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Effects of Lithium and Carbamazepine on Thyroid Hormone Metabolism in Rat Brain

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The effects of lithium (LI) and carbamazepine (CBM) on thyroid hormone metabolism were investigated in 11 regions of the brain and three peripheral tissues in rats decapitated at three different times of day (4:00 A.M., 1:00 P.M., and 8:00 P.M.). Interest was focused on the changes in the two enzymes that catalyze: (1) the 5'deiodination of T_4 to the biologically active T_3, i.e., type II 5'deiodinase (5'D-II) and (2) the 5 (or inner-ring) deiodination of T_3 to the biologically inactive 3'3-T_2, i.e., type III 5 deiodinase (5D-III). A 14-day treatment with both LI and CBM induced significant reductions in 5D-III activity. However, 5'D-II activity was elevated by CBM and reduced by LI, both administered in concentrations leading to serum levels comparable with those seen in the prophylactic treatment of affective disorders. The effects were dose dependent, varied according to the region of the brain under investigation, and strongly depended on the time of death within the 24-hour rhythm. The consequences of these complex effects of LI and CBM on deiodinase activities for thyroid hormone function in the CNS and also their possible involvement in the mechanisms underlying the mood-stabilizing effects of both LI and CBM remain to be investigated. ***[Neuropsychopharmacology 16:25–41, 1997]***

KEY WORDS: *Lithium, Carbamazepine; Thyroid hormones; Deiodinases; Rat brain*

Lithium (LI) and carbamazepine (CBM) are the most important drugs used for prophylaxis in patients with major affective disorder. Although many thousands of studies have been published on the clinical use and biochemical effects of both drugs, the mechanisms underlying their mood-stabilizing effects are as yet unknown (for reviews see Bunney and Garland-Bunney 1987; Jope and Williams 1994; Manji and Lenox 1994; Post 1987; Wood and Goodwin 1987).

The most relevant hypotheses on the mechanisms of action of lithium focus on the inhibition of myoinositol-l-phosphatase (Kendall and Whitworth 1990; Atack et al. 1993; for reviews, see Gani et al. 1993; Jope and Williams 1994). Further hypotheses concentrate on the lithium-induced inhibition of agonist-stimulated adenylate cyclase-mediated cAMP formation (Masana et al. 1991; Mørk and Geisler 1989). Yet another interesting hypothesis posits that the psychotropic properties of lithium may be due to its effects on G-protein function (Avissar et al. 1988; Avissar and Schreiber 1989, 1990; Carli et al. 1994; Newman et al. 1991). However, the validity of all these hypotheses and many others is still a matter of debate (Dixon et al. 1992; Ellis and Lenox 1991; Honchar et al. 1989; Jope et al. 1992; Mørk and Geisler 1989). Furthermore, common biochemical effects of both lithium and carbarmazepine have only very rarely been reported to date (for reviews, see Post 1987; Calabrese et al. 1994; Post and Weiss 1994).

It has also been known for more than 20 years that both LI and CBM may affect the peripheral production

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Received November 21, 1995; revised April 28, 1996; accepted May 13, 1996.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/97/$17.00
PII S0893-133X(96)00144-5

and serum concentrations of thyroid hormones (for reviews, see Kushner and Wartofsky 1988; Herman et al. 1991). Recently, several lines of evidence have emerged which suggest that both drugs may also have specific effects on intracellular thyroid hormone metabolism and that these effects may be related to their mood-stabilizing properties. On a clinical level, Roy-Byrne et al. (1984) reported that the serum concentrations of thyroxine (T_4) and free T_4 (fT_4) were lower in patients with affective disorders who responded to CBM treatment than in nonresponders. Hatterer et al. (1988) and we ourselves (Baumgartner et al. 1995) found higher serum concentrations of triiodothyronine (T_3) in patients with a favorable response to LI prophylaxis than in nonresponders to LI. Recently, Schöpf and Lemarchand (1994) reported higher levels of free T_3 in therapy-resistant depressed patients responding to lithium augmentation than in nonresponders.

In basic research, an interaction of LI with the effects of thyroid hormones in the central nervous system (CNS) has also been reported (e.g., Rastogi and Singhal 1977). Additionally, in vitro effects of LI on intracellular thyroid hormone metabolism in rat liver (Männistö 1974) and also in mouse neuroblastoma and GH3 cell lines (St. Germain 1987) have been reported. Bolaris et al. (1995) recently found an increase in nuclear binding of T_3 in cerebral hemispheres of rats treated either acutely or for 1 week with 5 mol/L/kg LI. In preliminary studies, we investigated the effects of LI and CBM on intracellular thyroid hormone metabolism, *i.e.*, their actions on the three deiodinase enzymes in the rat CNS. This issue would seem to be particularly interesting in as far as the metabolism of T_4 in the CNS (of the rat) seems to be subject to a highly specific regulation mechanism that differs substantially from that described in peripheral tissues such as the liver or kidney. In brief, in peripheral tissues most of the active iodothyronine compound (T_3) is taken up from the blood directly, whereas the T_3 supply of the brain depends almost completely on the cellular uptake and intracellular deiodination of T_4 (*e.g.*, Crantz et al. 1982). This implies that the supply of T_4 and intracellular deiodination are essential for T_3 function in the CNS. Furthermore, the mechanisms of deiodination in the CNS are also very different from those described in peripheral tissues. In the liver or kidney only one deiodinase isoenzyme, known as type I iodothyronine 5'-deiodinase (5'-D-I) catalyzes the deiodination of all iodothyronines (*e.g.*, T_4 to T_3 or rT_3, T_3 to the different T_2 compounds, etc.). In the rat CNS, however, two other deiodinases have been identified. Type II iodothyronine 5'-deiodinase (5'D-II) is a selective outer-ring deiodinase that catalyzes deiodination of T_4 to T_3 and of rT_3 to 3,3'T_2. Type III iodothyronine 5-deiodinase (5D-III) catalyzes inner-ring deiodination, thereby metabolizing T_4 to rT_3, and T_3 to the inactive compound 3,3'T_2 (see Visser et al. 1982; review in Köhrle et al. 1991). In recent studies we found that the antidepressants desipramine (DMI) and fluoxetine and also 24-hour sleep deprivation all enhance the activities of 5'D-II in various regions of rat brain, thereby enhancing CNS concentrations of T_3 (Baumgartner et al. 1994a; Campos-Barros et al 1993, 1994, 1995; Campos-Barros and Baumgartner 1994). In two preliminary studies we also found that 14-day treatment with LI and CBM enhanced the activity of 5'D-II and reduced that of 5D-III in the rat frontal cortex and hippocampus, respectively (Baumgartner et al. 1994b, 1994c). These effects were strongly dependent upon the time of day at which the rats were killed. In the present study we therefore set out to investigate the effects of LI and CBM on thyroid hormone metabolism in the rat CNS more thoroughly. We looked at the effects of different doses of the two drugs on deiodinase activity in 11 regions of the CNS and three peripheral tissues after both acute and subchronic administration in rats decapitated at three different times of day. The results have not previously been published, aside from those obtained on subchronic treatment with LI and CBM respectively in one brain region each, which have been presented in two brief reports (Baumgartner et al. 1994b, 1994c, see above).

MATERIALS AND METHODS

Materials

Thyroxine (T_4), 3,3',5'-triiodothyronine (rT_3), 3,5,3'-triiodothyronine (T_3), 3,5-diiodothyronine (3,5-T_2), and 3,3'-diiodothyronine (3 ,3'-T_2) of the highest available purity were obtained from Henning Berlin GmbH (Berlin, Germany). [5'-^{125}I]-T_4, [5'-^{125}I]-rT_3, and [3'-^{125}I]-T_3 were prepared for iodothyronine deiodinase assays and radioimmunoassays by radio iodination of T_3, 3,3'-T_2 and 3,5-T_2 respectively, as described by Meinhold (1986). The tracers with specific radio activities of 50 to 75 MBq/nmol were repurified immediately before use with disposable Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA), yielding a purity >99 % with ^{125}I- as the only contaminant (Mender et al. 1988). Inner-ring labeled [5-^{125}I]-T_3 (specific radioactivity 1.0-1.5 MBq/nmol) was produced by R. Thoma (Formula Berlin GmbH). Dithiothreitol (DTT) was purchased from Boehringer Mannheim GmbH (Mannheim, Germany); 6-*n*-propyl-2-thiouracil (PTU) was from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade. Pellets containing 0.15%, 0.25%, or 0.3% LI, *i.e.*, 1.5 g, 2.5 g, and 3 g LI/kg pellet feed, were purchased from Altromin. LiCl was purchased from Sigma Chemicals, and CBM was donated by CIBA Geigy (Basle). Pellets containing 0.3% or 0.4% CBM (3 g or 4 g CBM/kg pellet feed) and a LI- and CBM-free control diet were also obtained from Altromin.

Animal Treatments and Tissue Sampling

Adult male, euthyroid Sprague-Dawley rats (Tierversuchsanstalt, Hanover) weighing about 250 g were used throughout. They were housed in pairs with a 12-hour light-dark cycle (6:00 A.M. to 6:00 P.M.). All rats had free access to food and water. In the cages of the rats receiving LI, a second bottle of fluid containing 0.9% NaCl was installed. After allowing an adjustment period of at least 1 week in the new environment, the pharmacological studies were initiated. The details of the designs of all investigations conducted are given in Table 1. We first examined the effects of a 0.25% LI diet, given for 14 days, on deiodinase activity in different brain regions in rats decapitated during the day, i.e., as is conventional in psychopharmacological studies, at 1:00 P.M. As we observed only few effects of LI on deiodinase activity, and these were also difficult to interpret, we raised the dose slightly to 0.3% and killed the rats at 4:00 A.M. and 8:00 P.M. (group 2a). A further group of rats received a 0.3% CBM diet in the same design (group 2b). In these designs we found convincing effects of the two drugs on deiodinase activity. However, the serum levels of LI (but not those of CBM) were in the toxic range in some of the animals. We therefore further investigated the effects of a 0.15% LI diet on deiodinase activity (group 3a). As the effects of the 0.3% lithium diet on deiodinase activities were more pronounced at 8:00 P.M. than at 4:00 A.M., the effects of the 0.15% lithium diet were studied at 8:00 P.M. only.

When the rats were killed 12 or 24 hours after a single IP administration of CBM (in a 2% Tween 80 suspension), we found effects on deiodinase activity which were the reverse of those seen after 14-day treatment (group 6c). We therefore finally investigated the effects of CBM administered in the diet for an intermediate time period, i.e., for 7 days, raising the dose to 0.4% CBM (group 4a). As rats given a 0.3% LI-containing diet exhibited a significant weight loss in comparison with

Table 1. Drug Doses, Length of Application, Time of Death and Mean Lithium (LI) and Carbamazepine (CBM) Serum Concentrations in All Experimental Groups

Group	*n*	Drug/Mode of Application	Dose	Time or Length of Application (days)	Time of Decapitation	Mean Serum Concentration[a]	Range	Change of Body Weight[b]
1a	8	LI diet	0.25%	14 d	1 P.M.	1.12 ± 0.20	0.65–1.33	+17.0 ± 6.1%
b	8	control diet	—	14 d	1 P.M.	—	—	+22.5 ± 3.4%
2a	8	LI diet	0.30%	14 d	4 A.M.	1.30 ± 0.35	0.72 – 1.59	−20.2 ± 6.7%
a	8	LI diet	0.30%	14 d	8 P.M.	1.37 ± 0.31	0.97 – 1.77	−18.3 ± 6.5%
b	8	CBM diet	0.30%	14 d	4 A.M.	6.41 ± 1.50	5.30 – 8.21	+19.3 ± 2.5%
b	8	CBM diet	0.30%	14 d	8 P.M.	5.78 ± 1.35	3.01 – 7.14	+20.2 ± 2.3%
c	8	control diet	—	14 d	4 A.M.	—	—	+22.4 ± 2.3%
c	8	control diet	—	14 d	8 P.M.	—	—	+21.3 ± 3.5%
3a	6	LI diet	0.15%	14 d	8 P.M.	0.72 ± 0.08	0.58–0.85	+18.3 ± 3.4%
b	6	control diet	—	14 d	8 P.M.	—	—	+20.2 ± 4.5%
4a	6	CBM diet	0.40%	7 d	4 A.M.	5.99 ± 1.90	5.81–7.90	+ 8.5 ± 3.0%
a	6	CBM diet	0.40%	7 d	8 P.M.	5.40 ± 1.98	2.90–9.01	+ 7.9 ± 2.1%
b	6	control diet	—	7 d	4 A.M.	—	—	+12.4 ± 2.5%
b	6	control diet	—	7 d	8 P.M.	—	—	+11.3 ± 3.5%
5a	6	fasting	—	14 d	4 A.M.	—	—	−14.2 ± 4.5%
a	6	fasting	—	14 d	8 P.M.	—	—	−15.3 ± 3.9%
b	6	control diet	—	14 d	4 A.M.	—	—	+28.4 ± 2.5%
b	6	control diet	—	14 d	8 P.M.	—	—	+30.3 ± 3.5%
6a	6	LI IP	7.5 mmol/L/kg	10 P.M. (12 h)	10 A.M.	2.63 ± 0.18	2.45 – 2.95	—
a	6	LI IP	7.5mmol/L/kg	10 P.M. (24 h)	10 P.M.	1.91 ± 0.13	1.75 – 2.06	—
b	6	LI IP	3.0mmol/L/kg	10 P.M. (12 h)	10 A.M.	0.44 ± 0.07	0.35 – 0.55	—
c	6	CBM IP	40 mg/kg	10 P.M. (12 h)	10 A.M.	ND	—	—
c	6	CBM IP	40 mg/kg	10 P.M. (24 h)	10 P.M.	ND	—	—
d	6	complete fast	—	10 P.M. (12 h)	10 A.M.	—	—	—
e	6	control IP	NaCl	10 P.M. (12 h)	10 A.M.	—	—	—
e	6	control IP	NaCl	10 P.M. (24 h)	10 P.M.	—	—	—

[a]Given in mmol/L for LI and in mg/l for CBM.
[b]Percent change in body weight during treatment with LI or CBM and in control animals.
ND = not detectable.

controls, we also investigated the effect of fasting on deiodinase activities in two further "time groups" of rats (group 5a, 4:00 A.M. and 8:00 P.M.; see Table 1). On days 1 to 5, these rats received only the amount of feed consumed on the respective days by the rats in group 2a, which had received 0.3% LI in their diet. From days 6 to 14, the rats were weighed and their diet adjusted on a daily basis in order to achieve a percentage weight loss comparable with that seen in rats fed on a 0.3% LI diet (group 2a).

In the acute treatment groups (groups 6a to 6e) one toxic dose (7.5 mmol/L/kg) and one more physiologic dose (3 mmol/L/kg) and 40 mg/kg CBM were injected IP, at 10:00 P.M. in each case. A group of control rats received IP injections of saline. The rats were decapitated either 12 or 24 hours later. As the rats having received the high doses of LI (group 6a) took no feed at all during the night and the following day, we investigated a further group that was given no feed at all for 12 hours (group 6d).

The experiments on groups 1 to 6 were carried out consecutively over a period of more than 2 years.

All rats were decapitated without anesthesia. Various regions of their brains were dissected according to Glowinski and Iversen (1966). The pituitary gland, liver, and kidney were also dissected and all tissues immediately frozen at −70°C. Blood samples were taken from the decapitation wound and centrifuged. The serum was stored at−20°C.

Iodothyronine Deiodinase Assays

For measurement of deiodination, tissue samples were individually homogenized on ice in 5 to 6 vol of 0.25 mol/L sucrose, 10 mmol/L HEPES (pH 7.0) containing 10 mmol/L DTT and immediately frozen in a dry ice/acetone bath and stored at −80°C until assay. The measurement of types I and II 5′ deiodinase (5′D-I and 5′D-II) and type III 5 deiodinase (5D-III) was based on the release of radioiodide from the ^{125}I-labeled substrates (Leonard and Rosenberg 1980).

5′D-I and 5′D-II Assay. 5′D-I and S′D-II activities were determined simultaneously by measuring the release of radioiodide from 100,000 cpm (~2.5 kBq) [5′-^{125}I]-rT_3 at 5 nmol/L rT_3, 20 mmol/L DTT, in the presence (for 5′D-II) and absence (5′D-I + 5′D-II) of 5′D-I inhibiting PTU (Visser et al. 1982). 5′D-II was also determined using [5′-^{125}I]-T_4 as substrate in the presence of 6 nmol/L T_4, 30 mmol/L DTT, 1 mmol/L PTU, and 1 μmol/L T_3, in order to inhibit the inner ring deiodination of T_4 in those tissues containing significant type III deiodinase (5D-III) activity (Visser et al. 1982; Kaplan and Yaskoski 1980).

The measurement was conducted after 45 to 90 minutes (usually 60 min) of incubation at 37°C with 50 to 100 μg of protein from the crude homogenate in 100 μl of 0.1 mmol/L potassium phosphate buffer (pH 7.0), 1 mmol/L EDTA. The reaction was started by the addition of the tissue homogenate and stopped adding the 50 μl of ice-cold 5% BSA, 10 mmol/L PTU, followed by 400 μl of 10% ice-cold trichloroacetic acid. After centrifugation of 4000 *g* for 30 minutes, the supernatant containing the $^{125}I^-$ was further purified by cation exchange chromatography on 1.6-ml Dowex 50 WX 8 columns (mesh 100 to 200) (Serva GmbH and Co., Heidelberg, Germany). The iodide was then eluted with 2 mmol × 1 ml 10% acetic acid and counted in a gamma counter.

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

Preliminary experiments established that for each tissue: (1) the reaction rates were constant over time for up to 120 minutes in the presence and absence of PTU; (2) the reaction rates were proportional to protein concentrations in the ranges used (50 to 100 μg/tube in the 5′D-I and 5′D-II assays; 20 to 70 μg/tube in the 5D-III assay); and (3) after incubation, equal amounts of 3,3′-T_2 and I^- were produced from rT_3 (5,VD I+II assay) or from T_3 (5D-III assay) in homogenates from each of the different tissues, as determined by reversed phase high performance liquid chromatography (HPLC) (Eelkman-Rooda et al. 1989) 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% to 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 less than 10% to 15% of the substrate was consumed by enzymatic deiodination. Each experimental point was determined in triplicate with coefficients of variation of less than 5%. The deiodinase activities of the different experimental groups (1 to 6, see Table 1) were measured over a 2-year period (see above). Intra-assay coefficients of variation were regularly determined by measuring three samples from one homogenate in one assay. Almost all ranged between

1% and 3% for both 5'D-II and 5D-III activities. When the intra-assay coefficients of variation exceeded 5%, the experiment was repeated.

Inter-assay coefficients of variation could not be calculated for all experiments carried out in the whole 2-year period, as our experience has shown that deiodinase activities decrease after several months in both homogenates and tissue samples, even if they are stored at −70°C. We also had the impression that differences in the quality of several of the reagents (e.g., DTT), even when purchased from one and the same supplier, may have been responsible for some of the changes in absolute deiodinase values that became evident over the years. It is therefore not possible to make reliable between-group comparisons of our results on deiodinase activities (groups 1 to 6, see Table 1). However, within one experimental group all tests on a given brain region were always conducted in one assay and can therefore well be compared with each other. This applies in particular to those groups in which the rats were decapitated at different times of day. Each control group was killed at the same time as the respective drug treatment group. Likewise, the experiments on deiodinase activities were also performed together with those for the respective drug treatment group. Protein was assayed by the method published by Bradford (1976), using reagents from BioRad Laboratories (Richmond, CA) and bovine gamma globulin as standard.

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 radioimmunoassay as previously described for human serum (Meinhold 1986). For assaying total T_4 and T_3 in the rat sera, standards were set up in iodothyronine-free rat serum (Stringer and Wynford-Thomas 1982). The serum levels of TSH were measured by specific RIA developed for the rat, using immunoreactants kindly supplied by the National Institutes of Arthritis, Diabetes & Digestive and Kidney Diseases of the National Institutes of Health (Bethesda, MD). The serum concentrations of lithium were determined by flame photometry and those of CBM by an enzyme-linked immunoassay, both in the laboratory of the Klinikum-Benjamin-Franklin.

Statistical Analysis

The data are given as means ± SD in the text and, for greater clarity, as means ± SEM in the figures. p-values of less than .05 were considered significant. Where appropriate, comparisons of the results of the different groups of rats were performed using ANOVA with the factors "time" (morning vs evening) and "drug" (LI/CBM vs saline). Individual rankings were calculated with the aid of the Newman-Keuls test. Single comparisons were performed by the Mann-Whitney U test.

RESULTS

Serum Concentrations of LI and CBM

The serum concentrations of LI and CBM are shown in Table 1. The serum concentrations of CBM were within the lower "prophylactic" range after 7- and 14-day treatment with a 0.3% and 0.4% diet, respectively (groups 2b and 4a). The serum levels of LI were in the prophylactic range after 14-day administration of a 0.15% LI-containing diet (group 3a) and at the upper end of the therapeutic range after 14-day administration of 0.25% LI (group 1a). However, some rats that had received 0.3% LI diet for 14 days had serum concentrations of LI reaching the toxic range (group 2a). In the interests of greater clarity, this group (2a) will be referred to as the "toxic group."

The serum levels of CBM were not measurable 12 or 24 hours after a single IP injection of 40 mg/kg. The serum concentrations of LI were clearly in the toxic range both 12 and 24 hours after IP administration of the high dose of 7.5 mmol/L/kg (group 6a). They were at the lower end of the therapeutic range after a single administration of 3 mmol/L/kg LI (group 6b).

Effects of Subchronic Administration of LI and CBM, Respectively, on Deiodinase Activity

Group 1. Figure 1 reveals that administration of 0.25% LI in pellets over a 14-day period significantly inhibited 5'D-II activity in the hypothalamus and cerebellum. Nonsignificant trends toward an increase in 5'D-II activity were evident in the parieto-occipital cortex ($p = .06$) and hippocampus ($p = .09$). 5D-III activity was significantly inhibited by LI in the frontal cortex and hypothalamus ($p < .01$ in both cases, Figure 1). Administration of 0.25% LI failed to affect 5'D-I activity in 11 regions of the brain or in the pituitary (data not shown). However, 5'D-I activity was significantly reduced in both the liver and kidney in the lithium-treated rats ($p = .01$).

Group 2 (High-Dose LI ("Toxic") Treatment, CBM Treatment). The effects of 14-day treatment with a 0.25% LI diet on deodinase activity were, at least as regards 5'D-II, not completely consistent.

We therefore tried to clarify the effects of LI by investigating the effects of different doses of LI on deiodinase activity: one higher dose (0.3% LI diet) and one lower dose (0.15% LI diet), both administered for 14 days. The 0.3% LI dose resulted in toxic serum concentrations of LI in at least some of the rats (Table 1), whereas the rats having received the 0.15% LI diet had serum levels of LI well within the "prophylactic range" (Table 1). Both groups were killed during the "active period," as we had previously found that the effects of psychotropic drugs on deiodinase activities may heavily depend on the time of death during the 24-hour

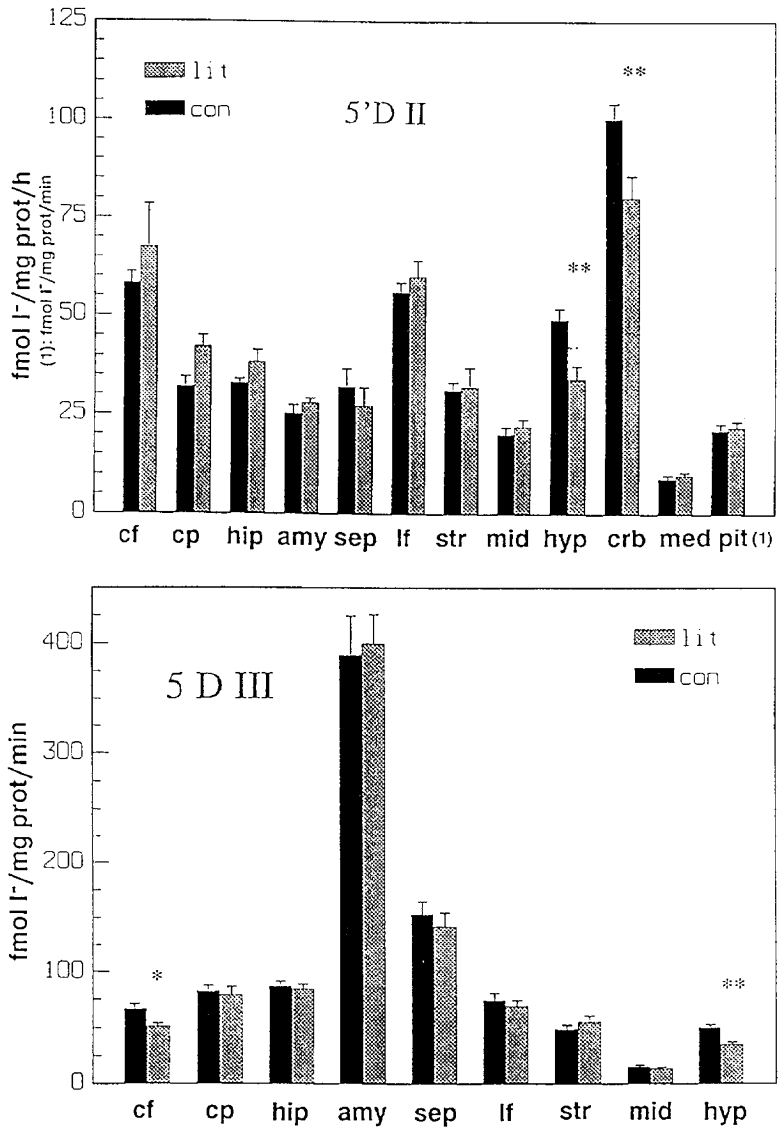

Figure 1. Activities of 5'II deiodinase (*top*) and 5III deiodinase (*bottom*) in different regions of the brain and pituitary in rats after 14-day administration of a 0.25% LI diet (time of death 1:00 P.M.; group 1). Abbreviations: CF = frontal cortex; CP = parieto-occipital cortex; HIP = hippocampus; AMY = amygdala; SEP = septum; LF = limbic forebrain; STR = striatum; MID = midbrain; HYP = hypothalamus; CRB = cerebellum; MED = medulla/pons; PIT = pituitary. * = $p < .05$, ** = $p < .01$, compared with controls.

circadian rhythm (Campos-Barros et al. 1994). The "toxic groups" (0.3% LI diet) were killed at 4:00 A.M. and 8:00 P.M., respectively. The "low-dose group" (0.15% LI diet) was sacrificed at 8:00 P.M. only. The effects of 0.3% LI ("toxic" group") and those of CBM were investigated using the same controls. As we had meanwhile found that the 5'D-I isoenzyme is not present in the human CNS (Campos-Barros et al. 1996) and because the above results failed to show any effect of LI on the activity of this enzyme in any region of the rat brain, in all further experiments 5'D-I was measured in the liver only. Figure 2 shows that the "toxic dose" of LI did not affect the activity of 5'D-II in the rats killed at 4:00 A.M., but significantly enhanced it in 7 out of the 10 brain regions investigated at 8:00 P.M. LI significantly inhibited 5D-III activity in six brain regions at 4:00 A.M. and in five at 8:00 P.M.

As shown in Figure 3, CBM induced a significant increase in the activity of 5'D-II in the hypothalamus ($p < .001$) and midbrain ($p < .05$) at 4:00 A.M. and also raised the activity of this enzyme in nine out of the 10 brain regions at 8:00 P.M. After CBM treatment, 5D-III activity was inhibited in the hippocampus at 4:00 A.M. only ($p = .02$) At 8:00 P.M. CBM inhibited 5D-III activity in the limbic forebrain ($p = .01$) and midbrain and septum ($p < .05$) (Figure 4). Administration of both LI and CBM inhibited 5'D-I activity in the liver at 4:00 A.M. ($p < .01$ in both cases), but only LI reduced the activity of 5'D-I at 8:00 P.M. ($p < .01$, data not shown).

Group 3 ("Low-Dose" LI Treatment). The effects of the lower dose of LI (0.15%) on deiodinase activities measured at 8:00 P.M. only are shown in Figure 5. LI treatment resulted in a significant decrease in 5'D-II activity in the parieto-occipital cortex, striatum, septum, and hypothalamus. No significant changes were seen in the other five brain regions. 5D-III activity was significantly reduced in the frontal cortex, parieto-occipital

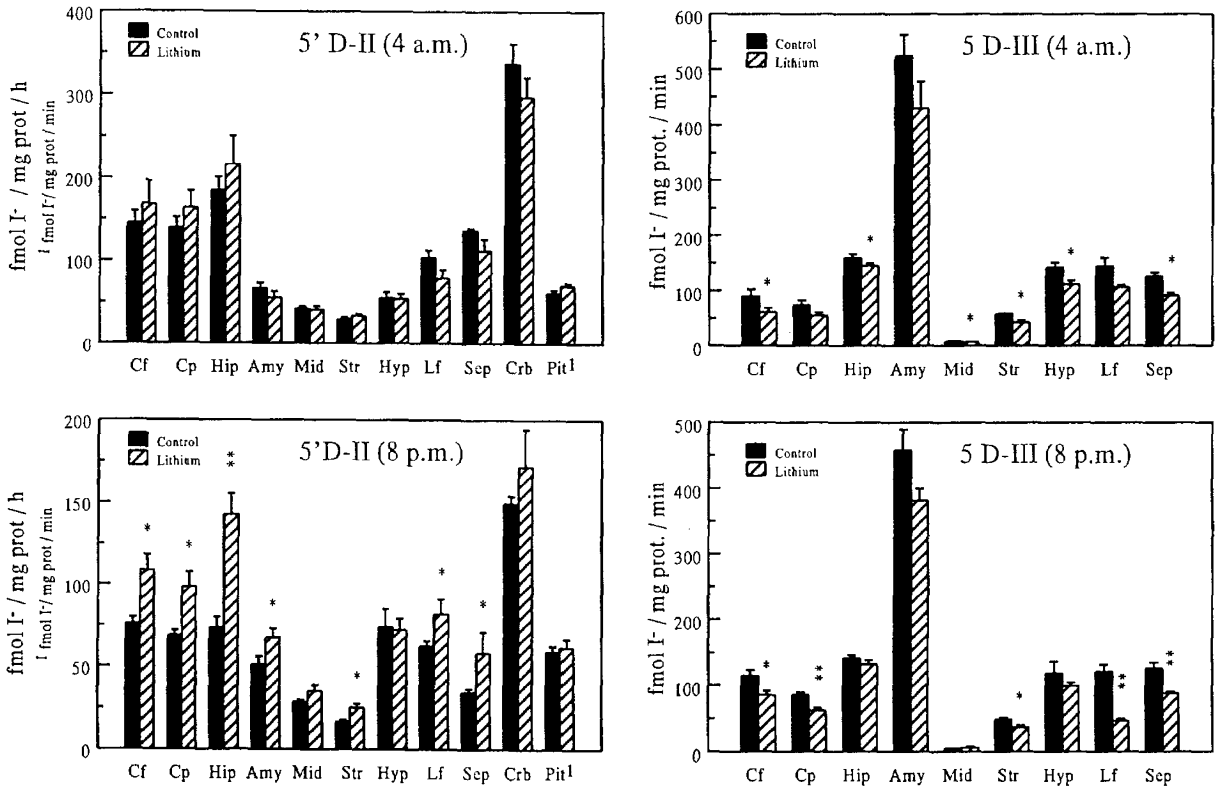

Figure 2. 5'D-II activity (*left*) and 5D-III activity (*right*) in rats treated with a 0.3% LI diet for 14 days and killed at 4:00 A.M. (*top*) and 8:00 P.M. (*bottom*) (group 2a). Abbreviations as in Figure 1.

cortex, and limbic forebrain but was enhanced in the hippocampus.

Groups 4 and 5 (Fasting 7-Day CBM Treatment). In the group that fasted for 14 days to an extent causing a weight loss comparable with that of the group that received 0.3% LI pellets, 5'D-II was significantly inhibited at 4:00 A.M. in the frontal cortex (Figure 6) and hippocampus. No differences were seen in the rats decapitated at 8:00 P.M. 5D-III was also significantly inhibited in the frontal cortex at 4:00 A.M. (19.1 ± 3.5 vs 26.2 ± 3.4 fmol I–/mg protein/min in the fasting and control rats, respectively), but not at 8:00 P.M. (28.6 ± 9.2 vs 25.1 ± 5.3 fmol I–/mg protein/min, Figure 6). In contrast, 5D-III activity was not inhibited in the hippocampus in the fasting rats at 4:00 A.M. but was reduced at 8:00 P.M. (19.6 ± 3.4 vs 24.4 ± 6.2 fmol I–/mg protein/min at 4:00 A.M. and 16.9 ± 1.4 vs 20.5 ± 2.8 fmol I–/mg protein/min at 8:00 P.M., $p = .12$ and .02, respectively). Seven days of treatment with pellets containing the 0.4% CBM diet did not significantly affect 5'D-II or 5D-III activity in the frontal cortex or hippocampus either at 4:00 A.M. or at 8:00 P.M.

Group 6 (Acute Treatment). Twelve hours after a single IP injection of 3 mmol/L/kg LI, the activities of 5'D-II and 5D-III were unchanged in the frontal cortex. Twelve and 24 hours after administration of the toxic dose of 7.5 mmol/L/kg LI, the levels of 5'D-II had risen dramatically in both the frontal and parietal occipital cortex, remained unchanged in the hypothalamus and even showed a trend toward a decrease in the pituitary after 12 hours ($p = .06$, Figure 7). No effect of this toxic dose was seen on 5D-III activity in the frontal cortex or hypothalamus at either measuring time (data not shown). IP administration of 40 mg/kg CBM failed to have an effect on 5'D-II activity in the frontal cortex, parieto-occipital cortex, or hypothalamus after 12 hours, but a significant reduction in the activity of this enzyme was found in two of these brain regions in the rats killed after 24 hours (Figure 7). The same treatment regime also failed to affect 5D-III activity in the same brain regions after 12 hours but had significantly enhanced the activity of this enzyme in the frontal cortex and hypothalamus after 24 hours. After 12 hours of total fasting (10:00 P.M. to 10:00 A.M.), 5'D-II activity was significantly elevated in the frontal cortex (125.9 ± 32.4 vs 95.2 ± 9.6 fmol I⁻/mg protein/h, $p = .02$, for the fasting and control rats, respectively). However, 5D-III activity was not affected by fasting (13.2 ± 2.5 vs 13.8 ± 2.0 fmol I–/mg protein/h, $p = .60$). No effects were found after 12 and 24 hours of administration of LI (7 mmol/L/kg) or CBM (40 mg/kg) on 5'D-I activity in the liver (data not shown).

The changes in body weight in all groups treated subchronically with LI or CBM and their respective

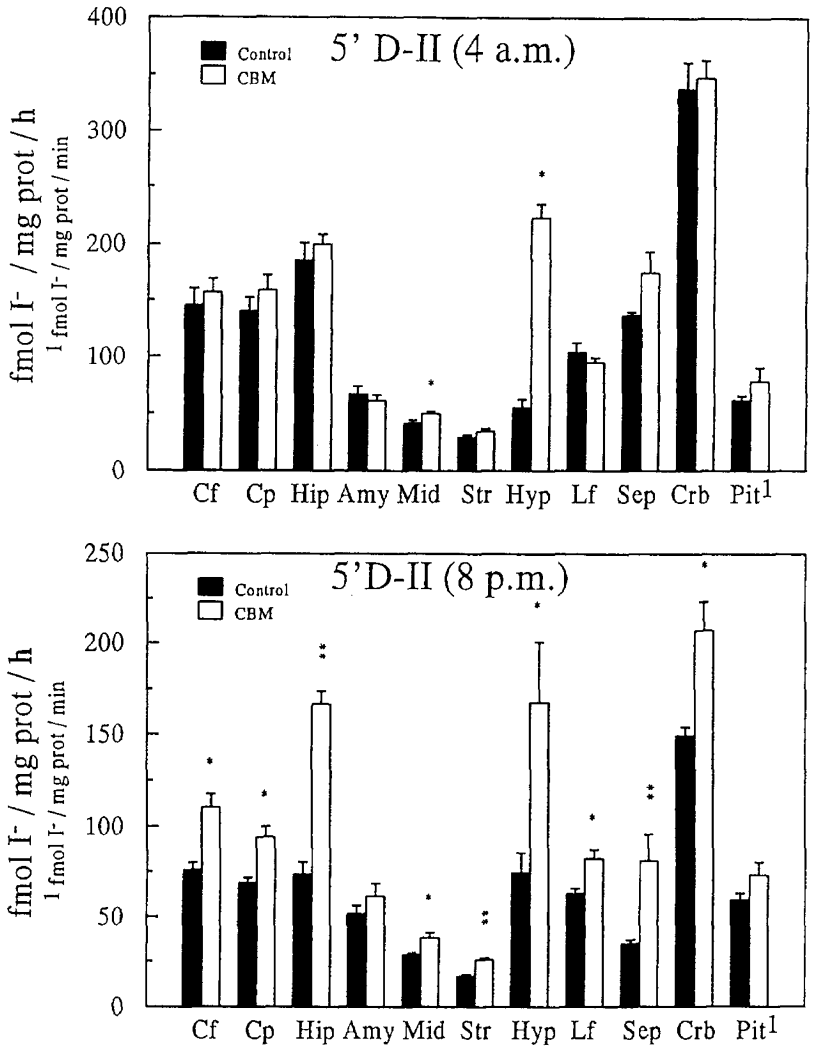

Figure 3. 5'D-II activity in rats treated with a CBM diet (0.3%) for 14 days and killed at 4:00 A.M. (*top*) and 8:00 P.M. (*bottom*) (group 2b). Abbreviations as in Figure 1.

controls are shown in Table 1 (groups 1 to 5). The body weights of the rats having received the 0.25% and 0.15% LI diets and the CBM diets, respectively (groups la, 2b, 3a, and 4a) did not differ from those found in the rats given the control diet. However, the rats receiving the 0.3% LI diet for 2 weeks exhibited a considerable weight loss: before death their weight was 31% lower than in the respective control groups (groups 2a and 2c). On the other hand, rats that had fasted for 14 days also weighed 32% less than their respective controls prior to decapitation (groups 5a and 5b).

The results for the serum levels of T_4, T_3, and TSH, which were not measured in all groups, are presented in Table 2. Fourteen days of administration with both the 0.3% LI and 0.3% CBM diets resulted in lower serum concentrations of T_4 and—at 4:00 A.M. at least—also of T_3. Surprisingly, the serum concentrations of T_4 and T_3 were elevated after 14-day administration of the low-dose LI diet (0.15%; group 3a). The serum levels of both T_3 and T_4 were dramatically reduced 12 and 24 hours after administration of the toxic dose of LI (7.5 mmol/L/kg). The concentrations of T_3 were also reduced in the rats having fasted completely for 12 hours. The levels of TSH were reduced at 8:00 P.M. after subchronic administration of lithium (group 2a).

DISCUSSION

Our results show that both LI and CBM alter thyroid hormone concentrations in the rat CNS. The two drugs seem to have a common effect on 5D-III activity, which was inhibited after 14-day administration of CBM and the "clinically relevant" dose of LI (0.15%). However, these effects were rather specific for brain region and time of day. The only exception was seen in the hippocampus, where LI (0.15%) stimulated 5D-III activity. On the other hand, the two drugs had contrasting effects on 5'D-II activity. Whereas CBM stimulated 5'D-II activity, LI—at least at "clinically relevant" doses—inhibited it (Figures 1, 3, and 4). Surprisingly, 14-day administra-

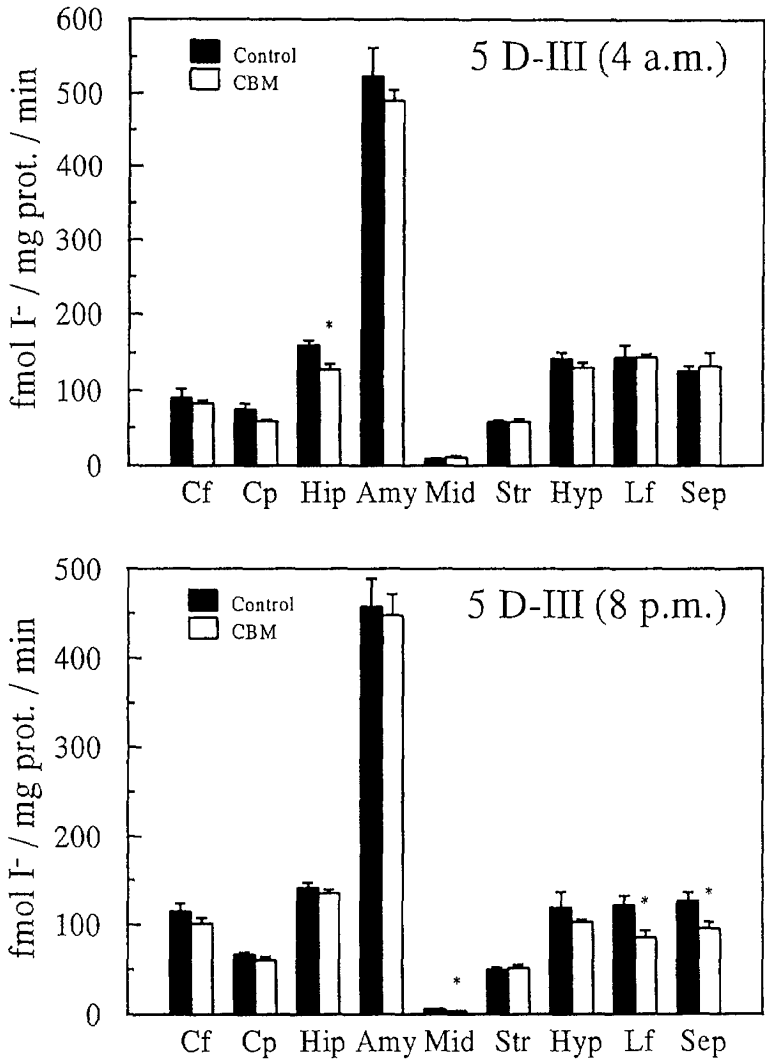

Figure 4. 5D-III activity in rats treated with a CBM diet (0.3%) for 14 days and killed at 4:00 A.M. (*top*) and 8:00 P.M. (*bottom*) (group 2b). Abbreviations as in Figure 1.

tion of the 0.3% LI diet, which led to toxic levels of LI in roughly half of the rats, enhanced 5'D-II activity in almost all brain regions, but at 8:00 P.M. only.

As regards the consequences of these changes in deiodinase activities for the production and function of T_3, it may be hypothesized that the effects of CBM may result in an increase in T_3 production. The "net" effect of treatment with "prophylactic" doses of LI on T_3 concentrations is however, unclear at present, as LI inhibited 5D-III activity, which should lead to a rise in T_3 concentrations and also inhibited 5'D-II activities, which should reduce the production of T_3. The fact that the 5D-III activity is about 100 times higher than 5'D-II activity in all brain regions may mean that the "net effect" of LI on T_3 concentrations is an increase. However, the same fact also strongly suggests that the two isoenzymes are most likely located in separate cells or subcellular compartments (because otherwise all T_3 produced by 5'D-II activity would be instantly degraded by 5D-III and the brain concentrations of T_3 could not be as high as previously observed [Campos Barros et al. 1995]). However, both enzymes have only recently been cloned (St. Germain et al. 1994; Davey 1995) and their cellular/subcellular location is yet unknown, whereas their exact role in regulating thyroid hormone function in the CNS is also only incompletely understood. Future studies on these issues may enable us to make more accurate interpretation of the functional significance of the effects of LI on deiodinase activities, which at first glance appear somewhat paradoxical. As regards the literature, to our knowledge as yet only one study has investigated the effects of LI on a "thyroid hormone parameter" in rat CNS. Bolaris et al. (1995) recently reported increased receptor binding of T_3 to the nucleus but not in the cytoplasm in rat cerebral hemispheres after 1 week of LI treatment. This result would be consistent with an increase in T_3 production.

In previous studies we had already found that administration of DMI (Campos-Barros and Baumgartner 1994, Campos-Barros et al. 1994, 1995) and sleep deprivation (Campos-Barros et al. 1993), respectively, also enhanced

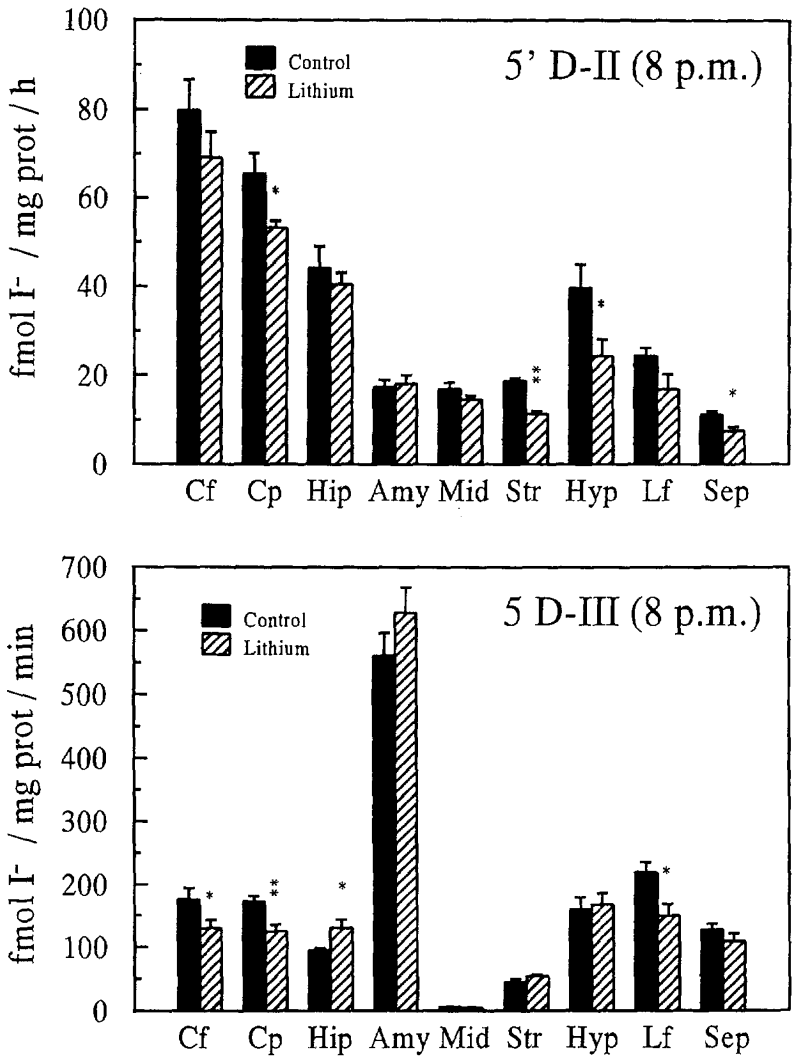

Figure 5. 5'D-II activity (*top*) and 5-III activity (*bottom*) in rats having received 0.15% LI for 14 days and killed at 8:00 P.M. (group 3a). Abbreviations as in Figure 1.

the activity of 5'D-II and did in fact raise the concentrations of T_3 in rat CNS. Fluoxetine, like LI, enhanced the activity of 5'D-II and inhibited 5D-III activity, but in a more limited number of brain regions (Baumgartner et al. 1994a). It would therefore seem that very different antidepressant treatments such as DMI, fluoxetine, and sleep deprivation all seem to enhance the concentrations of the active thyroid hormone T_3 in rat brain. Whether the same also applies to the prophylactic agents CBM and LI remains to be investigated. Based on the data presented in this study it can be hypothesized that the inhibition of 5D-III activity may somehow be involved in the prophylactic action of the two drugs. In contrast, drugs such as desipramine and treatments such as sleep deprivation, which are not prophylactic and may even induce mania, did not affect 5D-III activity but strongly enhanced 5'D-II activity. Studies measuring the effects of all these drugs and treatments, not only on deiodinase activities and T_3 concentrations but also on functional parameters influenced by T_3 in the CNS, are needed for further clarification. From a clinical point of view, as briefly pointed out in the introduction, three studies have all reported that serum levels of T_3 are higher in responders to prophylactic and/or therapeutic treatment with LI than in nonresponders (Baumgartner et al. 1995; Hatterer et al. 1988, Schöpf and Lemarchand 1994). One study has found lower serum concentrations of T_4 in responders to CBM (Roy-Byrne et al. 1984). These results may indicate that interactions of LI and CBM with thyroid hormone metabolism may indeed somehow be involved in their mechanism of action. It is also well known that severe hypothyroidism may mimic all symptoms of affective disorders (for a review, see Hall et al. 1986). Finally, thyroid hormones—both T_3 and T_4—have repeatedly been reported to be of value in different treatment regimes in patients with affective disorders (*e.g.*, Bauer and Whybrow 1990; Baumgartner et al. 1994d; Joffe and Singer 1990; Prange et al. 1969).

As shown by the results of basic research, thyroid hormones seem to influence widely varying biochemical functions in the CNS, which are also affected by LI, CBM, and other mood-stabilizing drugs, e.g., beta-recep-

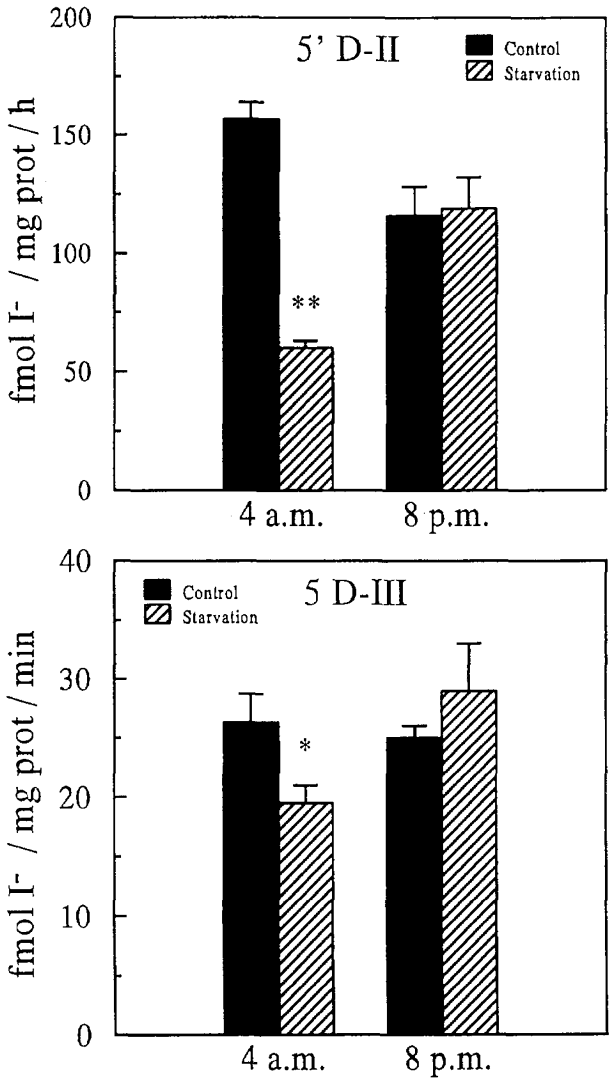

Figure 6. 5'D-II activity (*top*) and 5D-III activity (*bottom*) in the frontal cortex after 2 weeks of starvation (group 5a).

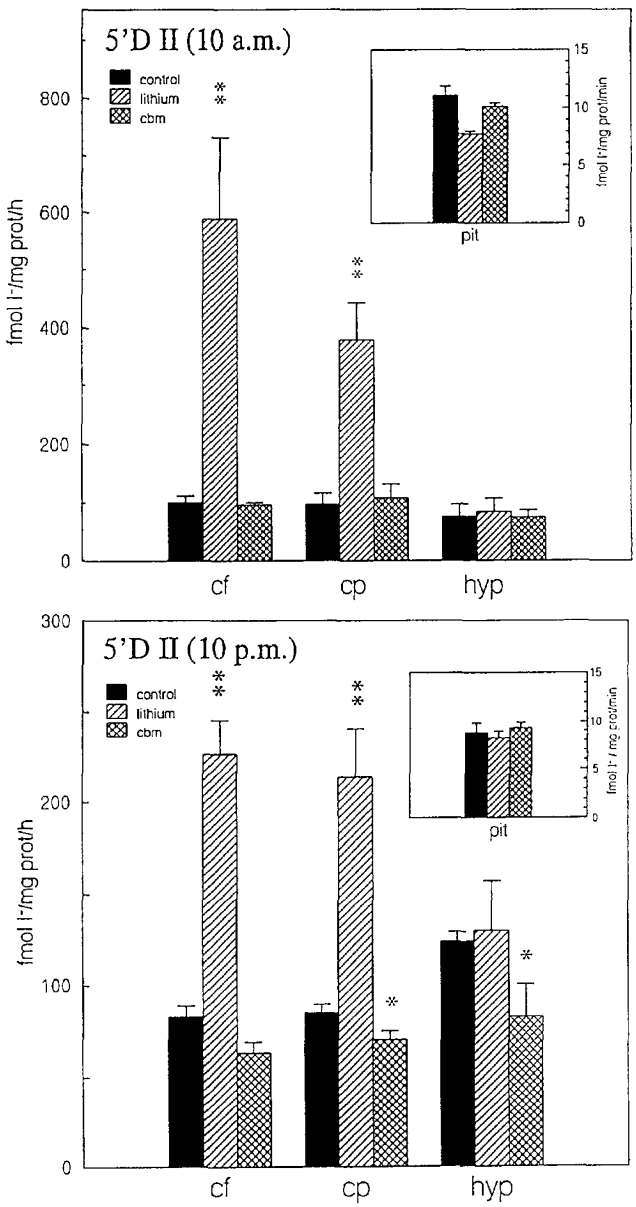

Figure 7. Effects of acute LI (7.5 mmol/L/kg, group 6a) or CBM (40 mg/kg, group 6c) on 5'D-II activity in different regions of the brain and pituitary at 10:00 A.M. (12 hours after intervention, *top*) and 10:00 P.M. (24 hours after intervention, *bottom*). Abbreviations as in Figure 1.

tor density (e.g., Schmidt and Schultz 1985), serotonergic function (e.g., Heal and Smith 1988; Tejani-Butt et al. 1993), dopaminergic function (e.g., Atterwill 1981), GABA uptake and release (e.g., Hashimoto et al. 1991, Mason et al. 1987), adenylate cyclase activity and G protein function (e.g., Michel-Reher et al. 1993; Walz and Howlett 1987), sodium-potassium-ATPase (e.g., Atterwill et al. 1985), fast synaptosomal calcium uptake (e.g., Mason et al. 1990), etc. As it has become increasingly evident in recent years that all our antidepressant and prophylactic therapies affect the functioning of several different neurotransmitter systems, future research strategies should probably focus on the search for common denominators in the mechanisms of action of all these forms of treatment. As already suggested earlier by other authors (e.g., Whybrow and Prange 1981), thyroid hormones may be a reasonable candidate for such a common denominator. However, more systematic research is needed to clarify whether thyroid hormones and antidepressants have common effects on relevant CNS functions.

The mechanism of action of the different anticonvulsant drugs is as yet largely unknown (for a review, see MacDonald and McLean 1986, MacDonald 1989). It is therefore somewhat strange that all relevant anticonvulsant drugs, however different their chemical profiles may be, affect serum concentrations of thyroid hormones in patients with convulsive disorder in the same direction. The serum levels of T_4 and fT_4 usually fall, whereas those of T_3 and TSH either remain unchanged or are also reduced by these drugs. These changes have been re-

Table 2. Thyroxine (T4), Triiodothyronine (T3), and Thyrotropine (TSH) Serum Concentrations in Different Experimental Groups

Group	Design				T4 (nmol/L)	T3 (nmol/L)	TSH (ng/ml)
2a	LI	−0.30%	−14 d	— 4 A.M.	45.8 ± 13.7[a]	1.1 ± 0.3[a]	1.7 ± 0.4
b	CBM	−0.30%	−14 d	— 4 A.M.	47.7 ± 5.1[a]	1.2 ± 0.2[a]	2.1 ± 0.7
c	control			— 4 A.M.	69.5 ± 11.4	1.5 ± 0.1	1.7 ± 0.7
a	LI	−0.30%	−14 d	— 8 P.M.	55.6 ± 12.1	1.0 ± 0.3	1.5 ± 0.7[a]
b	CBM	−0.30%	−14 d	— 8 P.M	45.1 ± 6.0[a]	1.0 ± 0.1	2.2 ± 0.8
c	control			— 8 P.M.	67.6 ± 12.2	1.2 ± 0.1	2.5 ± 0.7
3a	LI	−0.15%	−14 d	— 8 P.M.	78.5 ± 12.0[a]	1.4 ± 0.1[a]	
b	control		−14 d	— 8 P.M.	61.4 ± 8.4	1.2 ± 0.1	
4a	CBM	−0.40%	− 7 d	— 4 A.M.	60.7 ± 15.9	1.2 ± 0.3	
b	control		− 7 d	— 4 A.M.	56.8 ± 7.1	1.2 ± 0.2	
4a	CBM	−0.40%	− 7 d	— 8 P.M.	55.6 ± 6.1	1.0 ± 0.1[a]	
b	control		− 7 d	— 8 P.M.	61.8 ± 7.4	1.2 ± 0.1	
5a	fasting		−14 d	— 4 A.M.	54.4 ± 7.4	1.3 ± 0.2	
b	control		−14 d	— 4 A.M.	57.0 ± 7.4	1.2 ± 0.2	
5a	fasting		−14 d	— 8 P.M.	65.5 ± 5.7	1.6 ± 0.6	
b	control		−14 d	— 8 P.M.	61.4 ± 8.4	1.2 ± 0.1	
6a	LI	−7.5 mmol/L/kg	−12 h	— 10 A.M.	17.4 ± 5.9[a]	0.3 ± 0.1[a]	
b	LI	−3.0 mmol/L/kg	−12 h	— 10 A.M.	68.5 ± 6.5	0.7 ± 0.1	
c	CBM	−40 mg/kg	−12 h	— 10 A.M.	65.8 ± 6.5	0.8 ± 0.1	
d	fasting		−12 h	— 10 A.M.	67.6 ± 8.9	0.5 ± 0.2	
e	control			— 10 A.M.	57.4 ± 7.9	0.8 ± 0.1	
6a	LI	−7.5 mmol/L/kg	−24 h	— 10 P.M.	27.3 ± 1.8[a]	0.4 ± 0.03[a]	
c	CBM	−40 mg/kg	−24 h	— 10 P.M.	58.8 ± 4.2	0.6 ± 0.06	
e	control			— 10 P.M.	57.6 ± 4.4	0.7 ± 0.1	

[a] $p < .05$

ported after administration of diphenyl hydantoine (DPH) (Cavalieri et al. 1979; Isojärvi et al. 1992), CBM (see Herman et al. 1991 for a review), valproate (Bentsen et al. 1983; Fichsel and Knöpfle 1978), and primidone (Fichsel and Knöpfle 1978). The mechanism(s) underlying these effects are unclear. Furthermore, DPH has been found to inhibit nuclear binding of T_3 in the anterior pituitary (Franklyn et al. 1985) and liver (Mann and Surks 1983; Mann et al. 1983; Smith and Surks 1984), acting as a partial T_3 agonist. An increase in the uptake of T_4 by the erythrocytes (Oppenheimer and Tavernetti 1962) and a decrease in that of T_3 in GH3 cell lines (Zemel et al. 1988) have also been observed after administration of DPH. Furthermore, it has been found to induce an increase in 5'D-II deiodinase activity in the cerebellum and "brain," which is comparable to the data reported here for CBM (Schröder-Van der Elst and Van der Heide 1990). Finally, thyroid hormones seem to affect neuronal activity and seizure threshold (Timiras and Woodbury 1956). We therefore feel that more systematic research on the possibility that thyroid hormones may be involved in the mechanism of action of anticonvulsants is warranted.

No in vivo data have as yet been published on the neuronal regulation of the activities of the deiodinases in the CNS. However, stimulation of 5'D-II by norepinephrine, isoproterenol, and forskolin have been demonstrated in neonatal rat astrocyte cultures (e.g., Courtin et al. 1988). Activators of protein kinase C have also been shown to enhance 5'D-II activity in astrocytes (Courtin et al. 1989, 1991). Furthermore, there is definite evidence that norepinephrine has a stimulatory effect on 5'D-II activity in both brown adipose tissue and the pineal gland in the rat in vivo. This effect is, in fact, mediated by both α_1 and β-adrenergic receptor stimulation (for a review, see Raasmaja, 1990). However, to date no study has investigated the effects of the serotonergic, dopaminergic, GABAergic, or other transmitter systems on the regulation of the activities of the deiodinases. Therefore, further studies on the neuronal regulation of deiodinase isoenzymes are needed before our data can be interpreted.

As a 14-day fast reduces the activity of 5'D-II, which is the reverse of the effects of CBM, its effects on the activity of this enzyme cannot be caused by malnutrition. On the other hand, administration of 0.15% LI (group 3a) did not induce weight loss in comparison with control rats. It is therefore unlikely that the inhibition of 5'D-II activity seen after administration of 0.15% LI was due to weight loss. We found a reduction in 5D-III activity

in the fasting rats. We cannot therefore exclude the possibility that the inhibition of 5D-III activity found after 14-day administration of high-dosed (0.3%) LI may be induced (at least partly) by starvation. This interpretation does not, however, seem likely, for the following reason: 5D-III activity was also significantly inhibited in different brain regions in rats given low-dose (0.15%) LI for 14 days and also in CBM-treated rats (group 2b): however, the weight curves of these two groups did not differ from those found for the controls.

Diminished serum concentrations of thyroid hormones after administration of CBM have also been reported during short-term administration (weeks to months) of this drug to both healthy subjects and patients with affective disorders (e.g., Connell et al. 1984; Herman et al. 1991; Roy-Byrne et al. 1984). This indicates that this drug may have similar effects on thyroid hormone metabolism in both species.

The differing effects of two different dosages of LI on serum levels of thyroid hormones (see Table 2) could be explained by the different effects of the two diets on deiodinase activities. The 0.15% LI diet reduced 5'D-II and 5D-III activity, thereby inhibiting the intracellular deiodination of both T_4 and T_3. This could lead to an increase in the mean concentrations of T_4 and T_3 in the tissue and, secondarily, in the serum. After the 0.3% LI diet, however, 5'D- II activity was also enhanced, resulting in raised deiodination of T_4 and, as a result, a fall in tissue and serum levels of T_4.

The mechanisms underlying the dramatic decreases in the serum concentrations of both T_4 and T_3 seen 12 and 24 hours after IP administration of the toxic dose of 7.5 mmol/L/kg LI are currently unclear. As 5'D-II deiodinase activity was 300% to 500% enhanced in cortical areas in this experiment, it seems likely that the decreases in serum levels of T_4 reflect the dramatic increase in intracellular degradation of T_4, thus leading to a fall in tissue concentrations of T_4 and, secondarily, also in the serum levels of T_4.

It would also seem relevant that a single IP administration of 3.0 mmol/L/kg LI and 40 mg/kg CBM, respectively, either failed to affect the activity of the deiodinases in the CNS (LI) or even altered them in the reverse direction from the changes seen after 14-day administration (in the case of CBM). Also, our failure to find any changes in deiodinase activity after 7-day administration of CBM in a 0.4% diet emphasizes the fact that the effects of CBM on deiodinase activity seem to develop slowly over the course of weeks—as is also assumed for its therapeutic action. The highly toxic dose of 7.5 mmol/L/kg induced striking increases in 5'D-II activity in the frontal cortex and parieto-occipital cortex that were evident both 12 and 24 hours after IP administration. However, the activity of this enzyme was not affected in the hypothalamus and was even reduced in the pituitary. This latter result is consistent with those reported by St. Germain (1987), who found a decrease in 5'D-II activity in cultured pituitary tissue (and in mouse neuroblastoma cell lines) incubated with LI and also in rat pituitary 3 to 24 hours after injection of high doses of lithium. With respect to the dramatic increases in 5'D-II activity in the frontal and parieto-occipital cortex found at 12 and 24 hours after administration of the toxic dose of LI, we are unable to decide whether this is due to a toxic, but specific effect of LI, arising from different stress factors (abdominal pain after IP injection of a large dose), to fasting, or to a combination of all these factors.

The reductions in 5'D-I activity in the liver and kidneys of rats having received LI confirm previous findings by Männistö (1974). The decrease in 5'D-I activity after administration of CBM seems to be a new finding. These effects of the two drugs most likely reflect an adaptational response to the fall in serum levels of thyroid hormones (see Köhrle et al. 1992). In the case of LI an effect of weight loss is also conceivable (Balsam and Ingbar, 1978; Kaplan and Utiger, 1978).

As previously reported for desipramine, both LI and CBM had very different effects on deiodinase activity at different times of the light-dark cycle. For example, if we had investigated the effects of the 0.25% LI diet on deiodinase activity only in rats killed during their resting period (1:00 P.M., group 1), we would have obtained incomplete and misleading results. At 8:00 P.M. both the lower dose of 0.15% LI and the higher ("toxic") dose (0.3% LI) induced a significant decrease in 5D-III activity in four and five brain regions, respectively, three of which had not been affected at 1:00 P.M. However, effects of the "prophylactic" dose of LI on deiodinase activities were investigated at 8:00 P.M. only. Thus, more studies are needed to gain more insight into the circadian effects of "clinical relevant" doses of LI on deiodinase activities.

Several chronobiological effects of LI have been described in the literature (e.g., Kripke and Whyborney 1980; McEachron et al. 1982; Wilkinson et al. 1987; for a review, see Klemfuss 1992). Theoretically there may be several reasons for these effects: diurnal variations in drug intake and availability, variations in light conditions and activity during the light-dark cycle, a diurnal rhythm in the sensitivity of the CNS to different drugs, etc.

In group 2 the serum concentrations of LI and CBM were almost identical at 4:00 A.M. and 8:00 P.M. Thus, the time-dependent drug effects cannot be explained by diurnal variations in food intake.

We recently found that the activity of the 5'D-II isoenzyme has a pronounced circadian rhythm in all regions of the rat brain, with increases during the dark phase, peak values between midnight and 4:00 A.M. and decreases in activity levels between 4:00 A.M. and 8:00 A.M. (Campos-Barros et al., submitted). A glance at Figure 2 shows that 5'D-II activities measured in the control groups at 4:00 A.M. were significantly higher than those

determined at 8:00 P.M. in all brain regions, but not in the pituitary gland. The possibility cannot therefore be excluded that the changes found in the activities of this isoenzyme at 8:00 P.M. reflect a "phase shift" of the nocturnal peak, rather than a "true" change in the absolute amount of the 24-hour enzyme activity and production of T_3. In this case, CBM would induce a "phase advance" and the 0.15% LI dose a "phase delay" of the nocturnal peak of 5'D-II activity. More frequent measurement of both deiodinase activities and tissue concentrations of thyroid hormones are needed to clarify this.

However, the 5D-III isoenzyme activity has no diurnal rhythm in any region of the rat brain (Campos-Barros et al., submitted). As can also be seen from Figure 2, the 5D-III activities of the control rats were roughly the same at 4:00 A.M. and 8:00 P.M. in all brain regions. The inhibition of the activity of 5D-III by both drugs would therefore seem to reflect a "true" reduction in T_3 degradation per 24-hour cycle. (Note that the differences in baseline 5D-III activities in the controls between, for example, the 1:00 P.M. results of group 1 and the 4:00 A.M. or 8:00 P.M. results in group 2 do not reflect "true" diurnal variations but are exclusively due to the fact that the experiments on the different groups were performed over a period of more than 2 years and the absolute values are therefore no longer directly comparable (see Methods)).

We conclude that in light of these and our previous results (Campos-Barros et al. 1994), future research on the effects of psychopharmacologic drugs should carefully consider the circadian fluctuations in the susceptibility of the brain.

ACKNOWLEDGMENTS

This study was supported by Deutsche Forschungsgemeinschaft (DFG), Grant Ba932/5-2. The authors would like to thank CIBA Geigy for providing carbamazepine, the laboratory of the Klinikum Benjamin-Franklin for measuring the serum levels of lithium and carbamazepine, and Ines Frege, Alberto Musa, Marco Bachmann, and Stefan Kley for technical assistance.

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