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Effects of tranylcypromine on thyroid hormone metabolism and concentrations in rat brain

Hans Prengel, Oliver Brödel, Luis Hiedra, Graziano Pinna, Murat Eravci, Harald Meinhold, Andreas Baumgartner *

Department of Nuclear Medicine (Radiochemistry), Klinikum Benjamin Franklin, Free University of Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany

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Abstract

The effect of 14 days administration of the anti-depressant tranylcypromine (TCP) on iodothyronine deiodinase activities and the concentrations of thyroxine (T_4) and triiodothyronine (T_3) were investigated in homogenates of up to nine regions of the rat brain. The activity of the 5III deiodinase isoenzyme, which catalyses the inactivation of T_3 to 3,3′-diiodothyronine (3,3′-T_2), was enhanced in eight brain regions. However, the brain levels of T_4 were completely unchanged and the T_3 concentrations were significantly reduced in the frontal cortex only. Therefore, we also measured the T_3 concentrations of three subcellular fractions (nuclei, synaptosomes and mitochondria) of six brain regions. TCP induced a significant reduction in T_3 levels in the synaptosomes of the frontal cortex and significant increases in the mitochondrial T_3 concentrations in the amygdala. The latter effect was replicated after 14 days administration of 5 mg/kg desipramine. No effects of either drug on nuclear concentrations of T_3 were seen in any brain region. As the amygdala is critically involved in the affective coloring of sensory stimuli, the increase in T_3 concentrations in the mitochondria of this brain region may be of relevance for the mechanism of action of anti-depressant drugs.

Keywords: Rat brain; Tranylcypromine; Triiodothyronine; Deiodinases; Mitochondria; Synaptosomes

1. Introduction

Thyroid disorders such as hypo- or hyperthyroidism may mimic psychiatric symptoms found in the entire range of psychiatric diseases (for a review see Hall et al., 1986). On the other hand, both T_3 and T_4 have repeatedly been reported to enhance the action of anti-depressant drugs, particularly in treatment-resistant depressed patients (e.g. Joffe et al., 1993; Baumgartner et al., 1994a; Bauer et al., 1998). The question therefore arises as to whether thyroid function is disturbed in depressed patients. Almost all studies published to date have, however, reported that depressed patients are euthyroid, at least, in terms of their serum levels of thyroid hormones (for a review see Baumgartner et al., 1988). In recent years, however, several independent research groups have unanimously reported that serum concentrations of thyroxine (T_4) and free thyroxine (fT_4) decline during treatment with widely varying pharmacological and non-pharmacological anti-depressant therapies (for results and reviews see Baumgartner et al. 1996; Joffe et al. 1996). In nearly all of these studies the changes were significantly correlated to the degree of clinical improvement. These close correlations suggest that changes in thyroid hormone metabolism in specific tissues, such as the CNS, may be involved in the mechanisms of action of all these treatments.

We therefore conducted a series of animal studies in order to investigate the effects of anti-depressant and prophylactic medications on the metabolism and concentrations of thyroid hormones in rat brain. The metabolism of T_4 in the CNS is 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 directly from the blood, whereas the supply of T_3 to the

* Corresponding author. Tel.: +49-30-84452727; fax: +49-30-84452700.

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brain depends almost completely on cellular uptake and intracellular deiodination of T_4 (Van Doorn et al., 1984). This implies that the supply of T_4 and intracellular deiodination are essential for the function of T_3 in the CNS. The mechanisms of deiodination in the CNS are, however, 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) catalyses the deiodination of all iodothyronines. Two other deiodinases have been identified in both the rat and human CNS. Type II iodothyronine 5′-deiodinase (5′D-II) is a selective outer-ring deiodinase that catalyses deiodination of T_4 to T_3 and reverse T_3 (rT_3) to 3,3′-T_2. Type III iodothyronine 5-deiodinase (5D-III) catalyses inner-ring deiodination, thereby metabolising T_4 to rT_3 and T_3 to the inactive compound 3,3′-T_2 (for a review see Leonard and Köhrle, 1996).

In previous studies we found that the anti-depressant drug desipramine (DMI) significantly enhanced the activity of 5′II deiodinase in several regions of the rat brain (Campos-Barros et al., 1994). Consequently, there was a dose-dependent rise in tissue concentrations of T_3 (Campos-Barros and Baumgartner, 1994; Campos-Barros et al., 1995). Subchronic administration of the serotonin reuptake inhibitor fluoxetine also enhanced 5′D-II activities, but at the same time inhibited 5D-III activity in various areas of the rat brain (Baumgartner et al., 1994b). Theoretically, the combination of these synergistic effects should also lead to increases in tissue levels of T_3. Furthermore, sleep deprivation, a non-pharmacological anti-depressant therapy, also raised both 5′D-II activity and tissue concentrations of T_3 in rat frontal cortex (Campos-Barros et al., 1993). Finally, reductions in the activity of the 5D-III isoenzyme have been found after 14 days administration of the prophylactic drugs lithium and carbamazepine (Baumgartner et al., 1997).

In conclusion, these data lend strong support to the hypothesis that an increase in tissue concentrations of T_3 may be a common effect of different pharmacological and non-pharmacological anti-depressant or prophylactic treatments. For reasons outlined above, these increases may well be involved in the as yet unknown mechanism of action of anti-depressant therapies.

In order to further substantiate the evidence in favor of this hypothesis, in the present study we investigated the effects of the MAO inhibitor tranylcypromine (TCP) on thyroid hormone metabolism and concentrations in the rat CNS. As the initial results were somewhat unexpected, we finally conducted an extended series of studies on this issue. The results of all these studies are reported below.

2. Materials and methods

2.1. Materials

Thyroxine (T_4), 3,3′,5′-triiodothyronine (rT_3), 3,5,3′-triiodothyronine (T_3), 3,3′-diiodothyronine (3,3′-T_2) and 3,5-diiodothyronine (3,5-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, [3′-^{125}I]-T_3 were prepared for iodothyronine deiodinase assays and radioimmunoassay by radioiodination of T_3, 3,3′-T_2, 3,5-T_2, respectively, as described by Meinhold (1986). Separation and purification of ^{125}I labeled T_3 has been performed as previously described by Pinna et al. (1999). The tracers with specific radioactivity of 50–75 MBq/nmol were purified immediately before use with disposable Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA, USA) yielding a purity $>99\%$ with free iodide 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, Germany). Dithiothreitol (DTT) was purchased from Boehringer GmbH (Mannheim, Germany). Sucrose for density gradient centrifugation was obtained from Merck (Darmstadt, Germany). Iopanic acid (IOP), 6-*n*-propyl-2-thiouracil (PTU), aurothioglucose (ATG) and tranylcypromine hydrochloride (TCP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Bio-Rad AG 1×2 resin and columns were obtained from Bio-Rad Laboratories (Richmond, CA, USA). Polyallomer tubes (14×95 mm) and Ultraclear tubes (25×89 mm) and the L8-55 ultracentrifuge were from Beckman Instruments Inc. (Palo Alto, CA, USA). The TST 41.14 swing out rotors were from Kontron Instruments GmbH (Neufahn, Germany) and the Centrifuge 5415 C was from Eppendorf-Hintz-Netheler GmbH (Hamburg, Germany). Dowex 50 WX 8 columns (mesh 100–200) were obtained from Serva GmbH and Co. (Heidelberg, Germany).

2.2. Animal treatment and tissue samples

Euthyroid adult male Sprague–Dawley rats weighing approx. 300 g were employed throughout. They were housed in pairs with a 12-h light/dark cycle (06:00–18:00 h). Food and water were available ad libitum. Following an adjustment period of at least 1 week in the new environment the following studies were performed.

2.2.1. Group 1

Six rats received a daily dose of 5 mg/kg TCP i.p. dissolved in NaCl for 14 days. Six further rats received NaCl alone. All rats were decapitated without anesthesia at between 11:00 and 13:00 h, approx. 24 h after the last dose of the drug.

2.2.2. Group 2

Two further groups each consisting of six rats were treated as described for group 1, with the exception that they were decapitated at between 04:00 and 06:00 h, i.e. at the end of their active period. In the tissues of groups 1 and 2 we measured deiodinase activities.

2.2.3. Group 3

Two groups each containing six rats were treated and sacrificed exactly as described for group 1 (5 mg TCP/kg/day, decapitation at between 11:00 and 13:00 h). We measured T_4 and T_3 concentrations in the tissues of these rats.

2.2.4. Group 4

Two further groups of six rats received the lower dose of 1 mg TCP/kg/day for 14 days, as described for group 1, and were sacrificed at between 11:00 and 13:00 h. We measured T_4 and T_3 concentrations in the tissues of these rats.

2.2.5. Group 5

Two groups of six rats were treated and sacrificed as described for group 1. In these rats we determined T_3 concentrations in subcellular fractions of different brain regions.

2.2.6. Group 6

Two further groups each consisting of six rats were treated as described for group 1. This group was included for statistical validation of the results obtained for group 5.

2.2.7. Group 7

Six rats each received a daily dose of 5 mg/kg desipramine (DMI) dissolved in NaCl by gavage for 14 days. Six further rats received NaCl alone. The rats were decapitated at between 11:00 and 13:00 h.

After decapitation various areas of the brain were dissected by the method described by Glowinski and Iversen (1966) and stored immediately at -70°C. Blood was drawn from the decapitation wound and centrifuged, and the serum was stored at -20°C. The pituitary glands and livers were also dissected and frozen.

2.3. Iodothyronine deiodinase assays

Measurement of the deiodinases activities were performed as previously described in detail (Campos-Barros et al., 1994). Tissue samples were individually homogenized on ice in 5–6 vol. of 0.25 M sucrose, 10 mM HEPES (pH 7.0) containing 10 mM DTT and immediately frozen in a dry ice/acetone bath and stored at -80°C until assay. The measurement of the activities 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.

Assay of 5′D-I and 5′D-II. 5′D-I and 5′D-II activities were determined simultaneously by measuring the release of radioiodide from 100 000 cpm ($\sim$2.5 kBq) [5′-^{125}I]-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 PTU (Visser et al., 1982). 5′D-II was also determined using [5′-^{125}I]-T_4 as substrate in the presence of 6 nM T_4, 30 mM DTT, 1 mM PTU and 1 μM T_3, in order to inhibit the inner-ring deiodination of T_4 in those tissues containing significant type III deiodinase (5D-III) activity.

The measurement was conducted after 45–90 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 adding the 50 μl of ice-cold 5% BSA, 10 mM PTU, followed by 400 μl of 10% ice-cold trichloroacetic acid. After centrifugation at $4000 \times g$ 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 (mesh 100–200). The iodide was then eluted with 2×1 ml 10% acetic acid and counted in a gamma counter.

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 ($\sim$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-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 less than 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%.

2.4. Subcellular fractionation

All subsequent procedures were carried out at 4°C room temperature. In order to inhibit the deiodination activity, 25 μM IOP were added to all unbuffered sucrose solutions. For each subcellular fractionation we used tissue from only one rat.

The preparation of the subcellular fractions was performed according to Dodd et al. (1981), with slight modifications. Frozen tissue was placed in ice-cold 0.32 M sucrose at a final dilution of 1:10 (w/v). The tissue was homogenized mechanically with a motor driven glass–glass homogenizer, mortar-cooled in an ice–water mixture throughout, (difference in diameter between mortar and pestle 0.5 mm, motor speed 750 rpm, 12 strokes of the pestle).

The centrifugation steps were carried out in an L8-55 ultracentrifuge, using a TST41.14 swing out rotor. Subcellular fractions were washed once in 0.32 M sucrose before storage at −85°C.

The homogenate was centrifuged at $1800 \times g$ for 10 min to obtain the low speed supernatant (S1) and the crude nuclear pellet (P1). S1 was diluted with 0.32 M sucrose to a final volume of 9 ml, layered directly onto 4 ml 1.2 M sucrose and centrifuged at $200\,000 \times g$ for 15 min. The resultant high-speed pellet was composed of mitochondria. Synaptosomes were retained at the 1.2 /0.32 M sucrose suspension interphase. Following careful suction with a syringe, 0.5–1.5 ml of this material was diluted in 0.32 M sucrose to a final volume of 9 ml and then layered onto 4 ml of 0.8 M sucrose. This suspension was centrifuged under the same conditions as for the previous spin. The high-speed pellet was composed of synaptosomes.

The purity of the subcellular fractions was evaluated by electron microscopy in the Department of Neuroanatomy of the Klinikum Benjamin Franklin. Degrees of purity of 85, 80 and 85%, respectively were found for the nuclear, synaptosomal and mitochondrial fractions. We also measured the activity of acetylcholinesterase as marker enzyme for synaptosomes and succinate dehydrogenase as marker enzyme for mitochondria in all three subcellular fractions. The results confirmed the findings of the evaluation conducted by electron microscope.

2.5. Extraction and determination of T_4 and T_3

For measurement of T_4 and T_3 concentrations in homogenates the tissue samples were extracted and the concentrations determined according to the method developed by Morreale de Escobar et al. (1985) and modified by Campos-Barros et al. (1995). The tissue concentrations of T_4 and T_3 are given as pg per gram net weight.

Extraction of T_3 from subcellular fractions was performed as described by Pinna et al. (1999). Ice-cold 100% methanol (1.75 ml) containing 1mM PTU was added to each frozen subcellular fraction, following which the fraction was resuspended in an ultrasonic water bath for 20 s. After centrifugation of the probe at $14\,000 \times g$ for 20 min in a 5415 C centrifuge the extraction of the pellet was repeated.The supernatants were purified through Bio-Rad AG 1×2 resin columns. T_3 was eluted with 70% acetic acid, evaporated to dryness and taken up in the experimental buffer or stored at −20°C for no longer than 3 days. The organelle debris-containing pellet was used for protein determination (Bradford, 1976).

After addition of [^{125}I]-T_3 ($\sim$1000 cpm/ 100 μl) to each sample, recovery by extraction ranged between 84 and 90%. The concentrations of T_3 in the subcellular fractions were determined by a highly sensitive radioimmunoassay with an improved sensitivity in the lower range (Pinna et al., 1999). The limit of sensitivity was 0.6 pg/tube and allowed a detection limit of 1.3 pg T_3/mg protein, and the intra- and interassay coefficients of variation were always less then 10%. Each sample was determined in duplicate at one dilution. The results were corrected on the basis of individual recovery data.

In each experiment all samples of a single brain region were subfractionated, extracted and/or assayed together. The T_3 concentration in each subcellular fraction is given in pg/mg protein.

2.6. 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, as previously described (Campos-Barros et al., 1994). 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 Institute of Health (Bethesda, MD, USA).

2.7. Statistical analysis

The data are given as means ± SEM. Individual com-

parisons between the control group and each of the treatment groups were performed by the Mann–Whitney *U*-test. Altogether we conducted 62 statistical tests for groups 1–5. Strict application of Bonferroni's correction of the *P*-value would result in a limit of significance of $P < 0.0008$, in which case the results of only eight statistical calculations would remain significant. When applying a significance level of $P < 0.05$, in 62 calculations, approx. three 'significant' results would be expected to occur by chance. However, in our study we found 19 significant results. Thus, if Bonferroni's correction were strictly applied, several relevant findings would probably be lost. This problem was addressed as follows. We have marked those results which yielded *P*-values of between 0.05 and 0.0008, and discuss whether they seem plausible or may reflect statistical artefact. For example, after 5 mg/kg TCP 5D-III activities were reduced in four out of eight brain regions at a significance level of $P < 0.0008$, and in three further brain regions at significance levels between 0.05 and 0.0008. The latter results are unlikely to have occurred by chance. Furthermore, the effect of TCP on the concentrations of T_3 in mitochondria of the amygdala was not significant after Bonferroni's correction ($P = 0.02$, group 5). As this finding was considered to be of substantial importance, we validated it in a second, independent group (group 6). However, results which yielded *P*-values between 0.05 and 0.0008, and whose plausibility did not appear to be proven by other corresponding results, must be confirmed in future studies.

3. Results

3.1. Group 1

The effects of 14 days administration of 5 mg/kg TCP on 5′D-II and 5D-III activities are shown in Fig. 1. TCP reduced the activity of 5′D-II selectively in the frontal cortex ($P = 0.01$). TCP did not affect the activities of 5′D-II in the other eight brain regions.

The activity of the 5D-III isoenzyme was significantly enhanced in the frontal cortex, hippocampus, amygdala and hypothalamus. In the limbic forebrain, striatum and midbrain the activity of 5D-III was enhanced at a *P* level of between 0.05 and 0.0008 (Fig. 1). In this group we also measured 5′D-I and 5′D-II activities in the pituitary gland and the 5′D-I activity in the liver. In the pituitary, 5′D-I activity was unchanged and 5′D-II activity declined (33.4 ± 1.5 vs. 27.9 ± 3.0 fmol I^-/mg protein/min in controls and after administration of TCP, respectively, $P = 0.02$). No significant effects were seen on 5′D-I activity in the liver (52.1 ± 2.5 vs. 54.9 ± 2.9 pmol/mg protein/min).

The serum concentrations of T_4 were significantly lower in TCP-treated rats (62.7 ± 21.1 nmol/l) than in the controls (83.4 ± 11.1 nmol/l; $P = 0.0001$). No significant differences were found between the serum concentrations of T_3 and TSH of the two groups (data not shown).

3.2. Group 2

The results obtained for group 1 would predict a fall in tissue concentrations of T_3 after administration of TCP. This was unexpected, insofar as various anti-depressant and prophylactic treatments have to date been shown to induce increases in tissue levels of T_3 (see Section 1). As we had previously found that both deiodinase activities at baseline and also the effects of pharmacological drugs on the activities of these enzymes vary substantially during the 24-h cycle (Campos-Barros et al., 1994, 1997; Baumgartner et al., 1997), we next evaluated whether the effects of TCP on the activities of these enzymes are different at a different time of day. In group 2 the rats were therefore sacrificed at between 04:00 and 06:00 h. and deiodinase

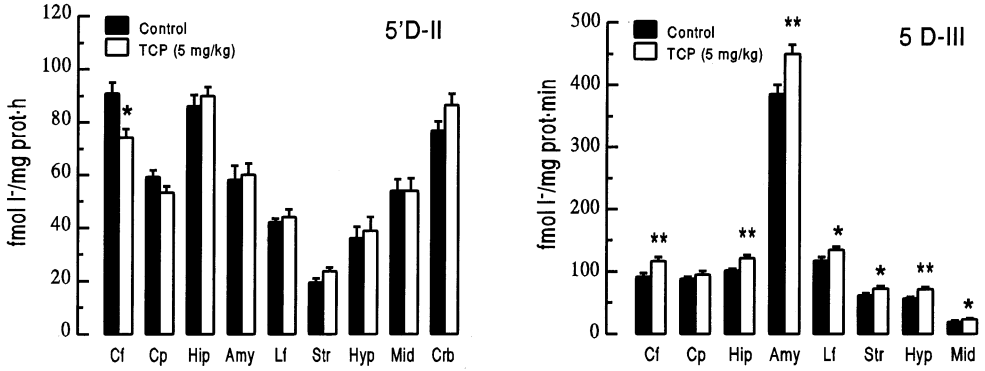

Fig. 1. Activities of the 5′-II deiodinase (5′D-II, left) and the 5-III deiodinase (5D-III, right) after 14 days administration of tranylcypromine (5 mg/kg/day, decapitation at between 11:00 and 13:00 h). Abbreviations: Cf = frontal cortex; Cp = parieto-occipital cortex; Hip = hippocampus; Amy = amygdala; Lf = limbic forebrain; Str = striatum; Mid = midbrain; Hyp = hypothalamus; Crb = cerebellum. * $P < 0.05$; ** $P < 0.0008$.

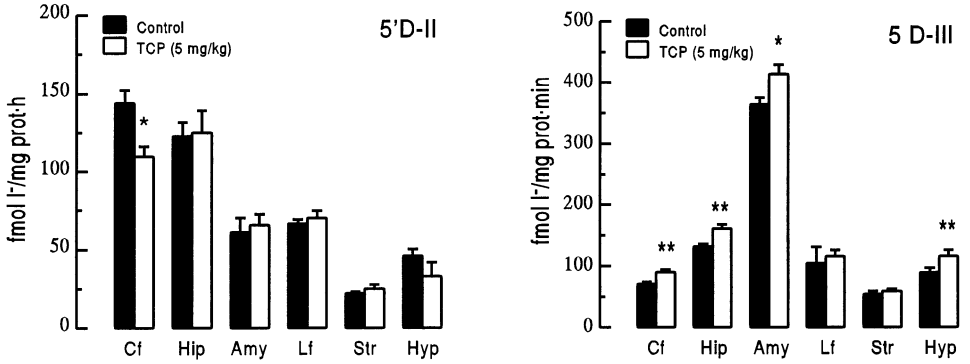

Fig. 2. Activities of the 5′-II deiodinase (left) and the 5-III deiodinase (right) after 14 days administration of tranylcypromine (5 mg/kg/day, decapitation at between 04:00 and 06:00 h). See Fig. 1 for an explanation of abbreviations. * $P < 0.05$; ** $P < 0.0008$.

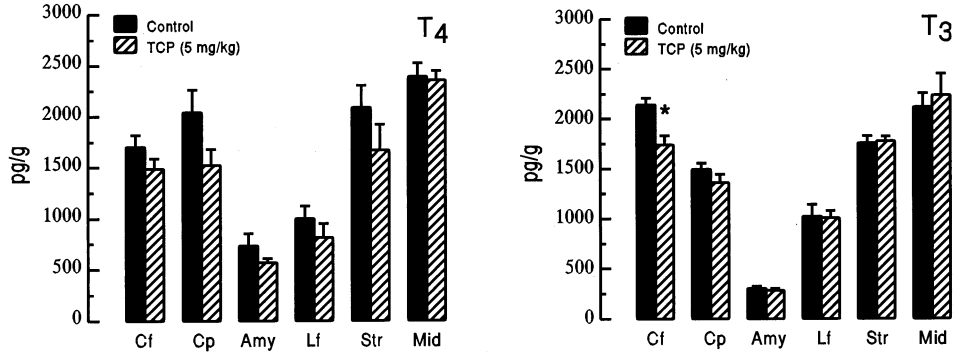

Fig. 3. Tissue concentrations of T_4 (left) and T_3 (right) in rat brain regions after 14 days administration of 5 mg/kg/day tranylcypromine. Decapitation at between 11:00 and 13:00 h. For an explanation of abbreviations see Fig. 1. * $P < 0.05$.

activities were measured in six selected brain regions. The results are shown in Fig. 2. As already seen in group 1, the activity of 5′D-II was inhibited in the frontal cortex ($P = 0.02$), but unchanged in other brain areas. Additionally, the activities of 5D-III were significantly enhanced in the frontal cortex, hippocampus, hypothalamus and in the amygdala.

3.3. Group 3

We next investigated whether the enhanced activities of 5D-III after TCP administration do, in fact, lower the tissue concentrations of T_4 and T_3. The effects of 14 days administration of 5 mg/kg TCP on the tissue levels of T_4 and T_3 measured in the homogenates of six selected brain regions are shown in Fig. 3. Surprisingly, the concentrations of T_4 were normal in all brain regions after administration of TCP. The levels of T_3 declined in the frontal cortex only ($P = 0.02$).

3.4. Group 4

We further investigated whether 14 days administration of the reduced dose of 1 mg/kg TCP has different effects on tissue levels of thyroid hormones from the 5 mg/kg dose. The results for this part of the study are presented in Fig. 4. No significant effects of TCP on the tissue concentrations of T_4 were seen. Again, the levels of T_3 were reduced in the frontal cortex only ($P = 0.01$).

3.5. Group 5

The concentrations of T_3 were measured in the nuclear, synaptosomal and mitochondrial fractions of six brain regions. As shown in Fig. 5, we found a highly significant decline in T_3 concentrations in the synaptosomal fraction of the frontal cortex ($P = 0.0001$). We also observed an increase in T_3 levels in the mitochondrial fraction of the amygdala ($P = 0.02$).

3.6. Group 6

As we considered the increases in T3 concentrations in the mitochondria of the amygdala potentially important, we repeated the experiment in a further group for the purpose of statistical validation. From Fig. 6 it can be seen that the concentrations of T_3 in the mitochondria of the amygdala were also elevated in the second experiment ($P = 0.01$).

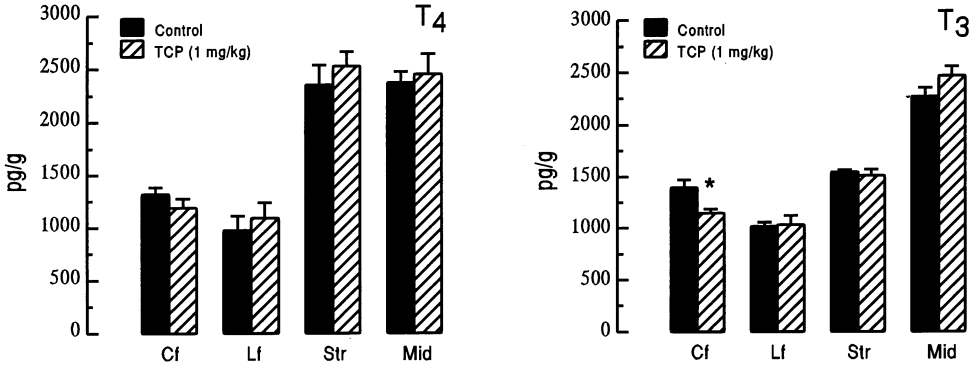

Fig. 4. Tissue concentrations of T_4 (left) and T_3 (right) in rat brain regions after 14 days administration of 1 mg/kg/day tranylcypromine. Decapitation at between 11:00 and 13:00 h. For an explanation of abbreviations see Fig. 1. * $P < 0.05$.

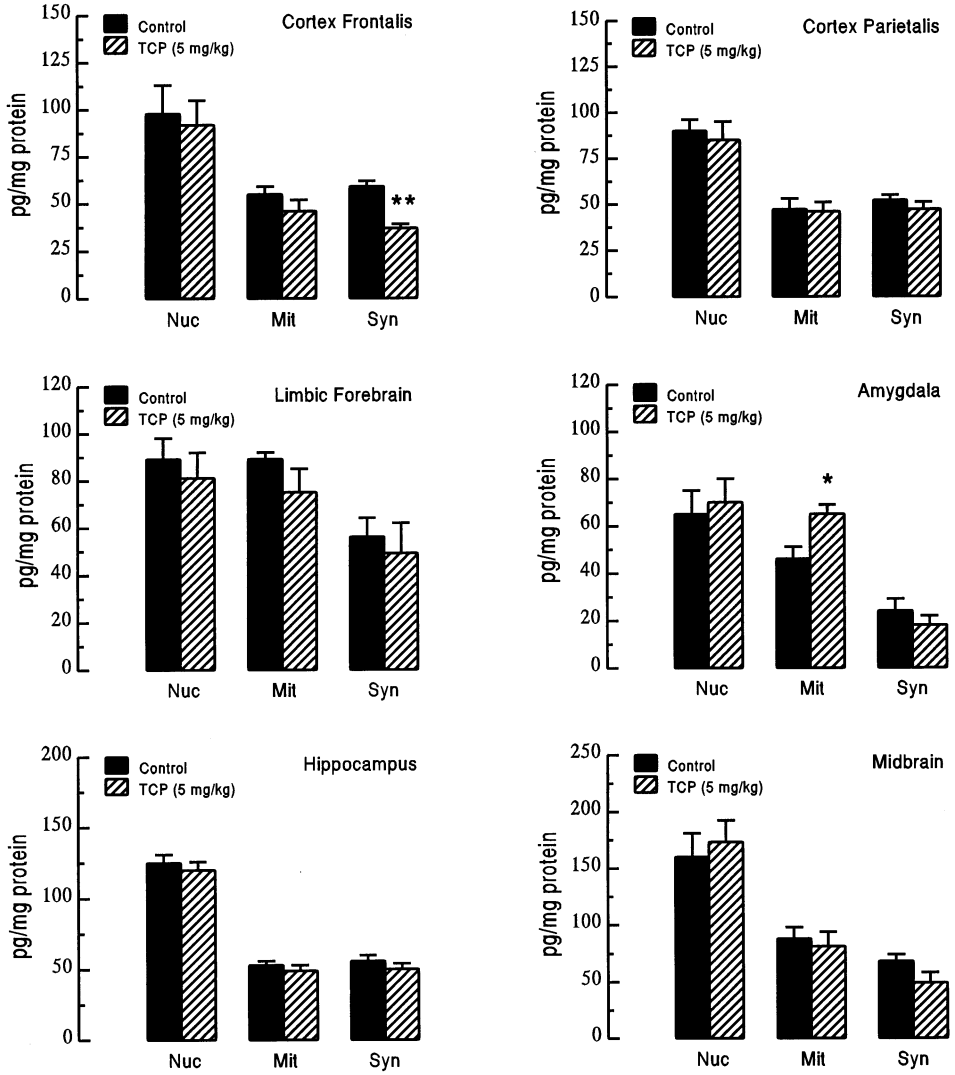

Fig. 5. Concentrations of T_3 in subcellular fractions of six rat brain regions after 14 days administration of tranylcypromine (5 mg/kg/day, decapitation at between 11:00 and 13:00 h). Abbreviations: Nuc = nuclei; Mit = mitochondria; Syn = synaptosomes. * $P < 0.05$; ** $P < 0.0008$.

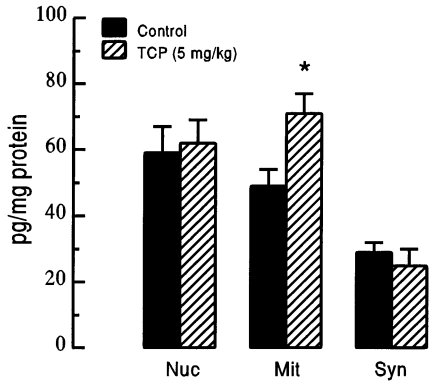

Fig. 6. Concentrations of T_3 in subcellular fractions of the amygdala after 14 days administration of tranylcypromine (5 mg/kg/day, decapitation at between 11:00 and 13:00 h, group 6). Abbreviations: Nuc = nuclei; Mit = mitochondria; Syn = synaptosomes. * $P < 0.05$.

3.7. Group 7

In order to investigate whether the effects of TCP on T_3 concentrations in subcellular fractions are also seen after administration of a different anti-depressant, we measured T_3 levels in subcellular fractions of the frontal cortex and amygdala after 14 days administration of 5 mg/kg desipramine (Fig. 7). We found no effects on T_3 levels in the synaptosomal fraction of the frontal cortex, but a significant increase in the mitochondrial fraction of the amygdala ($P = 0.01$).

4. Discussion

As mentioned in Section 1, our previous work showed increases in 5′D-II activity and/or declines in 5D-III activity in rat brain after subchronic administration of desipramine, fluoxetine, lithium and carbamazepine, and also sleep deprivation. The tissue concentrations of T_3 were measured after administration of desipramine and sleep deprivation, respectively, and were found to be enhanced.

Surprisingly, we found almost diametrically opposed changes after subchronic administration of TCP: an inhibition of 5′D-II in the frontal cortex and an enhancement of 5D-III activity in nearly all brain regions. These effects of TCP on deiodinase activities should induce a decline in tissue concentrations of T_4 and T_3. However, surprisingly, the T_4 levels were unaffected by administration of TCP in all brain regions and T_3 concentrations declined in the frontal cortex alone. Although neither the effect of TCP on 5′D-II activities nor that on the T_3 levels in the frontal cortex was significant after Bonferroni's correction, we believe that these findings are valid, as each was replicated in two independent experiments (groups 1 and 2, and groups 3 and 4, respectively).

We had already observed such an apparent 'mismatch' between changes in deiodinase activities and those in thyroid hormone concentrations in our previous studies (e.g. Baumgartner et al., 1998). This shows that it is not possible simply to infer changes in T_3 and T_4 from changes in deiodinase activities. One hypothetical explanation for this 'mismatch' is that TCP may have raised the tissue concentrations of T_3 by exerting an effect on parameters other than those affected by the deiodinases, e.g. by directly stimulating tissue uptake of T_3. In this case, a compensatory enhancement of 5D-III activity would be expected in order to restore physiological levels of T_3 in the brain (Leonard and Köhrle, 1996). However, this 'non-specific' hypothesis does not explain why we do not observe a compensatory inhibition of 5′D-II activity in the respective brain regions and why treatment with desipramine (Campos-Barros et al., 1994, 1995), and sleep deprivation (Campos-Barros et al,. 1993) both enhanced tissue levels of T_3 without affecting 5D-III activity. It is therefore more likely that the increase in 5D-III activities found in this study were specifically related to the pharmacological properties of TCP.

The mechanisms underlying these effects of TCP are unclear, mainly because the regulation of the deiodinase isoenzymes in the CNS has not yet been investigated in

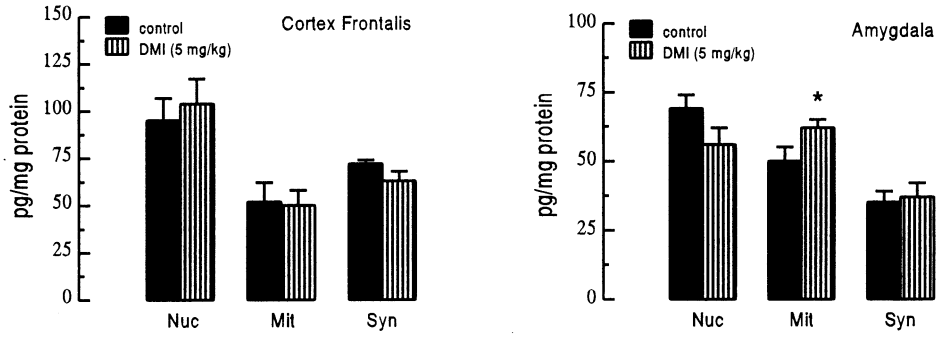

Fig. 7. Concentrations of T_3 in subcellular fractions of the frontal cortex (left) and amygdala (right) after 14 days administration of desipramine (5 mg/kg/day, decapitation at between 11:00 and 13:00 h, group 7). Abbreviations: Nuc = nuclei; Mit = mitochondria; Syn = synaptosomes. * $P < 0.05$.

vivo. Stimulation of the 5′D-II isoenzyme by norepinephrine, isoproterenol, cAMP and other stimulants of protein kinases A and C has been found in rat neonatal astrocytes in culture (Leonard, 1988). Type III deiodinase activity is stimulated mainly by activators of protein kinase C, and much less by those of protein kinase A (Courtin et al., 1991). Tranylcypromine is an MAO inhibitor which induces rises in the concentrations of norepinephrine, dopamine and serotonin in rat brain (Hampson et al., 1988). All of these neurotransmitters bind mainly to G-protein-coupled receptors, thereby inhibiting or enhancing the activity of protein kinases A or C. The same applies, however, to the norepinephrine reuptake inhibitor desipramine and the serotonin reuptake inhibitor fluoxetine, which have completely different effects on deodinase activity from those observed in the present study (Baumgartner et al., 1994b; Campos-Barros et al., 1994). The mechanisms underlying the effects of TCP on deiodinase activities therefore remain unclear.

In a further attempt to resolve the discrepancies between the results of the present study and those of our previous work we took into consideration one methodological weakness of all of these studies, namely, the fact that we measured both deiodinase activities and thyroid hormone concentrations in homogenates. These homogenates, however, not only contain different types of cells, such as glia and neurons, but also different subcellular components such as synaptosomal membranes, nuclei, mitochondria, microsomes, myelin, etc. According to current opinion the physiological effects of thyroid hormones are exerted mainly at specific nuclear receptors, which bind to thyroid response elements, thereby influencing gene expression (Oppenheimer et al., 1996). However, accumulation of thyroid hormones in and binding to synaptosomal membranes as well as transmitter-like properties of T_3 have repeatedly been reported (Dratman et al., 1976; Mason et al., 1990). Most interestingly, Martin et al. (1996) reported that T_3 modulates $GABA_A$ receptor function in a manner opposite to that described for some neuroactive steroids.

Furthermore, increases in oxygen consumption induced by thyroid hormones prompted several groups to investigate whether T_3 has direct effects at the mitochondria. Ardail et al. (1993) have found C-erb alpha and beta T_3 receptors in rat liver mitochondria. Furthermore, Iglesias et al. (1995) demonstrated a thyroid hormone receptor specific binding site in the mitochondrial NADH dehydrogenase subunit 3 gene. Also, an effect of T3 on the transcription of specific mitochondrial genes has now been reported in the adult rat brain (Vega-Nunez et al., 1995) and numerous effects of thyroid hormones on different mitochondrial functions such as oxidative phosphorylation and ATP synthesis have been reported (for a review see Soboll, 1993).

If T_3 concentrations are measured in homogenates only, drug effects on T_3 levels in specific subcellular compartments may be overlooked. An example of the differential effects of anti-depressants on the subcellular distribution of biochemical parameters has been given for protein kinase A (Nestler et al., 1989).

We therefore measured the effects of TCP on T_3 concentrations in nuclear, synaptosomal and mitochondrial fractions of six brain regions (Figs. 5 and 6). The results were most surprising for two reasons. First, we failed to find any effects of TCP on T_3 concentrations in the nuclear fraction of any brain region. Second, we found an increase in T_3 levels in mitochondria, but only in the amygdala. As this result would seem to be of potential importance, but was not significant after Bonferroni's correction, we repeated the experiment in another group of rats and also after administration of desipramine. The cross-validation after administration of TCP and the confirmation of the same results after desipramine show that the increases in T_3 levels in the mitochondrial fractions of the amygdala are a valid finding. The results are also surprising since both the amygdala as brain region, and the mitochondria as such have only rarely been considered in connection with the investigation of the mechanisms of action of anti-depressant drugs. This relative lack of interest in the amygdala would, however, seem surprising in light of current knowledge on the possible function of this brain structure. In brief, the amygdala seems to assign affective value to the incoming information from all sensory systems (LeDoux, 1992; Kandel et al., 1995). However, one of the key symptoms of depressive disorder is a disturbance of the affect associated with all kinds of sensory impressions. It is therefore very likely that the amygdala is at least partly involved in the pathogenesis of depression. Indeed, the few studies that have investigated a possible role of this brain region in depressive disorder have also confirmed a specific role of the amygdala, either in the pathogenesis of depression or in the mechanisms of action of anti-depressant treatments (Duncan et al., 1986; Ordway et al., 1991; Drevets et al., 1992; Sheline et al., 1998).

As regards the effects of anti-depressants on mitochondrial function, our study is, to the best of our knowledge, the first to indicate the possible importance of mitochondria for the mechanisms underlying the beneficial actions of anti-depressants. Previous studies on this issue are limited to a few investigations which were performed mostly in liver or heart tissue in vitro. They demonstrated an uncoupling of oxidative phosphorylation, an increase in oxygen consumtion or a decrease in ATP synthesis following administration of in, some cases, extremely high concentrations of antidepressants (Souza et al., 1994). In vivo studies showed an increase in stage 3 respiration and mitochondrial cytochrome content after 1 week of imipramine treat-

ment in rat liver (Katyare and Rajan, 1988). One more recent study showed an increase in stage 4 respiration and an uncoupling of oxidative phosphorylation in rat liver after 12 days treatment with fluoxetine (Souza et al., 1994). Investigations of the in vivo effects of anti-depressant drugs on well-defined mitochondrial functions in relevant regions of the CNS are needed to clarify a possible involvement of mitochondrial function in the mechanisms of action of anti-depressant drugs. Any increase in mitochondrial activity by anti-depressant drugs may reflect a secondary response of the mitochondria to enhanced neuronal activity, i.e. the need for enhanced ATP synthesis.

The decrease of 5′D-II activities in frontal cortex seen after TCP treatment (group 1 and 2) was reflected in decreased T_3 concentrations in tissue homogenates (group 3 and 4) and in synaptosomal fractions (group 5) of the same brain area. However, the significant increases in 5D-III activities in most of the brain areas were not reflected by changes in thyroid hormone concentrations. On the other hand the mechanism underlying the increases in T_3 levels in the mitochondria of amygdala could not be detected by measuring deiodinase activities. Therefore the measurement of deiodinase activities and thyroid hormone concentrations in tissue homogenates are of limited value. This implies that the effects of anti-depressant or prophylactic drugs, as well as those of sleep deprivation, on these parameters reported in our earlier papers (see Section 1) probably fail to reflect accurately enough the true changes in thyroid hormone concentrations induced by all these treatments. Measurement of thyroid hormone concentrations and (where interesting results are obtained) also thyroid hormone functions in subcellular fractions after administration of different anti-depressant drugs would currently seem a more appropriate approach to the problem.

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