Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 1489–1495
Commentary
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Effect of antidepressants on $GABA_B$ receptor function and subunit expression in rat hippocampus

Scott A. Sands, Scott A. Reisman, S.J. Enna*

Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical School, 3901 Rainbow Boulevard, Mail Stop 1018, Kansas City, KS 66160-7417, USA

Abstract

Laboratory and clinical studies suggest that depression is associated with changes in the hippocampus and that this brain region is a major target for antidepressant drugs. Given the data suggesting that $GABA_B$ receptor antagonists display antidepressant properties, the present study was undertaken to assess the effect of antidepressant administration on $GABA_B$ receptors in the rat hippocampus to determine whether changes in this regional receptor system may play a role in the response to these agents. Rats were administered (i.p.) the monoamine oxidase inhibitors tranylcypromine (10 mg/kg) or phenelzine (10 mg/kg), the tricyclic antidepressant desipramine (15 mg/kg), or fluoxetine (5 mg/kg), a selective serotonin re-uptake inhibitor, once daily for seven consecutive days. Two hours following the last drug treatment the hippocampal tissue was prepared for defining the distribution and quantity of $GABA_B$ receptor subunits using in situ hybridization and for assessing $GABA_B$ receptor function by quantifying baclofen-stimulated [^{35}S]-GTPγS binding. All of these antidepressants selectively increased the expression of the $GABA_{B(1a)}$ subunit in hippocampus, having no consistent effect on the expression of $GABA_{B(1b)}$ or $GABA_{B(2)}$. Moreover, except for fluoxetine, these treatments increased $GABA_B$ receptor function in this brain region. The results indicate that an enhancement in the production of hippocampal $GABA_{B(1a)}$ subunits may be a component of the response to antidepressants, supporting a possible role for this receptor in the symptoms of depression and the treatment of this condition.

Keywords: $GABA_B$ receptors; Hippocampus; Antidepressants; $GABA_B$ receptor subunits

1. Introduction

Pharmacologic manipulation of the γ-aminobutyric acid (GABA) system may be of benefit in the treatment of affective illness [1–2]. Thus, administration of antidepressants modifies $GABA_B$ receptor binding and function in rat brain [3–8], $GABA_B$ receptor antagonists have antidepressant activity in animal models of this disorder [9], and $GABA_{B(1)}$ null mice display an antidepressant-like phenotype in the forced swim test [2]. Moreover, clinical studies indicate that plasma GABA levels are altered in mania and depression [10,11], and drugs thought to affect GABAergic transmission are used for the treatment of bipolar disorder [12,13]. Questions remain, however, about the precise role of GABA and $GABA_B$ receptors in mediating symptoms of these conditions, and the extent to which antidepressant-induced changes in $GABA_B$ receptor number or function are a common characteristic of this drug class [3]. For example, while there have been numerous reports over the past two decades indicating that chronic administration of antidepressants increases $GABA_B$ receptor binding and function in rat and mouse brain cerebral cortex [4,6,7], others have been unable to confirm this finding [14–16]. These contradictory results make it difficult to develop a unifying theory on the relationship between $GABA_B$ receptors and affective illness.

Recent discoveries pertaining to neuroanatomical and neurochemical abnormalities associated with depression,

Abbreviations: GABA, γ-aminobutyric acid; $GABA_B$, γ-aminobutyric acid-B receptors; $GABA_{B(1a)}$, GABAB(1b); $GABA_{B(2)}$, γ-aminobutyric acid-B receptor subunits.

* Corresponding author. Tel.: +1 913 588 7533; fax: +1 913 588 7373.
E-mail address: senna@kumc.edu (S.J. Enna).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.07.027

and the composition and function of $GABA_B$ receptors, suggest new avenues of research for determining whether there may be a causal relationship between the clinical response to antidepressants and modifications in this receptor system. Included are laboratory and clinical research suggesting that changes in the volume of the hippocampus are associated with depression, and that antidepressant-induced modifications in this brain region may be a significant factor with regard to their clinical efficacy [17–20]. For example, chronic stress, a precipitating factor for depression, causes hippocampal atrophy, decreases cognitive abilities, and reduces the expression of neurotrophic growth factor in this brain region of the rat brain. A reduction in hippocampal volume has also been reported in depressed patients [18]. Taken together, these studies indicate that changes in the hippocampus may play a central role in mediating the symptoms of depression and the action of antidepressants.

Receptor cloning and expression studies have revealed that the $GABA_B$ site is a G protein-coupled heterodimer composed of $GABA_{B(1)}$ and $GABA_{B(2)}$ subunits [21–23]. Although numerous variants have been identified for these two gene products, not all are capable of forming functional receptors, with the $GABA_{B(1a)}$/$GABA_{B(2)}$ and $GABA_{B(1b)}$/$GABA_{B(2)}$ being the most extensively studied combinations. Gene knockdown and deletion experiments indicate that $GABA_B$ receptor assembly and function generally requires a union of $GABA_{B(1)}$ and $GABA_{B(2)}$ subunits, with some receptor activity detectable in the absence of $GABA_{B(2)}$ but not $GABA_{B(1)}$ [22,24–29]. While the subunit composition and stoichiometry of $GABA_B$ receptors is very limited, these two gene products are not always expressed in tandem, suggesting they may have functions separate from their role as constituents of the $GABA_B$ receptor [30–33]. It has also been found that certain drug treatments and physiological stimuli, such as pain, can regulate $GABA_B$ receptor subunit expression and function in defined regions of the central nervous system [30,34,35].

The present study was undertaken to assess the effect of antidepressant administration on the expression and function of $GABA_B$ receptors in the rat hippocampus in an attempt to determine whether there is a consistent modification in this brain region in response to this drug class. The results indicate that administration of tranylcypromine, desipramine, phenelzine or fluoxetine, representing three different classes of antidepressants, for seven consecutive days increases the expression of $GABA_{B(1a)}$, but not necessarily $GABA_{B(1b)}$ or $GABA_{B(2)}$, in the hippocampus. In addition, except for fluoxetine, all of these antidepressant treatments increase $GABA_B$ receptor activity in hippocampal tissue. These findings suggest a possible relationship between a selective increase in the expression of $GABA_{B(1a)}$ subunits in the hippocampus and the clinical response to antidepressants.

2. Materials and methods

2.1. Drug treatments

Harlan Sprague-Dawley male rats (150–200 g) (Indianapolis, IN) were used throughout the study. The animals were housed 3 to a cage under a 12 h light/dark cycle with food and water ad libitum. The animals were allowed to adapt to the home cage for at least 24 h prior to drug treatment. All drugs were administered i.p. between 8:00 and 10:00 am once daily for seven consecutive days. The drugs employed, and the doses administered, were tranylcypromine (10 mg/kg), desipramine (15 mg/kg), fluoxetine (5 mg/kg), and phenelzine (10 mg/kg). The selection of doses was made on the basis of earlier work demonstrating they induce behavioral changes or cause neurochemical alterations in the central nervous system [8,36–39].

Two hours following the last injection the animals were decapitated, the brains removed, blocked and immersion fixed in 4% paraformaldehyde for in situ hybridization, or the hippocampus was immediately dissected and homogenized for analysis of [^{35}S]-GTPγS binding.

2.2. In situ hybridization

The $GABA_{B(1a)}$ probe, a 400-base-pair *Sma*I cDNA fragment, the $GABA_{B(1b)}$ probe, a 310-base-pair *Ksp*I cDNA fragment, and the $GABA_{B(2)}$ probe, a 724-base-pair Nhe/*Sac*I cDNA fragment, were all sub-cloned into Bluescript (Stratagene, Amsterdam, The Netherlands). The plasmids were kindly donated by K. Kaupmann (Novartis Pharma, Basel, Switzerland). Riboprobes were synthesized with digoxigenin-11-UTP (Roche Molecular Biochemicals) using the 'MAXIscript' in vitro transcription kit (Ambion).

Hippocampal tissue was cryostat-sectioned at 12 μm thickness and thaw-mounted on silanized slides. The sections were covered with pre-hybridization buffer (50 ml deionized formamide; 20 ml 20× SSC; 0.2 ml 50× Denhardt's solution [Sigma Chemical Co., St. Louis, MO], 25 ml yeast tRNA [10 mg/ml, Gibco BRL, Rockville, MD], and 1.6 ml 50% dextran sulfate [Oncor, Gaithersburg, MD]), then incubated for 1 h at 60 °C. The sections were hybridized overnight at 60 °C in pre-hybridization buffer containing 1 μg/ml digoxigenin labeled probes. Non-specifically bound probes were removed with post-hybridization washing which consisted of 2 × 15 min in 2× SSC at 37 °C, 2 × 15 min in 1× SSC at 37 °C, and 2 × 30 min in 0.1× SSC at 37 °C. Following equilibration for 1 min in Buffer 1 (100 mM Tris–HCl, 150 mM NaCl, pH 7.5) the tissues were pre-incubated at room temperature for 1 h with blocking reagent consisting of Buffer 1 with 2% normal sheep serum and 0.1% Triton X-100. Immunohistochemical detection of digoxigenin labeled hybrids was accomplished using an anti-digoxigenin antibody

conjugated to alkaline phosphatase (Roche Molecular Biochemicals, Indianapolis, IN) diluted 1:1000 with Buffer 1 containing 1% normal sheep serum and 0.1% Triton X-100. After 1 h at 37 °C, the antibody-containing solution was removed and the tissue sections washed for 10 min in Buffer 1 and 10 min in Buffer 2 (100 mM Tris–HCl, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5). A chromogen solution (45 μl of 75 mg/ml nitroblue tetrazolium in 70% dimethylformamide, 35 μl of 50 mg/ml 5-bromo-4-chloro-3-indolyl phosphate in dimethylformamide in 10 ml Buffer 2) was used as a substrate for detecting the alkaline phosphatase marker. The reaction, which results in a purple precipitate, was terminated with Buffer 3 (10 mM Tris–HCl containing 1 mM EDTA, pH 8.0). Sections from different groups of animals were processed simultaneously to allow for visual comparison of precipitate densities. The tissue sections were dehydrated through graded ethanol, cleared in xylene, and mounted with Permount. Controls included hybridization with sense probes or omission of either the labeled probe or the anti-digoxigenin-alkaline phosphatase conjugated antibody. No staining was observed in either case. Tissues from three different animals for each treatment, with at least five sections per animal, were evaluated by image analysis.

Grayscale values (0–255) of the nitroblue tetrazolium salt precipitate were used to compare levels of $GABA_B$ receptor subunits between groups. Hippocampal tissue obtained from vehicle-treated control animals was analyzed with each batch of tissue from drug-treated subjects to control for variation in the optical densities between groups. The image analysis system consists of a Dage/MTI 72 CCD camera mounted on the trinocular port of Zeiss Axioplan microscope (Carl Zeiss Inc., Thornwood, NJ). The camera is connected to a Matrox MVP-AT array processor installed in a 486-based PC running IM3000B image processing and analysis software (Belvoir Consulting, Long Beach, CA). All preparations were measured using a single illumination setting. The lamp was warmed and a 10× magnification selected. The lamp intensity, camera and software settings were manipulated to produce maximum contrast (spread of grayscale values) in the images.

Image analysis was performed using Scion Image (Frederick, MD). One section in each of the five different regions of the hippocampus (dentate gyrus, CA1–CA4 regions) containing immunoreactive product was outlined using a circle drawing command. The designated area was measured and the density of immunoreactivity analyzed within it. The five circular regions were pooled to obtain a final mean value for that tissue sample. The results are displayed as the overall average of the percent change in drug-treated animals as compared to vehicle-treated controls. The data were compared by SuperAnova using a one-way ANOVA with Fisher's PLSD post hoc. Differences between means were considered significant when $P \leq 0.05$.

2.3. GTPγS binding assay

Rat hippocampi were dissected, weighed, and homogenized in 50 mM Tris buffer, then centrifuged at 39,000 × *g* for 10 min. The resultant pellet was resuspended in 50 mM Tris to a final concentration of 100 mg/ml. Portions (1.1 ml) of this homogenate were stored (−80 °C) individually until assayed.

The assay used for quantifying [^{35}S]-GTPγS binding is a modified version of that reported by Alper [40]. Briefly, the frozen samples were thawed and suspended in 10 volumes of 50 mM Tris buffer and centrifuged at 39,000 × *g* for 10 min, after which the resultant pellet was again suspended in 10 volumes of 50 mM Tris buffer and centrifuged as before. The final pellet was suspended in 18 ml assay buffer (4 mM $MgCl_2$, 160 mM NaCl, 0.267 mM EGTA, 67 mM Tris, pH 7.4) with 200 μl of the suspension added to 200 μl of 1.2 mM GDP, 200 μl [^{35}S]-GTPγS (55,000–70,000 cpm), and 200 μl of 1 mM baclofen, a saturating concentration of this $GABA_B$ receptor agonist. Nonspecific binding was defined by replacing baclofen with 200 μl of 40 μM unlabeled GTPγS. Basal levels of GTPγS binding were examined by replacing baclofen with water. The mixture was incubated at 37 °C for 20 min, after which the reaction was terminated by rapid filtration over presoaked glass fiber filters using a Brandel cell harvester. The filters were placed in 7 ml liquid scintillation vials with 3 ml scintillation fluid (EconoSafe, Research Products International, Mount Prospect, IL). Radioactivity was quantified using a liquid scintillation counter. The data are displayed as the overall average of the percent stimulation over basal [^{35}S]-GTPγS binding in drug-treated as compared to vehicle-treated controls, with specific binding of [^{35}S]-GTPγS analyzed using SigmaPlot. The results are compared by SuperAnova using a one-way ANOVA with Fisher's PLSD post hoc. Differences between means were considered significant when $P \leq 0.05$.

2.4. Materials

Desipramine HCl, fluoxetine HCl, tranylcypromine HCl, phenelzine sulfate and guanosine diphosphate were all purchased from Sigma Chemical Co. (St. Louis, MO). Unlabeled GTPγS was purchased from CalBiochem (San Diego, CA), and [^{35}S]-GTPγS (1250 Ci/mmol) from Amersham Pharmacia Biotech (Piscataway, NJ).

3. Results

3.1. $GABA_B$ receptor subunit expression

In situ hybridization analyses revealed that all three $GABA_B$ receptor subunits are expressed in the hippocampal pyramidal cell layer (CA1–CA4) and the granule cell

layer of the dentate gyrus (Fig. 1A–C). Sparse labeling was also observed for all three subunits in the stratus oriens and stratus radiatum of the hippocampus. This pattern of distribution for $GABA_{B(1a)}$, $GABA_{B(1b)}$, and $GABA_{B(2)}$ in the hippocampus is qualitatively similar to that reported by others [41–43]. Daily administration of tranylcypromine, fluoxetine, phenelzine or desipramine for 1 week had no significant effect on the relative distribution of any of these subunits in the hippocampus (data not shown).

Image analysis revealed that antidepressant treatment selectively modifies the expression of the $GABA_B$ receptor subunits in the rat hippocampus in comparison with control tissue (Fig. 2). Thus, all of the antidepressants examined significantly ($P < 0.05$) increased the expression of the $GABA_{B(1a)}$ subunit approximately 20% as compared to control tissue, while none had any effect on the expression of hippocampal $GABA_{B(1b)}$. As for $GABA_{B(2)}$, only tranylcypromine administration had any significant ($P < 0.05$) effect on this subunit, increasing its expression nearly 50% (Fig. 2).

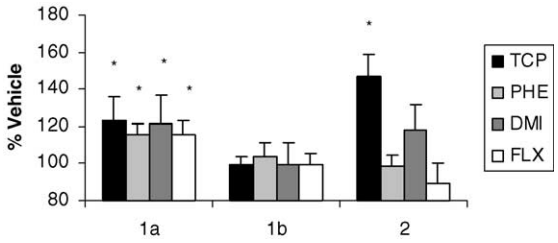

Fig. 2. Image analysis of in situ hybridization of $GABA_{B(1a)}$, $GABA_{B(1b)}$, and $GABA_{B(2)}$ receptor subunits in rat hippocampus taken from animals treated with desipramine (DMI; 15 mg/kg, i.p.), tranylcypromine (TCP; 10 mg/kg, i.p.), fluoxetine (FLX; 5 mg/kg, i.p.), or phenelzine (PHE; 10 mg/kg, i.p.) for seven consecutive days. The analysis was graphed from five hippocampal regions from each animal, and the results combined for a single measurement. The height of each bar represents the mean ± S.E.M. of the results from three different animals. The average value, ±S.E.M., for all subunits in vehicle-treated animals was 144 ± 8 grayscale units. Statistically significant from vehicle-treated controls ($^*P < 0.05$).

3.2. $GABA_B$ receptor function

The effect of antidepressant administration on $GABA_B$ receptor function was assessed by quantifying [^{35}S]-GTPγS binding to hippocampal membrane in the presence of a saturating concentration (1 mM) of baclofen, a $GABA_B$ receptor agonist (Fig. 3). While daily injections of tranylcypromine, phenelzine or desipramine for 1 week significantly ($P < 0.05$) enhanced the response to baclofen in the hippocampal tissue, treatment with fluoxetine did not. The extent of the enhancement in [^{35}S]-GTPγS binding resulting from the administration of the two monoamine oxidase inhibitors and the tricylic antidepressant varied from 40 to 60% as compared to vehicle-treated controls (Fig. 3).

4. Discussion

Modification of hippocampal $GABA_B$ receptor activity is known to influence behavior, cognitive performance, and endocrine function [44–51]. Thus, whereas $GABA_B$ receptor antagonists improve performance in both active and passive avoidance tests, baclofen, a $GABA_B$ receptor agonist, impairs spatial learning and working memory in rats. Indeed, a number of agents found to enhance cognitive performance in laboratory animals are believed to reduce $GABA_B$ receptor function [48–50]. Activation of hippocampal $GABA_B$ receptors increases the activity of the hypothalamic-pituitary-adrenal axis, an endocrine system intimately associated with mood disorders [51]. Further evidence linking $GABA_B$ receptors to affective illness are the reports that baclofen administration exacerbates learned helplessness in rats, and that $GABA_B$ receptor antagonists display antidepressant activity in this behavioral test [2,9,52,53]. Given the evidence linking changes

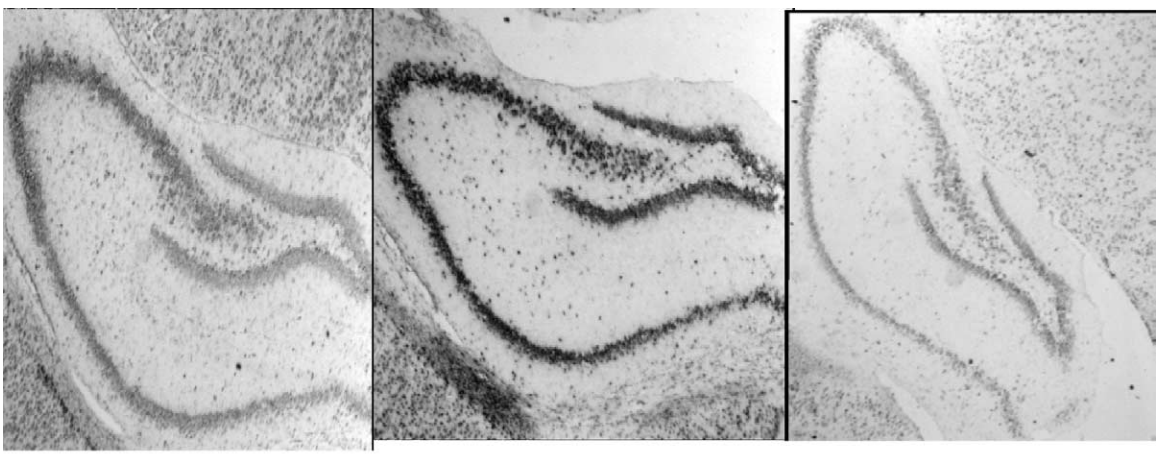

Fig. 1. Distribution of $GABA_B$ receptor subunits in the rat hippocampus as measured using in situ hybridization. (A) $GABA_{B(1a)}$; (B) $GABA_{B(1b)}$; (C) $GABA_{B(2)}$. Magnification = 10×. The data are representative of results obtained from six rats injected (i.p.) with water once daily for seven consecutive days (vehicle controls).

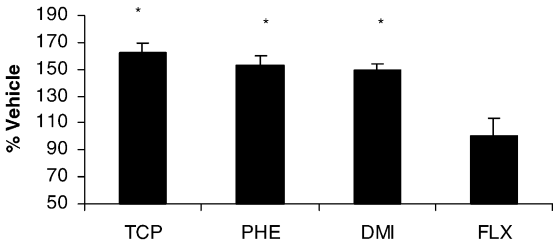

Fig. 3. Baclofen (1 mM)-stimulated [^{35}S]-GTPγS binding in hippocampal membranes taken from rats treated with tranylcypromine (TCP; 10 mg/kg, i.p.), desipramine (DMI; 15 mg/kg, i.p.), phenelzine (PHE; 10 mg/kg, i.p.), or fluoxetine (FLX; 5 mg/kg, i.p.) for seven consecutive days. The height of each bar represents the mean ± S.E.M. of results obtained from six different animals. The average value, ±S.E.M., for baclofen-stimulated [^{35}S]-GTPγS binding in control animals was 952 ± 74 cpm. *Statistically significant from vehicle-treated controls ($P < 0.05$).

in hippocampal structure and function with depression, and the widespread distribution of $GABA_B$ receptors in this brain region, it is possible that modifications in this receptor system may contribute to the symptoms of this disorder and the clinical response to antidepressants. Inasmuch as the earlier work aimed at examining a relationship between antidepressants and $GABA_B$ receptors focused on cerebral cortex and yielded conflicting data [3,4,6,7,14,16], the present study was undertaken to determine whether a consistent change in the function or expression of this receptor and its subunits might be more evident in the hippocampus following administration of these drugs.

To examine whether modifications in the hippocampal $GABA_B$ receptor system are a characteristic of this drug class, three different types of antidepressants were tested. Included were tranylcypromine and phenelzine, monoamine oxidase inhibitors, desipramine, a tricyclic antidepressant that inhibits both norepinephrine and serotonin re-uptake into neuronal tissue, and fluoxetine, a selective serotonin re-uptake inhibitor. The results of the present study confirmed that all three $GABA_B$ receptor subunits are present in the pyramidal and granule cell layers of the rat hippocampus [41,43]. Moreover, it was found that daily administration of these antidepressants for 1 week had no obvious effect on the apparent pattern of distribution for any of these subunits in this brain region. However, while the overall distribution remained unchanged, the level of expression of some $GABA_B$ receptor subunits was significantly influenced by the administration of these agents. Most consistent was the finding that all four drugs caused small, but statistically significant, increases in the expression of the $GABA_{B(1a)}$ subunit in the hippocampus. The selectivity of this effect is evident by the finding that none of these agents increased the expression of $GABA_{B(1b)}$, and administration of only one, tranylcypromine, increased the expression of the $GABA_{B(2)}$ subunit. This targeted effect on the $GABA_{B(1a)}$ subunit is intriguing given the reports that other types of manipulations, such as inflammatory pain and electroshock, selectively increase the expression of $GABA_{B(1b)}$, but not $GABA_{B(1a)}$, in the spinal cord and cerebral cortex, respectively [34,54]. Moreover, inflammatory pain has been reported to enhance the expression of the $GABA_{B(2)}$ subunit in the rat spinal cord dorsal horn [30,34].

It is noteworthy that in the present study the dose of fluoxetine is significantly less, and the duration of treatment with all of the antidepressants substantially shorter, than in other studies aimed at examining their effects on neurochemical markers in brain [36,38,55]. The results of the present experiments therefore suggest that $GABA_{B(1a)}$ expression in the hippocampus may be a particularly sensitive indicator of antidepressant activity.

While tranylcypromine, phenelzine or desipramine administration all increased $GABA_B$ receptor function as measured by quantifying baclofen-stimulated [^{35}S]-GTPγS binding in hippocampal membranes, fluoxetine was inactive in this regard. However, it is possible that administration of a higher dose of fluoxetine may also have been effective in this regard. The results with tranylcypromine are similar to those reported in for rat cerebral cortex following chronic administration of this antidepressant [8].

The present findings suggest that the drug-induced changes in $GABA_{B(1a)}$ subunit expression are not necessarily accompanied by an increase in receptor activity. This is in accord with earlier work indicating that the expression of $GABA_B$ receptor subunits is differentially regulated, and that there is not necessarily a correlation between subunit expression and receptor function [29]. In the case of antidepressant administration, it remains possible that the drug-induced increase in $GABA_{B(1a)}$ subunit expression contributes to an increase in $GABA_B$ receptor function in those cases where receptor activity is enhanced, although it may also represent some other type of change in cellular activity. For example, it has been reported that $GABA_B$ receptor subunits are capable of interacting with the transcription factors CREB2 and ATFx, suggesting these proteins may influence cellular function in ways other than through formation of $GABA_B$ receptors [33,56]. Recent data suggest that $GABA_B$ receptor subunit expression is regulated by attachment of CREB to alternative promoters rather than to alternative splicing [33], possibly explaining the selective subunit response to antidepressants observed in the present study.

It was suggested in the past that an antidepressant-induced increase in $GABA_B$ receptor binding or function may indicate that an underactive $GABA_B$ system contributes to the symptoms of depression, and that antidepressants act, in part, to enhance GABAergic transmission [4]. However, since it is now known that $GABA_B$ receptor agonists worsen behavior in animal models of depression, and $GABA_B$ receptor antagonists display antidepressant activity [2,9,52,53], this interpretation seems unlikely. Rather, it appears that an antidepressant-induced increase in $GABA_B$ receptor function may indicate that these drugs diminish $GABA_B$ synaptic activity and that, over time, this is compensated for by an up-regulation in receptor number

or sensitivity. Indeed, it has been reported that chronic treatment with $GABA_B$ receptor antagonists increases $GABA_B$ receptor binding the rat brain frontal cortex and spinal cord, supporting the notion that a reduction in the activity of this receptor system leads to a compensatory increase in receptor number [7,57]. Accordingly, depression, or perhaps certain forms of this disorder, may be associated with an overactive $GABA_B$ receptor system that is indirectly inhibited by antidepressants, leading to overexpression of hippocampal $GABA_{B(1a)}$ subunits and, in some cases, an increase in receptor sensitivity.

While the results of these experiments suggest that antidepressants as a class share a common mechanism in increasing $GABA_{B(1a)}$ receptor subunit expression in the rat hippocampus, without further work this conclusion remains highly speculative. To assign this property to antidepressants it must first be demonstrated that psychotherapeutics without antidepressant efficacy are inactive in this regard. It would also be worthwhile to screen a larger battery of antidepressants to ensure that the selective increase in the expression of this subunit is a characteristic of all clinically effective agents. Confirmatory experiments such as these could provide important new insights into neurochemical abnormalities associated with depression, might yield a biochemical measure for screening antidepressant candidates, and could lead to the design and development of novel agents for the treatment of this condition.

Acknowledgements

This work was supported, in part, by a grant from the Lied Foundation. We thank Ms. Lynn LeCount for her editorial assistance.

References

[1] Brambilla P, Perez J, Barale F, Schettini G, Soares JC. GABAergic dysfunction in mood disorders. Mol Psychiatr 2003;8:721–37.
[2] Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF. Genetic and pharmacological evidence of a role for $GABA_B$ receptors in the modulation of anxiety- and antidepressant-like behavior. Neuropsychopharmacology 2004;29:1050–62.
[3] Enna SJ, Bowery NG. $GABA_B$ receptor alterations as indicators of physiological and pharmacological function. Biochem Pharmacol 2004;68:1541–8.
[4] Lloyd KG, Thuret F, Pilc A. Upregulation of gamma-aminobutyric acid (GABA) B binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock. J Pharmacol Exp Therap 1985;235:191–9.
[5] Gray JA, Green AR. Increased GABAB receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive seizures. Br J Pharmacol 1987;92:357–62.
[6] Szekely AM, Barbaccia ML, Costa E. Effect of protracted antidepressant treatment on signal transduction and [3H](-)-baclofen binding at GABAB receptors. J Pharmacol Exp Therap 1987;243:155–9.
[7] Pratt GD, Bowery NG. Repeated administration of desipramine and a GABAB receptor antagonist, CGP 36742, discretely up-regulates GABAB receptor binding sites in rat frontal cortex. Br J Pharmacol 1993;110:724–35.
[8] Sands SA, Reisman SA, Enna SJ. Effects of stress and tranylcypromine on amphetamine-induced locomotor activity and $GABA_B$ receptor function in rat brain. Life Sci 2003;72:1085–92.
[9] Nakagawa Y, Sasaki A, Takashima T. The GABA(B) receptor antagonist CGP36742 improves learned helplessness in rats. Eur J Pharmacol 1999;381:1–7.
[10] Petty F, Kramer GL, Dunnam D, Rush AJ. Plasma GABA in mood disorders. Psychopharmacol Bull 1990;26:157–61.
[11] Swann AC, Petty F, Bowden CL, Dilsaver SC, Calabrese JR, Morris DD. Mania: gender, transmitter function, and response to treatment. Psychiat Res 1999;88:55–61.
[12] Fesler FA. Valproate in combat-related posttraumatic stress disorder. J Clin Psychiat 1991;52:361–4.
[13] Davis LL, Ryan W, Adinoff B, Petty F. Comprehensive review of the psychiatric uses of valproate. J Clin Psychopharmacol 2000;20(Suppl 1):1S–17S.
[14] Cross JA, Horton RW. Effects of chronic oral administration of the antidepressants desmethylimipramine and zimelidine on rat cortical GABAB binding sites: a comparison with 5-HT2 binding site changes. Br J Pharmacol 1988;93:331–6.
[15] Cross JA, Horton RW. Are increases in $GABA_B$ receptors consistent findings following chronic antidepressant administration. Eur J Pharmacol 1987;141:159–62.
[16] McManus DJ, Greenshaw AJ. Differential effects of antidepressants on $GABA_B$ and β-adrenergic receptors in rat cerebral cortex. Biochem Pharmacol 1991;42:1525–8.
[17] Magariños AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. Neuroscience 1995;69:83–8.
[18] Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 1996;93:3908–13.
[19] Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3-mRNAs in the hippocampus. J Neurosci 1995;15:1768–77.
[20] Conrad CD, Galea LA, Kuroda Y, McEwen BS. Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. Behav Neurosci 1996;110:1321–34.
[21] Enna SJ. GABAB receptors. Encycl Biol Chem 2004 [in press].
[22] Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, et al. $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. Nature 1998;396:683–7.
[23] Bowery NG, Bettler B, Froestl W, Gallagher J, Marshall F, Raiteri M, et al. Mammalian γ-aminobutyric acid B receptors: structure and function. Pharmacol Rev 2002;54:247–64.
[24] Schuler V, Loscher C, Blanchet C, Klix N, Sansig G, Klebs K, et al. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic $GABA_B$ responses in mice lacking GABAB(1). Neuron 2001;31:47–58.
[25] Chronwall BM, Davis TD, Severidt MW, Wolfe SE, McCarson KE, Beatty DM, et al. Constitutive expression of functional $GABA_B$ receptors in mIL-tsA58 cells requires both GABA(B(1)) and GABA(B(2)) genes. J Neurochem 2001;77:1237–47.
[26] Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, et al. $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. Nature 1998;396:674–9.
[27] White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, et al. Heterodimerization is required for the formation of a functional $GABA_B$ receptor. Nature 1998;396:679–82.
[28] Ng GY, Clark J, Coulombie N, Ethier N, Hebert TE, Sullivan R, et al. Identification of a $GABA_B$ receptor subunit, gb2, required for functional $GABA_B$ receptor activity. J Biol Chem 1999;274:7607–10.

[29] Gassman M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, et al. Redistribution of $GABA_{B(1)}$ protein and atypical $GABA_B$ responses in $GABA_{B(2)}$-deficient mice. J Neurosci 2004;24:6066–97.
[30] Sands SA, McCarson KE, Enna SJ. Differential regulation of $GABA_B$ receptor subunit expression and function. J Pharmacol Exp Therap 2003;305:191–6.
[31] Fritschy JM, Sidler C, Parpan F, Gassmann M, Kaupmann K, Bettler B, et al. Independent maturation of the GABAB receptor subunits GABAB(1) and GABAB(2) during postnatal development in rodent brain. J Comp Neurol 2004;477:235–52.
[32] Sands SA, Purisai MG, Chronwall BM, Enna SJ. Ontogeny of $GABA_B$ receptor subunit expression and function in the rat spinal cord. Brain Res 2003;972:197–206.
[33] Steiger JL, Bandyopadhyay S, Farb DH. Russek SJ. cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal $GABA_B$ R1a and $GABA_B$ R1b subunit gene expression through alternative promoters. J Neurosci 2004;24:6115–26.
[34] McCarson KE, Enna SJ. Nociceptive regulation of $GABA_B$ receptor gene expression in rat spinal cord. Neuropharmacology 1999;38: 1767–73.
[35] Castro-Lopes JM, Malcangio M, Pan BH, Bowery NG. Complex changes of $GABA_A$ and $GABA_B$ receptor binding in the spinal cord dorsal horn following peripheral inflammation or neurectomy. Brain Res 1995;679:289–97.
[36] Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995;15:7539–47.
[37] Sands SA, McCarson KE, Enna SJ. Relationship between the antinociceptive response to desipramine and changes in $GABA_B$ receptor function and subunit expression in the dorsal horn of the rat spinal cord. Biochem Pharmacol 2004;67:743–9.
[38] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000;20:9104–10.
[39] Mura VS, Molina VA. Antidepressants reduce inactivity during both inescapable shock administration and shuttle-box testing. Eur J Pharmacol 1991;204:187–92.
[40] Alper RA. Agonist-stimulated [^{35}S]GTPγS binding. Curr Prot Pharmacol 1998;1:2.6.1–2.6.10.
[41] Durkin MM, Gunwaldsen CA, Borowsky B, Jones KA, Branchek TA. An in situ hybridization study of the distribution of the GABA(B2) protein mRNA in the rat CNS. Brain Res 1999;71:185–200.
[42] Bischoff S, Leonhard S, Reymann N, Schuler V, Shigemoto R, Kaupmann K, et al. Spatial distribution of $GABA_B$R1 receptor mRNA and binding sites in the rat brain. J Comp Neurol 1999;412:1–16.
[43] Furtinger S, Bettler B, Sperk G. Altered expression of $GABA_B$ receptors in the hippocampus after kainic-acid-induced seizures in rats. Mol Brain Res 2003;113:107–15.
[44] Mondadori C, Jaekel J, Preiswerk G. CGP 36742: the first orally active $GABA_B$ blocker improves the cognitive performance of mice, rats, and rhesus monkeys. Behav Neural Biol 1993;60:62–8.
[45] Getova D, Bowery NG. The modulatory effects of high affinity $GABA_B$ receptor antagonists in an active avoidance learning paradigm in rats. Psychopharmacology 1998;137:369–73.
[46] McNamara RK, Skelton RW. Baclofen, a selective $GABA_B$ receptor agonist, dose-dependently impairs spatial learning in rats. Pharmacol Biochem Behav 1996;53:303–8.
[47] DeSousa NJ, Beninger RJ, Jhamandas K, Boegman RJ. Stimulation of $GABA_B$ receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze.. Brain Res 1994;641:29–38.
[48] Galeotti N, Ghelardini C, Bartolini A. Piracetam and aniracetam antagonism of centrally active drug-induced antinociception. Pharmacol Biochem Behav 1996;53:943–50.
[49] Shimidzu T, Itoh Y, Oka M, Ishiima T, Ukai Y, Yoshikuni Y, et al. Effect of a novel cognition enhancer NS-105 on learned helplessness in rats: possible involvement of $GABA_B$ receptor up-regulation after repeated treatment. Eur J Pharmacol 1997;338:225–32.
[50] Ogasawara T, Itoh Y, Tamura M, Mushiroi T, Ukai Y, Kise M, et al. Involvement of cholinergic and GABAergic systems in the reversal of memory disruption by NS-105, a cognition enhancer. Pharmacol Biochem Behav 1999;64:41–52.
[51] Hausler A, Monnet G, Peter O. Involvement of $GABA_B$ receptors in the regulation of the hypothalamic-pituitary-adrenocortical (HPA) axis in rats. J Steroid Biochem Mol Biol 1993;46:767–71.
[52] Nakagawa Y, Ishiima T, Ishibashi Y, Tsuji M, Takashima T. Involvement of GABAB receptor systems in experimental depression: baclofen but not bicuculline exacerbates learned helplessness in rats.. Brain Res 1996;741:240–5.
[53] Nakagawa Y, Ishiima T, Ishibashi Y, Tsuji M, Takashima T. Involvement of GABAB receptor systems in the action of antidepressants: II. Baclofen attenuates the effect of desipramine whereas muscimol has no effect in learned helplessness paradigm in rats. Brain Res 1996;728:225–30.
[54] Billinton A, Stean TO, Bowery NG, Upton N. $GABA_{B(1)}$ splice variant mRNAs are differentially affected by electroshock induced seizure in rats. NeuroReport 2000;11:3817–22.
[55] Nestler EJ, McMahon A, Sabban EL, Tallman JF, Duman RS. Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus. Proc Natl Acad Sci USA 1990;87:7522–6.
[56] White JH, McIllhinney RA, Wise A, Ciruela F, Chan WY, Emson PC, et al. The $GABA_B$ receptor interacts directly with the related transcription factors CREB2 and ATFx. Proc Natl Acad Sci USA 2000;97:13967–72.
[57] Malcangio M, Da Silva H, Bowery NG. Plasticity of $GABA_B$ receptors in spinal cord detected by autoradiography. Eur J Pharmacol 1993;250:143–56.