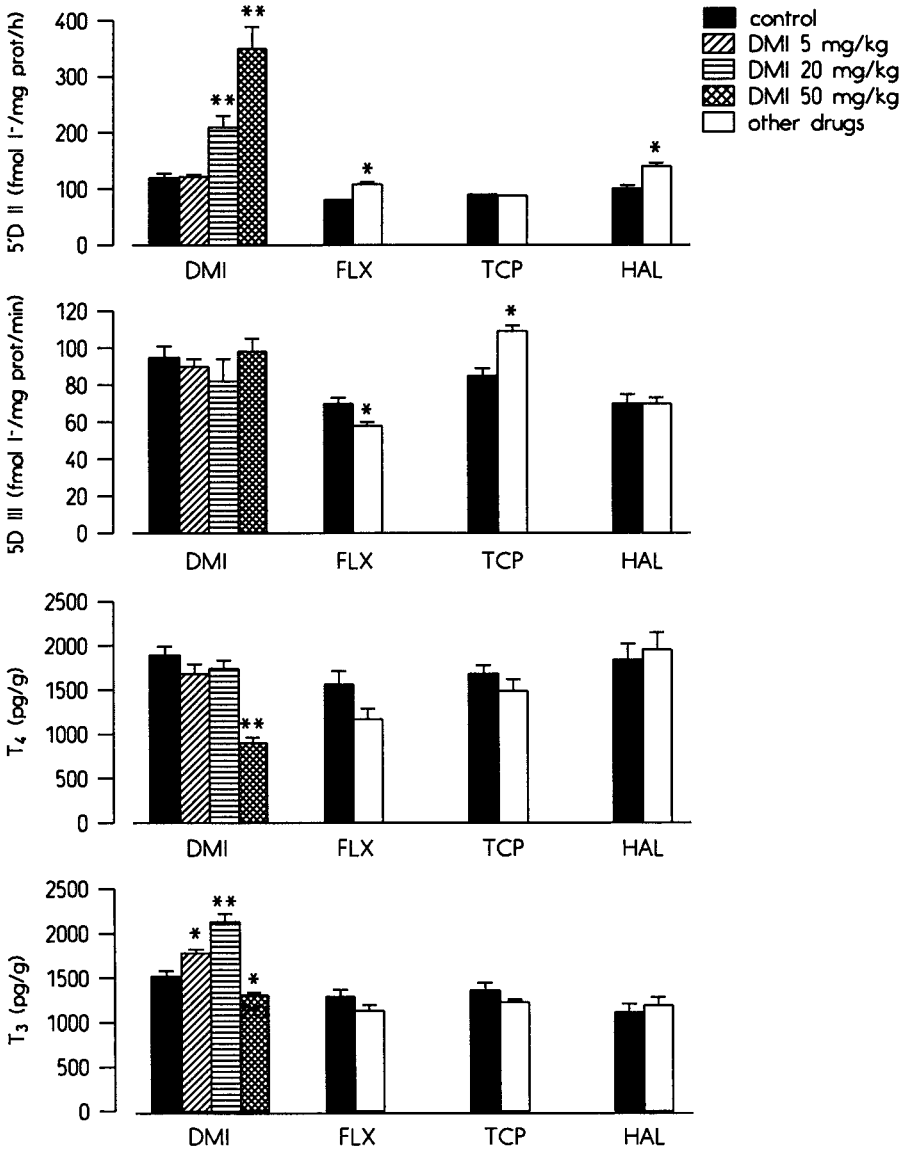

FIG. 7. Deiodinase activities and thyroid hormone concentrations in the cortex in different groups of rats having received different doses of desipramine, 15 mg/kg fluoxetine, 5 mg/kg tranylcypromine (and 1 mg/kg haloperidol) for 14 days. DMI, Desipramine; FLX, fluoxetine; TCP, tranylcypromine; HAL, haloperidol.

T_4 remained unaltered, and after a 14-day calorie-reduced diet the activity of 5′D-II was diminished although the tissue concentrations of T_4 had fallen. It therefore seems likely that these treatments influence the activity of 5′D-II directly in the CNS. Many of our data may therefore be interpreted in the following ways. First, conditions such as administration of a toxic lithium dose or chronic carbamazepine treatment, where the experimental pattern of deiodinase activities and tissue hormone levels fit an understandable pattern: in these conditions, depressed tissue levels of T_4 may have triggered an increase in 5′D-II activity with maintenance of relatively normal tissue concentrations of T_3. Second, in conditions such as sleep deprivation, 14 days' fasting or desipramine treatment the observed changes in deiodinase activity do not appear to have been triggered by changes in tissue levels of thyroid hormone, but would rather seem to be responsible for alterations in tissue concentrations of T_3. One may speculate that in these circumstances the unexplained changes in deiodinase activity may be a direct or indirect effect of the treatment itself. Conceivably this is of physiological benefit (*e.g.* in the case of sleep deprivation) or related to the therapeutic effects of drugs (*e.g.* desipramine). Third, in some experiments the observed changes in deiodinase activities are small in magnitude (*e.g.* Groups 18 to 20) or are not consistent with any of the above-mentioned patterns (*e.g.* Groups 5 and 9).

One finding, in particular, is currently rather difficult to interpret, namely, the decreases in both 5′D-II and 5D-III activities in the same brain region in several treatment groups (14 days on a low-calorie diet, low-dose, subchronic administration of lithium, and administration of the antidepressant mianserin and the neuroleptic clozapine). The physiological meaning of these changes is not clear because they result in a decrease in both the production and the degradation of T_3. 5′D-II has recently been located in astrocytes and tanycytes (30), whereas the 5D-III isoenzyme has recently been located in neurons *in vivo* (31), but has also been demonstrated in astrocytes in culture (32). Even if both deio-

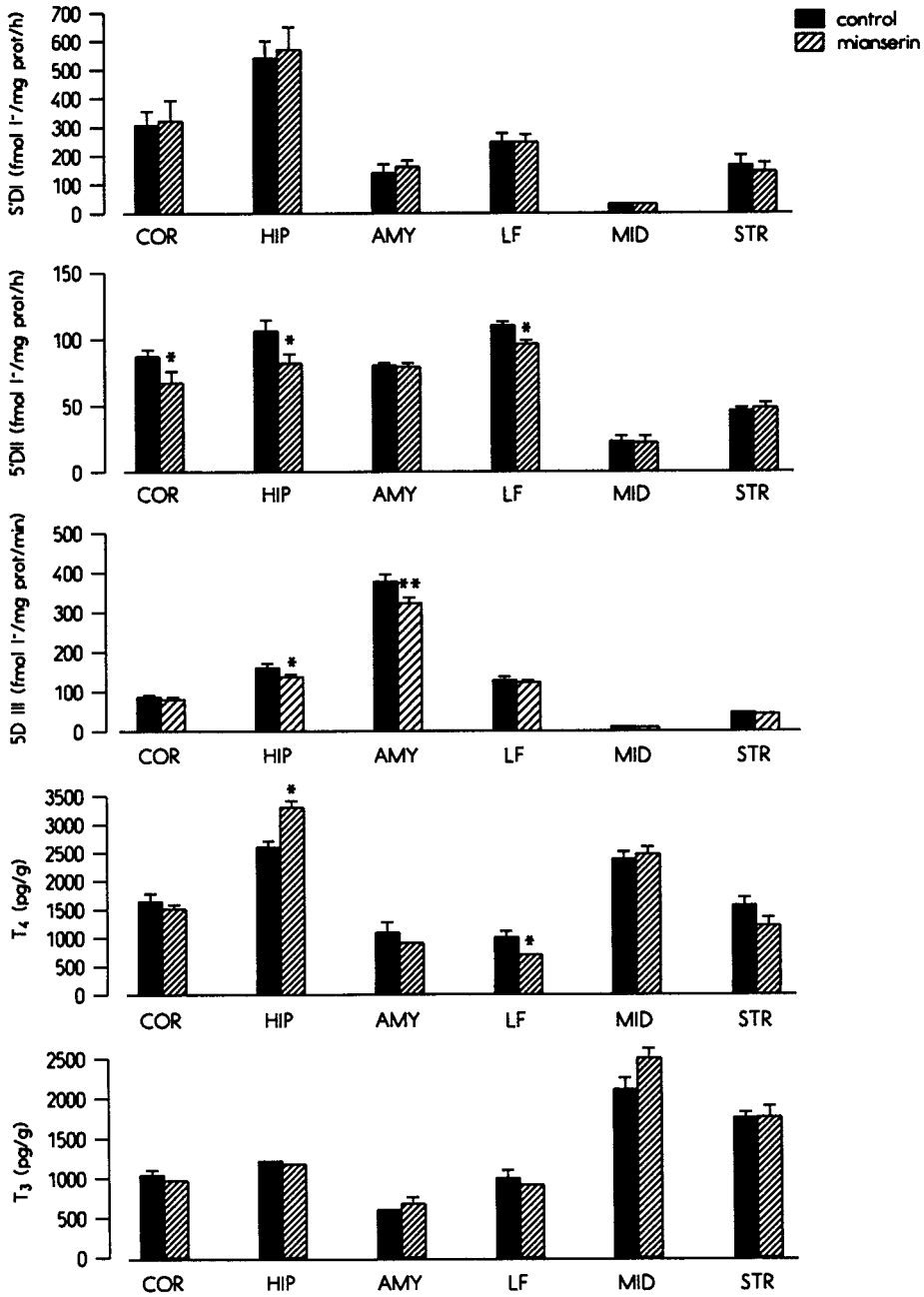

FIG. 8. Effects of 14 days of administration of 20 mg/kg mianserin on deiodinase activities and thyroid hormone concentrations in different regions of the rat brain. For an explanation of the abbreviations, see Fig. 1.

dinase isoenzymes were in fact located in different types of cell or subcellular compartments, the relevance of such constellations is not readily apparent. It is also hard to understand why we found lowered tissue concentrations of T_4 in an experimental group in which the activities of 5′D-II and 5D-III were both significantly reduced (*e.g.* 14 days of calorie-reduced diet), whereas the T_4 levels were elevated in other groups in which the activities of the two deiodinases were also reduced (Groups 15 and 20).

The prompt effect of ethanol on 5D-III activity (Fig. 3) was surprising, as this enzyme tends to react very slowly to peripheral hypo or hyperthyroidism, *i.e.* over a period of several days (12, 32). The effects of ethanol would therefore seem to be more direct and it is unlikely that they are mediated by changes in tissue concentrations of T_3.

With respect to the mechanisms underlying the decreases in serum levels of T_4 seen after subchronic treatment with different psychotropic drugs (15–20), both enhanced tissue uptake with subsequent deiodination to T_3 and an increase in peripheral enzyme induction seem possible, as, for example, a rise in UDP-glucuronyl transferase activity in the liver was reported after 3 weeks of treatment with the anticonvulsant diphenylhydantoin (33). Furthermore, it should be noted that we measured protein-bound serum levels of T_4 and T_3. However, the serum concentrations of free thyroid hormone may be more relevant for the supply of thyroid

hormones in the brain. We cannot, therefore, exclude the possibility that our results do not adequately reflect the relationships between the serum levels of free T_4 on the one hand, and tissue levels of T_4 and the deiodinase activities, on the other.

The activity of the 5′D-I isoenzyme was not affected by any of the treatments. This is consistent with the finding of a previous study that different forms of stress had no effect on 5′D-I activity (15). In light of these results, and also of the fact that this isoenzyme has a considerably higher substrate preference for rT_3 than T_4 (5) and is not found in the human brain (6), it seems doubtful that it is of physiological importance in the rat CNS.

Several of our findings show that factors such as drug dosage, length of treatment, and time of decapitation strongly influence the effects on thyroid hormone homeostasis. Effects of subchronic treatments on thyroid hormone homeostasis may slowly develop in some cases, reflecting underlying neuronal adaptation mechanisms. Moreover, a complete and valid evaluation of thyroid hormone metabolism and probably also function in the CNS should take into account the circadian components, as the results of a previous study revealed circadian variations both in 5′D-II activity and in the effects of desipramine, lithium, and carbamazepine on this activity (14, 16–18).

The biochemical mechanisms underlying the different changes in deiodinase activities seen in these experiments remain unexplained. Although it is well known that the thyroid hormone concentrations regulate deiodinase activities (12, 13, 32), it is unclear how all the different forms of treatment described in this study induced the observed changes in tissue concentrations of thyroid hormones. Furthermore, little is known about the neurotransmitter-related regulation of deiodinase isoenzymes in the CNS *in vivo*. It has repeatedly been demonstrated in astrocytes in culture that cAMP, isoproterenol, norepinephrine, and other factors such as TSH, FGF glucocorticoids, or protein kinase C activators stimulate 5′D-II and/or 5D-III activity (34–37). Our data suggest that the regulation of deiodinase activities *in vivo* is highly complicated. The norepinephrine reuptake inhibitor desipramine enhanced 5′D-II activity and the serotonin reuptake inhibitor fluoxetine both enhanced 5′D-II activity and inhibited 5D-III activity. Tranylcypromine is an MAO inhibitor which enhances norepinephrine, serotonin, and dopamine concentrations and the corresponding neuronal activities in the CNS. This drug should therefore have at least some similar effects to desipramine and fluoxetine. However, it had no effect on 5′D-II activity and enhanced that of 5D-III. In light of these results, it seems most likely that the deiodinase isoenzymes are not regulated by a single specific transmitter system, *e.g.* 5′D-II by norepinephrine, etc. The regulation of these enzymes is probably more complex and as yet not at all understood.

In conclusion, thyroid hormone metabolism and concentrations in the CNS are affected by many physiological or pharmacological influences that may change neuronal activity. Each specific effect on brain function seems to induce a specific "pattern" of changes in thyroid hormone homeostasis in terms of the direction and type of isoenzyme and hormone involved and the brain region affected. The data presented here also indicate that mechanisms other than the two deiodinase isoenzymes may be operating in the regulation of thyroid hormone metabolism in the CNS. Sulfur transferases, for example, are possible candidates.

As regards the physiological significance of these findings, it is very unlikely that all the highly complex and specific changes in thyroid hormone homeostasis in the CNS are not functionally important. In recent years, effects of thyroid hormones on the expression of a large number of genes have also been reported in the adult CNS (*e.g.* 38–40). It is, however, as yet unclear whether T_3 has a direct or indirect effect on these genes. Furthermore, it has been demonstrated that thyroid hormones have a large number of effects on a large variety of parameters of the adult CNS. These effects range from direct modulation of neuronal activity (*e.g.* 41) to numerous influences on the characteristics of many kinds of G protein-coupled neurotransmitter receptors (*e.g.* 42) or even morphological changes (43).

The question that currently remains unanswered is by what mechanisms thyroid hormones affect all these CNS parameters. Such mechanisms may well include the "classical" effects of T_3 and nuclear receptors. However, it is not unlikely that others are mediated directly at the mitochondrial level, since α and β T_3 receptors have recently been identified in mitochondria (44), a thyroid hormone response element has been identified in mitochondrial genes (45), and effects of hypothyroidism on the expression of mitochondrially encoded genes have been reported in the adult CNS (46). The hypothesis that thyroid hormones may be critically involved in the adjustment of the mitochondrial production of ATP to the current ATP requirement seems worthy of further investigation. The same applies to that of a possible specific effect of thyroid hormones directly at synaptic membranes, because effects of both T_4 (*e.g.* 47) and T_3 (41) at plasma membranes of CNS tissue have been reported.

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