Influence of thyroid hormone on 5-HT$_1A$ and 5-HT$_2A$ receptor-mediated regulation of hippocampal BDNF mRNA expression

V.A. Vaidya *, M.E. Castro, Q. Pei, M.E. Sprakes, D.G. Grahame-Smith

University Department of Clinical Pharmacology, University of Oxford, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, UK

Received 23 February 2000; received in revised form 12 May 2000; accepted 18 May 2000

Abstract

The aim of the present study was to determine the influence of thyroid hormone, T3, on the regulation of hippocampal BDNF expression by 5-HT receptor agonists. Chronic T3 administration prior to treatment with the 5-HT$_1A$ agonist, 8-OH-DPAT, significantly decreased BDNF mRNA in the dentate gyrus region of the hippocampus. Administration of 8-OH-DPAT did not alter hippocampal BDNF mRNA expression in naive, euthyroid rats. Pretreatment with the 5-HT$_1A$ antagonist, WAY 100635, completely blocked the 8-OH-DPAT-induced down-regulation of BDNF mRNA in chronic T3-treated rats. Acute T3 administration prior to 8-OH-DPAT treatment led to a small, but significant, decrease in hippocampal dentate gyrus BDNF mRNA. Acute or chronic administration of T3 did not alter the decrease in hippocampal BDNF mRNA induced by the 5-HT$_2A/C$ receptor agonist, DOI. The influence of 8-OH-DPAT and DOI on hippocampal BDNF mRNA was also unaltered in rats rendered hypothyroid by propylthiouracil administration. Chronic T3 treatment or hypothyroidism did not influence the basal expression of hippocampal BDNF mRNA. The affinity and density of 5-HT$_1A$ receptors, and the hippocampal expression of 5-HT$_1A$ mRNA were also not influenced by chronic T3 treatment. The results of this study clearly demonstrate a powerful interaction between thyroid hormone and the 5-HT$_1A$ receptor in the regulation of hippocampal BDNF expression. Crosstalk between signal transduction cascades influenced by T3 and 5-HT$_1A$ receptors may mediate the synergistic effects of these systems on hippocampal BDNF expression. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: BDNF (brain-derived neurotrophic factor); 5-HT (serotonin); T3 (tri-iodothyronine); Hyperthyroidism; 5-HT$_1A$ receptor; 5-HT$_2A$ receptor

1. Introduction

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family of growth factors, which also includes nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). BDNF influences the development, survival, maintenance and plasticity of neurons within the immature and adult nervous system (Thoenen, 1995; Lewin and Barde, 1996) and has recently been shown to also elicit rapid action potentials thus influencing neuronal excitability (Kafitz et al., 1999). In the adult brain BDNF is expressed at highest levels within the hippocampus (Ernfors et al., 1990; Gall and Lauterborn, 1992). BDNF mRNA expression, in particular within the hippocampus, is regulated by physiological activity, neuronal insults such as seizures and stress, and therapeutic agents such as antidepressants (Lindvall et al., 1994; Smith et al., 1995; Nibuya et al., 1995). Several neurotransmitter and neurohormonal systems have been implicated in the regulation of BDNF mRNA (Thoenen et al., 1991; Lindholm et al., 1994).

Recent work has shown that serotonin (5-HT) regulates basal hippocampal BDNF mRNA expression (Vaidya et al., 1997; Zetterström et al., 1999) and also contributes to the stress-induced down-regulation of BDNF mRNA in the hippocampus (Vaidya et al., 1999). Elevation of brain 5-HT levels significantly decreases hippocampal BDNF mRNA levels whilst depletion of brain 5-HT increases hippocampal BDNF mRNA (Zetterström et al., 1999). 5-HT mediates its actions through a large family of receptors of which the 5-HT$_1A$ and 5-HT$_2A$ receptors are the best characterized (Boess and Martin, 1994). Stimulation of the 5-HT$_2A$, but not the 5-HT$_1A$, receptor has been shown to decrease hippo-
campal BDNF mRNA levels (Vaidya et al., 1997). In addition, chronic administration of 5-HT selective re-uptake inhibitors, clinically used as antidepressants, enhances hippocampal BDNF mRNA (Nibuya et al., 1995; Russo-Neustadt et al., 1999). These studies taken together indicate that 5-HT plays an important role in regulation of basal hippocampal BDNF mRNA expression and may contribute, at least in part, to the regulation of BDNF mRNA by stress and antidepressant treatments.

Thyroid hormone has also been shown to regulate BDNF expression. Transient postnatal treatment of rats with thyroid hormone enhances hippocampal BDNF mRNA levels (Luesse et al., 1998) however BDNF mRNA expression in the adult brain appears to remain unaltered by thyroid hormone manipulations (Giordano et al., 1992; Alvarez-Dolado et al., 1994; Kim et al., 1998). In addition, thyroid hormone also exerts a powerful influence on 5-HT neurotransmission, enhancing 5-HT metabolism and levels (Ito et al., 1976; Cleare et al., 1992; Alvarez-Dolado et al., 1994; Kim et al., 1998). The aim of this study was to examine the influence of thyroid hormone on the regulation of BDNF mRNA by 5-HT. The action of these manipulations on the regulation of hippocampal BDNF mRNA by 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists was assessed using in situ hybridization analysis.

2. Methods

2.1. Animal treatment paradigms

Male Sprague–Dawley (CD) rats (225–250 g, Charles River, Kent, UK) were used for all experiments. Animals were group housed and maintained on a 12 h light–dark cycle with access to food and water ad libitum. All animal experiments were carried out in accordance with the UK Animal Scientific Procedures Act of 1986.

To determine the influence of acute and chronic triiodothyronine (T3) administration on 5-HT$_{1A}$ and 5-HT$_{2A}$-mediated regulation of BDNF mRNA, animals were administered 500 µg/kg T3 or vehicle (0.02N NaOH) s.c. once or once daily for 8 consecutive days followed by 5-HT$_{1A}$ or 5-HT$_{2A}$ agonist treatment 30 min after administration of last dose of T3. To determine the influence of hypothyroidism on 5-HT$_{1A}$- and 5-HT$_{2A}$-mediated regulation of BDNF mRNA, animals were rendered hypothyroid by allowing them free access to 0.05% 6-n-propyl-2-thiouracil (PTU) (roughly equivalent to 30 mg/kg body weight per day) in the drinking water for 3 weeks followed by 5-HT$_{1A}$ or 5-HT$_{2A}$ agonist treatment. For treatment with the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), animals were administered 1 mg/kg of 8-OH-DPAT or vehicle (0.9% saline) via an i.p. injection and sacrificed 2 h later. For treatment with the 5-HT$_{2A}$ agonist 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI), animals were administered 2 mg/kg of DOI or vehicle (0.9% saline) via i.p. injection and sacrificed 2 h later. For the 5-HT$_{1A}$ receptor antagonist experiment, all groups were administered chronic T3 (500 µg/kg, s.c.) once daily for 8 consecutive days followed by administration of 1 mg/kg i.p. of the 5-HT$_{1A}$ receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyrindinyl)cyclohexane-carboxamide trihydrochloride (WAY 100635) or vehicle (0.9% saline) 20 minutes prior to treatment with 1 mg/kg i.p. of 8-OH-DPAT or vehicle (0.9% saline) and sacrificed 2 h after the last injection.

The different experimental groups (n=5/group) were as follows: (1) Acute T3 experiment: Vehicle, Acute T3, DOI, Acute T3+ DOI, 8-OH-DPAT, Acute T3+8-OH-DPAT; (2) Chronic T3 Experiment: Vehicle, Chronic T3, DOI, Chronic T3+ DOI, 8-OH-DPAT, Chronic T3+8-OH-DPAT; (3) PTU Experiment: Vehicle, PTU, DOI, PTU+ DOI, 8-OH-DPAT, PTU+8-OH-DPAT; (4) 5-HT$_{1A}$ Antagonist Experiment: Chronic T3+ Saline, Chronic T3+ WAY 100635, Chronic T3+8-OH-DPAT, Chronic T3+ WAY 100635+8-OH-DPAT. All rats were sacrificed 2 h after the last injection with an i.p. adminis-
treatment of sodium pentobarbital (200 mg/kg) and then transcardially perfused with 50 ml of saline solution (0.9%), brains were removed, frozen in isopentane on dry ice and then stored at -70°C prior to use. The drugs utilised in this study were obtained as follows: 8-OH-DPAT and DOI were purchased from Research Biochemicals Inc. (UK); T3, PTU was purchased from Sigma (UK) and WAY 100635 was a gift from Wyeth-Ayerst (UK).

2.2. Serum T3 assay

Trunk blood was collected from chronic T3 and PTU administered animals at the time of sacrifice. Sera were separated out and stored at -20°C until assayed. T3 levels were analysed in all animals using the commercially available Technicon Immuno 1® system (Bayer, UK) which is based on a heterogeneous competitive magnetic separation assay. The intra-assay coefficient of variation is <10%.

2.3. In situ hybridization

Coronal cryostat brain sections (12 μm) through the hippocampus were thaw mounted onto gelatine-subbed slides. Half of the sections from acute and chronic T3 administration experiments were stored at -20°C for autoradiographic analysis and the remaining slides were pretreated for in situ hybridization using a previously described protocol (Pei et al., 1997). Oligonucleotides complementary to bases 642–686 (5’ GGG CTC GTA GAA ATA TTG GTT CAG TTG GCC TTT TGA TAC CGG GAC 3’ ) of the rat BDNF gene and bases 204–245 (5’ CAG AGA GGT GAT CAC TTG GTA GCT GAC GTC GCA GAT 3’ ) and 988–1029 (5’ CTT TGG AGT TGC CCA CTC GGT GCA CTT CGA TCA CCT CCA 3’ ) of the 5-HT1A receptor gene were 3’-tail labeled with [35S]-dATP using terminal deoxynucleotide transferase. The oligonucleotide (45mer) complementary to BDNF recognizes all forms of mRNAs for BDNF. The labeled oligonucleotide probes (approximate specific activity >10⁶ cpm/μg) were added to each section (1×10⁶ cpm/section) in hybridization buffer as previously described (Pei et al., 1997). After incubation in humid chambers at 35°C for 14–16 h, slides were washed in 1×SSC buffer (BDNF gene) or 0.5x×SSC (5-HT1A receptor gene) at 55°C three times for 20 min followed by two 60-min washes at room temperature. Sections were air-dried and exposed to Hyperfilm β-max (Amersham, Buckinghamshire, UK) for 3 weeks at room temperature. Controls included hybridization with sense oligonucleotides and displacement with unlabelled probes. A search of Genbank and other databases using the BLAST search revealed no significant homology of the oligonucleotides used in the present study with any other previously identified gene sequences.

2.4. Receptor autoradiography

Saturation assays were performed using five concentrations (0.3–5 nM) of [3H] 8-OH-DPAT (218 Ci/mmol). In brief, sequential cryostat sections (12 μm) were preincubated for 30 min at room temperature in a 0.17 M Tris–HCl buffer (pH 7.6) containing 4 mM CaCl₂ and 0.01% ascorbic acid. Sections were then incubated for 1 h at room temperature in the same buffer (in the presence of 10 μM pargyline) containing different concentrations of the radioligand. Non-specific binding was defined by co-incubation with 10 μM unlabelled 5-HT. Autoradiograms were generated by exposure of sections to 3H-Hyperfilm (Amersham, UK) along with tritiated microscales (Amersham, UK) for 1 month at 4°C.

2.5. Quantitation and data analysis

The levels of BDNF and 5-HT1A mRNA and the density of [3H]8-OH-DPAT binding were analysed using a Macintosh-based NIH-Image program, Scion Image 1.62. For each animal, densitometric measurements from both hippocampi of three sections were analysed and averaged. To correct for non-linearity 14C microscales or 3H microscales (Amersham, UK) were used for calibration. Kd and Bmax values were calculated using the program InPlot (GraphPad Software). Experiments with two groups were analysed for differences using the unpaired Student’s t-test, with significance determined at P<0.05. Experiments with three or more groups were subjected to statistical analyses using the two-way ANOVA, followed by the post hoc Scheffé test with a significance level of P<0.05.

3. Results

3.1. Influence of 5-HT1A and 5-HT2A receptor agonists on hippocampal BDNF mRNA expression following acute and chronic thyroid hormone administration

The effect of acute and chronic T3 administration on regulation of hippocampal BDNF mRNA expression by the 5-HT1A receptor agonist 8-OH-DPAT and the 5-HT2A/2C receptor agonist DOI was examined using in situ hybridization analysis. DOI regulation of hippocampal BDNF mRNA levels has been previously shown to be mediated via the 5-HT2A, and not the 5-HT2C, receptor (Vaidya et al., 1997). Acute T3 administration did not significantly alter the influence of 5-HT1A or 5-HT2A receptor stimulation on hippocampal BDNF mRNA expression (Fig. 1). Treatment with 8-OH-DPAT did not significantly regulate BDNF expression in the hippocampus of naive rats as previously shown (Vaidya et al., 1997). Although there is a small trend towards a decrease in dentate gyrus BDNF mRNA levels in the 8-
Influence of acute T3 administration on the regulation of hippocampal BDNF mRNA by the 5-HT$_{1A}$ agonist 8-OH-DPAT and the 5-HT$_{2A}$ agonist DOI.

Rats were administered 500 mg/kg of T3 or vehicle 30 min prior to treatment with vehicle, 8-OH-DPAT (1 mg/kg) or DOI (2 mg/kg) and levels of BDNF mRNA were determined 2 h later by in situ hybridization analysis. Results are expressed as percent of vehicle and are the mean±S.E.M. ($n$=5). *$P$<0.05 compared with vehicle (ANOVA; post hoc Scheffe’s test).

In the 8-OH-DPAT-treated group it is not significant in comparison to vehicle-treated group. Acute T3 treatment prior to 8-OH-DPAT administration did not significantly alter hippocampal BDNF mRNA levels in comparison to those in naive animals administered 8-OH-DPAT. However, the levels of BDNF mRNA in the dentate gyrus region of animals acutely administered T3 prior to 8-OH-DPAT treatment showed a small, but significant decrease when compared to the vehicle group. Acute T3 administration had no effect on the DOI-induced significant down-regulation of BDNF mRNA in the dentate gyrus (Fig. 1). In addition, acute T3 administration did not influence the basal expression of BDNF mRNA in the hippocampus (Fig. 1).

Chronic administration of T3 led to significantly elevated serum T3 levels in comparison to vehicle-treated animals (Vehicle group=$41.13\pm1.25$, Chronic T3 group=$105.09\pm7.38$; Results are expressed as ng/dl and are the mean±S.E.M., *$P$<0.05 Student’s $t$-test). Chronic T3 administration prior to treatment with 8-OH-DPAT and DOI significantly altered the influence of 8-OH-DPAT, but not DOI, on BDNF mRNA expression in the dentate gyrus (Fig. 2). Levels of BDNF mRNA in the dentate gyrus were significantly decreased in chronic T3-treated animals administered 8-OH-DPAT in comparison to both vehicle-treated, as well as naive animals administered 8-OH-DPAT (Fig. 2, Fig. 3). 8-OH-DPAT administration in the absence of chronic T3 treatment did not influence dentate gyrus BDNF mRNA expression. Chronic T3 administration did not alter the DOI-induced down-regulation of BDNF mRNA in the dentate gyrus and in addition did not influence the basal expression of BDNF mRNA (Fig. 2, Fig. 3). Expression of BDNF mRNA was also examined in parietal cortex and piriform cortex, however no effects of acute or chronic T3 administration were observed on BDNF mRNA levels in vehicle- and agonist-treated animals (data not shown).

3.2. Influence of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists on hippocampal BDNF mRNA expression following experimental hypothyroidism induced by propylthiouracil administration

Serum T3 levels in rats chronically administered PTU were significantly reduced confirming their experimentally hypothyroid state (Vehicle group=$39.40\pm3.48$, PTU group=$10.26\pm1.29$; Results are expressed as ng/dl and are the mean±S.E.M., *$P$<0.05 Student’s $t$-test). PTU administration prior to treatment with 8-OH-DPAT and DOI did not significantly alter their influence on hippocampal BDNF mRNA expression (Fig. 4). 8-OH-DPAT administration to naive and PTU-treated animals did not influence hippocampal BDNF mRNA expression. In addition, DOI treatment produced an equivalent significant down-regulation of BDNF mRNA in both naive and PTU-treated animals. Experimental hypothyroidism through PTU administration did not appear to affect basal hippocampal BDNF mRNA levels (Fig. 4). No effects of experimental hypothyroidism were observed on BDNF mRNA levels in the parietal and piriform cortex of vehicle- and agonist-treated animals (data not shown).

3.3. Regulation of BDNF mRNA by chronic T3 and 8-OH-DPAT is mediated via the 5-HT$_{1A}$ receptor

The pharmacological specificity of the 8-OH-DPAT-induced down-regulation of BDNF mRNA in the dentate gyrus region of chronic T3-treated rats was examined using the specific 5-HT$_{1A}$ antagonist, WAY100635. 8-OH-DPAT also acts as an agonist of the 5-HT$_{1}$ receptor...
Fig. 3. Regulation of hippocampal BDNF mRNA by chronic T3 and 8-OH-DPAT treatment. Rats were administered T3 (500 μg/kg) or vehicle for 8 days followed by treatment with vehicle or 8-OH-DPAT (1 mg/kg) and levels of BDNF mRNA were determined by in situ hybridization analysis. Representative autoradiograms through hippocampus of vehicle, chronic T3, 8-OH-DPAT and chronic T3+8-OH-DPAT-treated groups are shown. An arrow indicates the down-regulation of BDNF mRNA seen in the dentate gyrus (DG) of chronic T3+8-OH-DPAT-treated rats.

Fig. 4. Influence of experimental hypothyroidism on regulation of hippocampal BDNF mRNA by 8-OH-DPAT and DOI. Rats were administered PTU (0.05%) or vehicle for 3 weeks in drinking water followed by treatment with vehicle or 8-OH-DPAT (1 mg/kg) or DOI (2 mg/kg) and levels of BDNF mRNA were determined 2 h later by in situ hybridization analysis. Results are expressed as percent of vehicle and are the mean±S.E.M. (n=5). *P<0.05 compared with vehicle (ANOVA; post hoc Scheffe’s test).

(Lovenberg et al., 1993) raising the possibility that this receptor may be involved in the influence of 8-OH-DPAT on hippocampal BDNF expression. Pretreatment with the selective 5-HT1A antagonist WAY 100635, prior to 8-OH-DPAT administration, completely blocked the 8-OH-DPAT-induced down-regulation of BDNF mRNA levels in the dentate gyrus of chronic T3-treated rats (Fig. 5). WAY 100635 did not influence the basal hippocampal expression of BDNF mRNA in chronic T3 administered (Fig. 5), as well as naive rats (Vaidya et al., 1999). These findings indicate that 8-OH-DPAT regulation of hippocampal BDNF mRNA in chronic T3 administered animals is mediated via activation of the 5-HT1A receptor.

Fig. 5. Effect of WAY 100635 pretreatment on chronic T3 and 8-OH-DPAT regulation of hippocampal BDNF mRNA. Chronic T3-treated rats (500 μg/kg for 8 days) were administered WAY 100635 (1 mg/kg) or vehicle 10 min after last T3 treatment and 20 min prior to treatment with 8-OH-DPAT (1 mg/kg) or vehicle. Levels of BDNF mRNA were determined 2 h later by in situ hybridization analysis. Results are expressed as percent of chronic T3+vehicle and are the mean±S.E.M. (n=5). *P<0.05 compared with chronic T3+vehicle; **P<0.05 compared with chronic T3+WAY+8-OH-DPAT (ANOVA; post hoc Scheffe’s test).

3.4. Regulation of BDNF mRNA expression by chronic T3 and 8-OH-DPAT does not involve an enhanced expression of 5-HT1A mRNA or an enhanced density and/or affinity of 5-HT1A receptors

The influence of chronic T3 treatment on the hippocampal expression of 5-HT1A mRNA was examined using in situ hybridization. Hippocampal 5-HT1A mRNA expression was unaltered in response to chronic T3 administration (Fig. 6). There was a non-significant trend towards a decrease in 5-HT1A mRNA in response to 8-OH-DPAT treatment observed in both naive and chronic T3-treated rats (Fig. 6).
Table 1
Influence of acute and chronic T3 administration on $K_d$ and $B_{max}$ of $[^{3}H]8$-OH-DPAT binding to 5-HT$_{1A}$ receptors in the dentate gyrus region of the hippocampus

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Acute treatment</th>
<th>Chronic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$</td>
<td>$B_{max}$</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.6±0.22</td>
<td>135.0±18.9</td>
</tr>
<tr>
<td>T3</td>
<td>0.5±0.17</td>
<td>178.8±19.7</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>0.5±0.13</td>
<td>165.0±8.3</td>
</tr>
<tr>
<td>T3+8-OH-DPAT</td>
<td>0.5±0.02</td>
<td>171.9±7.9</td>
</tr>
</tbody>
</table>

* Effect of acute and chronic T3 treatment on affinity and density of hippocampal 5-HT$_{1A}$ receptors. Rats were administered T3 (500 µg/kg) or vehicle once (acute treatment) or once daily for 8 consecutive days (chronic treatment). $K_d$ and $B_{max}$ values of $[^{3}H]8$-OH-DPAT binding to 5-HT$_{1A}$ receptors were determined using receptor autoradiography. $K_d$ is expressed in nM and $B_{max}$ as fmol/mg tissue. The results are the mean±S.E.M. (n=5). There are no significant differences between the treatment groups (ANOVA).

4. Discussion

The results of the present study demonstrate that chronic administration of thyroid hormone interacts with 5-HT$_{1A}$ receptor stimulation to induce a significant down-regulation of BDNF mRNA expression in the dentate gyrus region of the hippocampus. Acute treatment with T3 prior to administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT produces only a small decrease in BDNF mRNA levels in the dentate gyrus suggesting that chronic administration of T3 is required to induce a significant down-regulation of BDNF mRNA in response to 5-HT$_{1A}$ receptor stimulation. BDNF mRNA expression in the hippocampus is unaltered by administration of thyroid hormone or the 5-HT$_{1A}$ agonist 8-OH-DPAT alone indicating that it is the synergistic interaction of these systems which induces a down-regulation of dentate gyrus BDNF mRNA. This down-regulation of BDNF mRNA was completely blocked by pretreatment with the 5-HT$_{1A}$ selective antagonist WAY 100635 indicating that the actions of 8-OH-DPAT are indeed mediated via the 5-HT$_{1A}$ receptor. In addition, the results of this study also indicate that experimental hypothyroidism produced by depletion of thyroid hormone through PTU administration does not interact with 5-HT$_{1A}$ receptor stimulation to regulate hippocampal BDNF mRNA expression.

Consistent with previously reported results (Giordano et al., 1992; Alvarez-Dolado et al., 1994; Kim et al., 1998) basal hippocampal BDNF mRNA levels are unaltered by thyroid hormone manipulations. Hypothyroidism increases BDNF mRNA within the pituitary and paraventricular hypothalamic nuclei, but does not influence BDNF expression in limbic regions (Kim et al., 1998). Hyperthyroidism also does not appear to influence basal BDNF mRNA levels in the adult brain as confirmed by our results (Giordano et al., 1992; Alvarez-Dolado et al., 1994). However, transient postnatal thyroid hormone treatment increases BDNF mRNA expression in the developing hippocampus suggesting that regulation of basal BDNF expression by thyroid hormone may be restricted to discrete developmental stages (Luesse et al., 1998).

We have also examined the influence of thyroid hormone on the regulation of BDNF mRNA by the 5-HT$_{2A/2C}$ receptor agonist DOI. DOI has been previously shown to decrease BDNF mRNA levels in the hippocampus whilst increasing them in the parietal cortex (Vaidya et al., 1997). These effects of DOI on BDNF...
mRNA expression are mediated via stimulation of the 5-HT$_{2A}$, but not the 5-HT$_{2C}$, receptor (Vaidya et al., 1997). The results of the present study demonstrate that acute and chronic thyroid hormone administration, as well as experimental hypothyroidism, does not influence the regulation of BDNF mRNA by the 5-HT$_{1A}$ receptor agonist DOI. Thyroid hormone manipulations did not alter the DOI-induced down-regulation of BDNF mRNA expression within the dentate gyrus region of the hippocampus (Fig. 1, Fig. 2, Fig. 4). In addition, thyroid hormone manipulations did not influence the DOI-induced regulation of BDNF mRNA levels within the parietal cortex (data not shown). These results indicate that thyroid hormone does not appear to interact with 5-HT$_{2A}$ receptor stimulation to influence BDNF mRNA expression.

The main finding of this study indicates that increased levels of thyroid hormone cause 5-HT$_{1A}$ receptor stimulation to produce a down-regulation of hippocampal dentate gyrus BDNF mRNA expression. The actions of thyroid hormone are mediated via nuclear thyroid hormone receptors, which are expressed at high levels throughout the brain, in particular within the hippocampus (Gould et al., 1991; Calza et al., 1997). Thyroid hormone receptors are DNA-binding transcription factors that can repress or induce transcription of specific target genes, which vary according to cell type and developmental stage. In addition, to expressing thyroid hormone receptors the hippocampus also expresses amongst the highest levels of the 5-HT$_{1A}$ receptor subtype (Boess and Martin, 1994; Wright et al., 1995). Overlapping expression of thyroid and 5-HT$_{1A}$ receptors in the hippocampus provides for the possibility of a local interaction between these systems in the regulation of hippocampal BDNF mRNA expression. Thyroid hormone may influence the sensitivity of target regions, such as the dentate gyrus hippocampal subfield, to 5-HT$_{1A}$ receptor stimulation through several possible mechanisms such as: (1) regulation of hippocampal 5-HT$_{1A}$ receptor expression, density and/or affinity; (2) influencing 5-HT neurotransmission; (3) altered coupling of agonist-receptor binding to second messenger cascades; (4) altered regulation of enzymes influencing second messenger cascades; (5) promoter specific interactions to influence transcription directly.

Results of the present study indicate that chronic thyroid hormone administration does not alter either the hippocampal expression of 5-HT$_{1A}$ receptor mRNA (Fig. 6) or the density and affinity of [${}^{3}$H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in the hippocampus (Table 1). These findings are consistent with previous reports, which indicate that hyperthyroidism does not influence the affinity and density of 5-HT$_{1A}$ receptors (Mason et al., 1987; Tejani-Butt et al., 1993; Kulikov et al., 1999). In contrast, previous studies show that depending on the rat strain hypothyroidism can produce either an increase (Tejani-Butt et al., 1993) or no change (Kulikov et al., 1999) in [${}^{3}$H]8-OH-DPAT binding. Taken together these studies suggest that the down-regulation of BDNF mRNA induced by 5-HT$_{1A}$ receptor stimulation in hyperthyroid rats is unlikely to result from a thyroid hormone induced increase in the density or affinity of 5-HT$_{1A}$ receptors.

Thyroid hormone administration has been shown to increase 5-HT levels and turnover in the cortex but not in the hippocampus (Ito et al., 1976; Sandrini et al., 1996; Gur et al., 1999). Elevated thyroid hormone levels enhance both the 5-HT behavioural syndrome induced by precursor loading and the locomotor responses to 5-HT receptor agonists (Atterwill, 1981; Heal and Smith, 1988). In contrast, chronic T$_3$ administration markedly attenuates the hypothermia induced by 8-OH-DPAT (Heal and Smith, 1988). These kind of attenuated hypothermic responses to 8-OH-DPAT have been observed following repeated treatment with several drugs that enhance 5-HT function, including 5-HT re-uptake inhibitors and monoamine oxidase inhibitors (Heal and Smith, 1988). There is considerable evidence that thyroid hormone influences 5-HT neurotransmission as well as 5-HT-mediated behaviours. The present study is the first evidence that thyroid hormone also interacts with 5-HT to regulate gene expression. It is possible that a thyroid hormone induced enhancement of 5-HT neurotransmission may play a role in the 5-HT$_{1A}$ receptor-mediated decrease of BDNF mRNA seen in hyperthyroid rats.

Thyroid hormone may also modulate the sensitivity of hippocampal BDNF mRNA to 5-HT$_{1A}$ receptor stimulation through changes in post-receptor responsiveness. The 5-HT$_{1A}$ receptor is coupled to inhibition of adenylyl cyclase. Thyroid hormone has been shown to interact with multiple components of the adenylyl cyclase signal transduction cascade. Brief treatment with high levels of T$_3$ decreases G, in cortex and cerebellum (Orford et al., 1992). Hypothyroidism attenuates forskolin-stimulated adenylyl cyclase activity in forebrain synaptosomes, (Mazurkiewicz and Saggerson, 1989), whereas T$_3$ administration increases adenylyl cyclase activity in neuroblastoma cell cultures (Walz and Howlett, 1987). It is possible that chronic T$_3$ treatment may alter the sensitivity of hippocampus to 8-OH-DPAT receptor stimulation by influencing components of 5-HT$_{1A}$ transmembrane signaling. Finally, thyroid hormone may interact with 5-HT$_{1A}$ receptor stimulation, directly at the level of the BDNF promoter to influence transcription of the BDNF gene. There is a cAMP response element (Shieh et al., 1998) but no thyroid response element in the BDNF promoter region (Giordano et al., 1992; Alvarez-Dolado et al., 1994) suggesting that a direct interaction between the thyroid and 5-HT$_{1A}$ systems within the promoter of the BDNF gene may be unlikely. Although several potential sites for synergistic interactions between 5-HT$_{1A}$ receptors and thyroid hormone have
been discussed, the actual mechanisms mediating the down-regulation of BDNF by these systems remain unclear and will require further study.

Thyroid dysfunction may be associated with depression and thyroid hormone has been shown to act as an effective adjunct to antidepressant treatment (Henley and Koehnle, 1997). Although there is substantial clinical literature supporting the role of thyroid hormone in mood disorders, little is known about the effects of thyroid hormone on the mature mammalian brain and the mechanisms that may underlie its mood-elevating properties. The results of the present study indicate that interactions which influence gene expression may exist between thyroid hormone and the 5-HT$_{1A}$ receptor system, itself a target in the treatment of depressive disorders (Delgado et al., 1994). Since 5-HT plays a critical role in the actions of antidepressant treatments, understanding the influence of thyroid hormone on 5-HT-mediated gene expression will help further elucidate the mechanisms underlying the augmentation of antidepressant treatments by thyroid hormone.

**References**


Sandrini, M., Vitale, G., Vergoni, A.V., Ottani, A., Bertolini, A., 1996. Effect of acute and chronic treatment with triiodothyronine on sero-
tonin levels and serotonergic receptor subtypes in the rat brain. Life Sciences 58 (18), 1551–1559.