Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 515 (2005) 94 – 98

www.elsevier.com/locate/ejphar

Short communication

Up-regulation of $GABA_B$ receptors by chronic administration of the $GABA_B$ receptor antagonist SCH 50,911

Fabio Pibiri[a], Giovanni Carboni[a], Mauro A.M. Carai[a], Gian Luigi Gessa[a,b], M. Paola Castelli[a,*]

[a]*B.B. Brodie Department of Neuroscience, University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (CA), Italy*
[b]*Center of Excellence 'Neurobiology of Addiction', University of Cagliari, Italy*

Received 25 November 2004; received in revised form 15 March 2005; accepted 30 March 2005
Available online 12 May 2005

Abstract

Chronic treatment of mice with the specific γ-aminobutyric $acid_B$ ($GABA_B$) receptor antagonist (2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50,911) increased both the number of $GABA_B$ receptors in the whole brain (measured as [^{3}H]CGP 54626 [*S*-(*R*,*R*)]-3-[[1-(3,4-dichlorophenyl)amino]-2-hydroxypropyl](cyclohexylmethyl)phosphinic acid hydrochloride binding) and the ability of baclofen to activate $GABA_B$ receptor coupled G-protein (measured as % reduction of the EC_{50} of baclofen to activate [^{35}S]GTPγS binding).

The results indicate that persistent blockade of $GABA_B$ receptors leads to their compensatory up-regulation and suggest that $GABA_B$ receptors are tonically activated by endogenous GABA.

Keywords: $GABA_B$ receptor; GTPγS binding; SCH 50,911; Chronic treatment; (Mice)

1. Introduction

The $GABA_B$ receptor is a G-protein coupled receptor composed of the $GABA_{B1}$ and $GABA_{B2}$ subunits. Activation of the $GABA_B$ receptor coupled G-protein mediates both opening of the inwardly rectifying potassium channels and inactivation of voltage gated calcium channels. These mechanisms in turn lead to the inhibition of neurotransmitter release from presynaptic terminals (see Bowery et al., 2002; Bettler et al., 2004).

Up-regulation of $GABA_B$ receptors has been shown to occur after chronic treatment with antidepressants, lithium, carbamazepine and valproate (see Enna and Bowery, 2004). However, the increase observed in $GABA_B$ receptor density was not always associated with increased $GABA_B$ receptor function, measured as physiological responses to baclofen or, in vitro, as $GABA_B$ coupled G-protein mediated effects, such as the ability of baclofen to inhibit forskolin-stimulated adenyl-cyclase activity (McManus and Greenshaw, 1991a; Szekely et al., 1987). Moreover, following chronic administration of antidepressant drugs, binding of [^{3}H]GABA to $GABA_B$ receptors has been reported to be unaltered in cortical tissue (Cross and Horton, 1987; McManus and Greenshaw, 1991b).

Differences in experimental design, methods used and different brain regions analysed may account for the conflicting results obtained in different laboratories.

However, to ascertain whether modifications to the $GABA_B$-receptor are produced by chronic treatment with $GABA_B$ receptor ligands is an issue of particular interest, in view of the promising therapeutic applications of $GABA_B$ receptor agonists and antagonists of these receptors in pain control, drug addiction, mood disorders, epilepsy and cognitive disorders (see Bowery et al., 2002; Bowery and Enna, 2000).

Chronic administration of baclofen has been shown to decrease the number of $GABA_B$ receptors associated with tolerance to the pharmacological effects of this agent (Enna et al., 1998; Malcangio et al., 1993) and the loss of baclofen-stimulated [^{35}S]GTPγS binding, a marker of G-protein activation, indicating $GABA_B$ receptor desensitisation (Sands et al., 2003).

* Corresponding author. Tel.: +39 070 6754065; fax: +39 070 6754320.
E-mail address: castelli@unica.it (M.P. Castelli).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.03.040

In contrast, no reduction of $GABA_B$ receptors was observed by Motohashi et al. (1989) after chronic baclofen in different brain regions. Moreover, a recent paper by Lehmann et al. (2003) has reported that chronic administration of baclofen inducing tolerance respect to hypothermia, failed to modify $GABA_B$ receptors binding sites and mRNA levels for the subunit $GABA_{B(1a)}$ and $GABA_{B(1b)}$. Conversely, the only study on $GABA_B$ receptor antagonists has shown that in rats chronic treatment with the $GABA_B$ receptor antagonist CGP 36742 produced an increase in the number of $GABA_B$ receptors restricted to the outer lamina of the frontal cortex, associated to an enhanced G-protein mediated $GABA_B$ receptor function, measured as the ability of baclofen to inhibit forskolin-stimulated adenyl-cyclase. However, chronic treatment with CGP 35348, another $GABA_B$ receptor antagonist, failed to increase $GABA_B$ receptor numbers and to influence forskolin-stimulated cyclic AMP production (Pratt and Bowery, 1993).

In the present study, we investigated the effect of chronic treatment with the selective and potent $GABA_B$-receptor antagonist SCH 50,911 ((2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid) on $GABA_B$-receptor density and function, measured as [^{3}H]CGP 54626 binding and baclofen-stimulated [^{35}S]GTPγS binding, respectively, in the brain of DBA mice.

2. Materials and methods

2.1. Animals

Male DBA/J2 mice (Charles River, Como, Italy), weighing 20–25 g, were used in all experiments and maintained on ad libitum food and water. Animals, randomly divided into two treatment groups, received daily intraperitoneal (i.p.) injections of SCH 50,911 (Tocris, Bristol, UK) (100 mg/kg) or sterile saline for 15 consecutive days. Another group was acutely administered i.p. with a single dose of SCH 50,911 (100 mg/kg) and control mice received sterile saline. Mice were killed by decapitation on day 15 of treatment 1 h after the last injection in both acutely and chronically treated animals, their brains rapidly removed and placed on ice.

2.2. Binding experiments

2.2.1. Tissue preparation

Brains were processed as described in Castelli et al. (2003). Briefly, brains were homogenized using a homogenizer system (Glass-Col, Terre Haute, IN, USA) in 20 volumes (v/w) tissue of ice-cold 0.32 M sucrose. The homogenate was centrifuged at $1000 \times g$ for 10 min at 4 °C and the supernatant collected and recentrifuged at $20{,}000 \times g$ for 20 min. The pellet was resuspended in 20 volumes (v/w) of ice-cold water, homogenized using a Polytron homogenizer and centrifuged at $8000 \times g$ for 20 min. The supernatant, together with the buffy layer on the pellet, was then centrifuged at $48{,}000 \times g$ for 1 h at 4 °C. The final pellet was frozen and stored at − 80 °C for at least 18 h before use. On the experiment day, for both [^{3}H]CGP 54626 and [^{35}S]GTPγS binding, membrane pellets were thawed at 4 °C, resuspended in 20 volumes (v/w) of ice-cold Krebs Henseleit buffer (NaCl 143 mM, Tris 50 mM, KCl 5.9 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM, pH 7.4) and then homogenized. To remove SCH 50,911 and/or its metabolites, membranes underwent three cycles of washing, incubation at 22 °C for 15 min and centrifugation at $48{,}000 \times g$ for 15 min at 4 °C. The Bradford (1976) protein assay was used for protein determination using bovine serum albumin as a standard according to the supplier protocol (Bio-Rad, Milan, Italy).

2.2.2. [^{3}H]CGP 54626 and [^{35}S]GTPγS binding assay

[^{3}H]CGP 54626 binding was carried out as previously described (Bittiger et al., 1992), using 50 μg of membrane proteins and increasing concentrations (from 0.07 to 5 nM) of the labelled compound; *R*-(+)baclofen (10 μM) was used to define non-specific binding. The maximal number of binding sites (B_{max}) and apparent equilibrium dissociation constant (K_d) values of the radioligand were determined by computerized Scatchard analysis using non-linear regression (GraphPad Prism Program, San Diego, CA).

[^{35}S]GTPγS binding was performed with 4–6 μg of membrane protein, 0.2 nM [^{35}S]GTPγS and increasing concentration of baclofen (from 0.1 μM to 1.0 mM) in 96-well Packard-Picoplates as described previously (Castelli et al., 2003).

Non-specific binding was measured in presence of 10 μM unlabelled GTPγS. Basal binding was assayed in the absence of agonist and presence of 30 μM GDP.

Agonist stimulation was defined as a percentage increase above basal levels (i.e., {[dpm (agonist) − dpm (no agonist)]/dpm (no agonist)} × 100).

Non-linear regression analysis of concentration–response data was performed using Prism 3.0 software (GraphPad Prism Program, GraphPad, San Diego, CA, USA) to calculate E_{max} and EC_{50} values. Data were statistically evaluated by one-way analysis of variance (ANOVA), followed by the Newman–Keuls test for multiple comparison when appropriate.

3. Results

Acute treatment with SCH 50,911 failed to alter either $GABA_B$ receptor density (B_{max}) or affinity (K_d) measured with [^{3}H]CGP 54626 binding and $GABA_B$ receptor function, measured as baclofen-stimulated [^{35}S]GTPγS binding (Figs. 1A,B and 2A,B). Conversely, daily administration of SCH 50,911 for 15 consecutive days increased the number of $GABA_B$ receptor binding sites for [^{3}H]CGP 54626 by 23.8 ± 5.4% and enhanced the K_d value of this ligand by 37.2 ± 9.4%.

Fig. 1A,C evidentiates a significant increase in the B_{max} of mice chronically treated with SCH 50,911 compared to chronic saline- and acute SCH 50,911- or saline-treated mice. Moreover, K_d values (Fig. 1B,C) are significantly increased in SCH 50,911 chronically treated mice compared to animals acutely or chronically treated with saline.

To determine the functional relevance of baclofen-induced increase in $GABA_B$ receptor density associated with a reduction in receptor affinity, the effect of chronic SCH 50,911 on $GABA_B$ receptor coupled G protein binding was examined.

Basal levels of [^{35}S]GTPγS binding were similar in brain homogenates of control (385 ± 57 fmol/mg protein) and chronic

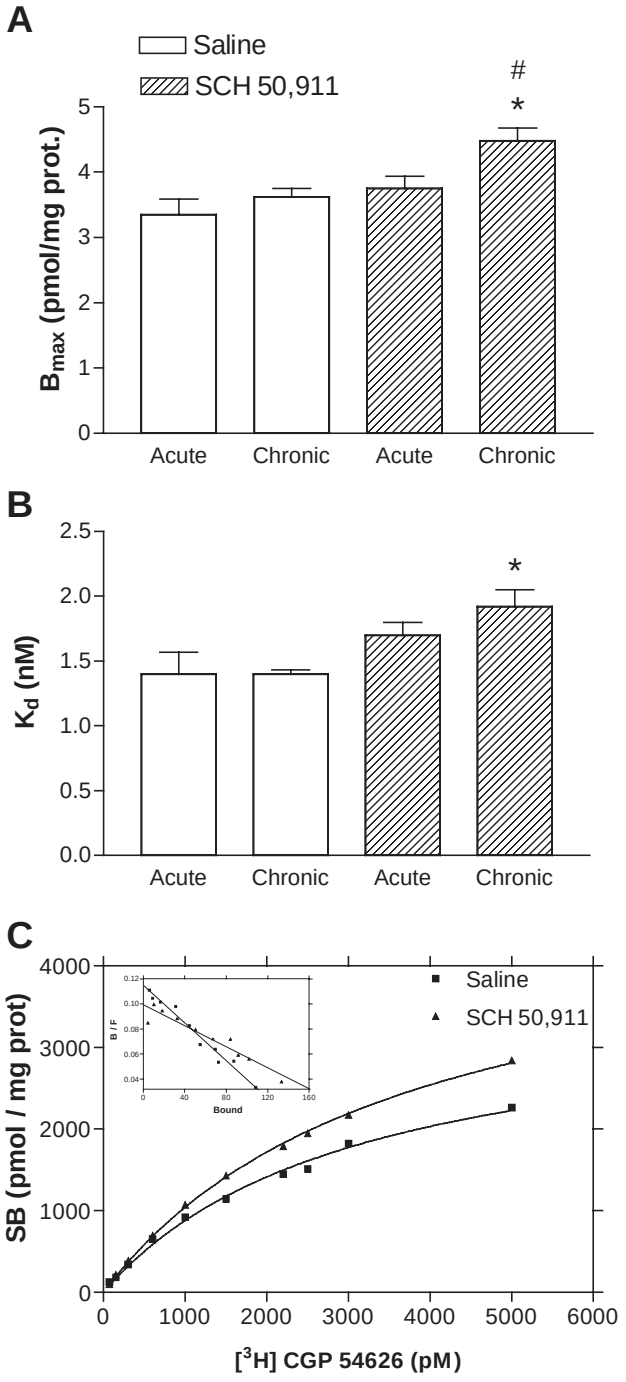

Fig. 1. B_{max} and K_d of [^{3}H]CGP 54626 binding in saline- and SCH 50,911-treated group. Mice, treated as described in the text, were sacrificed 1 h after treatment. Data of B_{max} and K_d are expressed as the mean±S.E.M. of at least four experiments, each performed in duplicate (4 to 6 mice for each group). (A) B_{max}—ANOVA: $F(3,17)=6.908$, $P<0.005$; $^*P<0.05$ chronic SCH 50,911 versus chronic saline- and acute SCH 50,911-treated mice, $^{\#}P<0.01$ chronic SCH 50,911 with respect to acute saline-treated mice (Newman–Keuls test). (B) K_d—ANOVA: $F(3,17)=5.12$, $P<0.02$; $^*P<0.05$ chronic SCH 50,911 versus chronic and acute saline-treated group (Newman–Keuls test). (C) Concentration-binding isotherms and Scatchard plot (inset) of [^{3}H]CGP 54626 binding to $GABA_B$ receptor. $K_d=1.30$ nM, $B_{max}=3.20$ pmol/mg protein (chronic saline); $K_d=2.30$ nM, $B_{max}=4.90$ pmol/mg protein (chronic SCH 50,911). The data represent a typical experiment out of 2 independent experiments from 2 different mice.

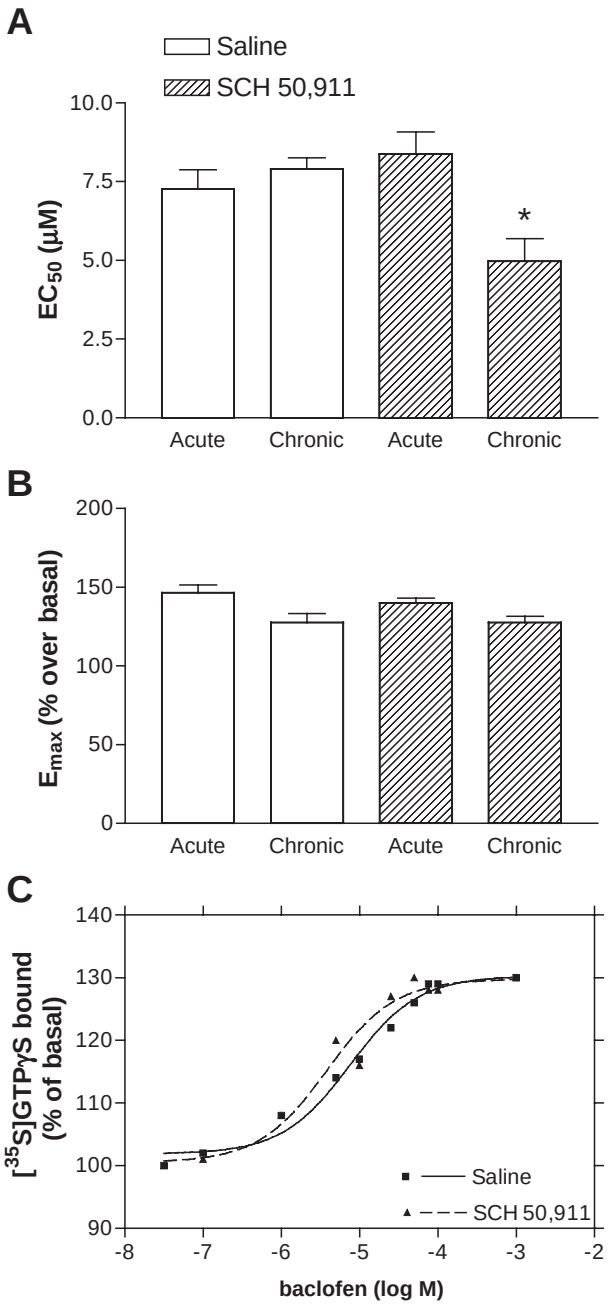

Fig. 2. Effect of acute and chronic treatment with SCH 50,911 on baclofen-stimulated [^{35}S]GTPγS binding. Mice were treated as described in Fig. 1 legend. Data from EC_{50} (A) and E_{max} (B) are expressed as the mean±S.E.M. of at least three experiments, each performed in triplicate (3 to 5 mice for each group). EC_{50}—ANOVA: $F(3,15)=6.98$, $P<0.05$; $^*P<0.05$ chronic SCH 50,911 with respect to chronic, acute saline-treated group and acute SCH 50911-treated group (Newman–Keuls test). E_{max}—ANOVA: $F(3,15)=4.47$, $P<0.02$ (Newman–Keuls test=NS). Baclofen concentration–effect curves for the $GABA_B$ receptor-mediated stimulation of [^{35}S]GTPγS binding to mice brain membranes (C) Basal binding is defined as 100% on the y-axis. $EC_{50}=7.7$ μM, $E_{max}=130\%$ (chronic saline); $EC_{50}=3.8$ μM, $E_{max}=130\%$ (chronic SCH 50,911). The data represent a typical experiment out of 2 independent experiments from 2 different mice.

SCH 50,911-treated mice (286±58 fmol/mg protein). In brain homogenates from saline-treated mice, baclofen-stimulated [^{35}S]GTPγS binding with an EC_{50} of 7.90±0.35 μM and an E_{max} of 127.00±5.60% the basal value. As shown in Fig. 2A, chronic exposure to SCH 50,911 induced a decrease of 37.3±9.0% in EC_{50} of baclofen for stimulating [^{35}S]GTPγS binding. The EC_{50} from chronically SCH 50,911-treated mice was significantly lower respect to both saline-treated mice and those treated with a single dose of SCH 50,911. However, chronic treatment failed to modify E_{max} values of baclofen for stimulating [^{35}S]GTPγS binding (Fig. 2B,C).

4. Discussion

The present findings demonstrate that chronic treatment with SCH 50,911 increases the number of $GABA_B$ receptors and $GABA_B$-receptor function in the whole mouse brain. The increase in $GABA_B$ receptor density reported in the present study pertains to the high affinity component of the $GABA_B$ receptor, as the high affinity antagonist ligand CGP 54626 labels a single high affinity site of the $GABA_B$ receptor, as previously reported (Green et al., 2001). Moreover, in our saturation studies, we obtained K_d and B_{max} values that are consistent with previously published data (Bittiger et al., 1992; Green et al., 2001).

Our data show that chronic treatment with SCH 50911 also increases the potency of baclofen in activating $GABA_B$ receptor coupled G proteins. This increased potency is associated with a decreased binding affinity suggesting that sensitization of $GABA_B$ receptors might occur at the G-protein level (e.g. number and/or changes of their conformational structure). In our study, increased B_{max} was not accompanied by an enhanced maximal response (E_{max}) to baclofen in GTPγS assay, indicating that $GABA_B$ receptors up-regulation might include G-protein uncoupled receptors. Accordingly, Sim et al. (1995) reported that the relative levels of receptors may not correspond completely to the levels of activated G-proteins.

Our results are consistent with previous results reported by Pratt and Bowery (1993) in rats, showing an increase in $GABA_B$ receptor number and function in the frontal cortex after chronic treatment with the $GABA_B$ receptor antagonist CGP 36742. However, the authors examined $GABA_B$ receptors by autoradiography and found that up-regulation was restricted only to the outer lamina of the frontal cortex.

The increase in $GABA_B$ receptor population produced by SCH 50,911 in the whole brain homogenate was of comparable magnitude to the increase observed in previous studies in discrete brain areas, such as the frontal cortex or hippocampus following different treatments (Lloyd et al., 1985; Motohashi, 1992).

Accordingly, if SCH 50,911-induced changes in the mouse brain were confined to discrete brain areas, our results should indicate a much higher degree of up-regulation than that revealed in the whole brain homogenate.

However, the possibility exists that $GABA_B$ receptor up-regulation is not restricted to focal brain regions but may involve $GABA_B$ receptors throughout the brain.

The up-regulation mechanism of $GABA_B$ receptors is not known. It has been suggested that persistent occupation of the receptor by the antagonist would eventually lead to a compensatory increase in $GABA_B$ receptor density and sensitivity.

This interpretation implies that $GABA_B$ receptors are tonically activated by an endogenous GABAergic activity.

Future studies should be directed at correlating $GABA_B$ receptors up-regulation to specific behavioural or physiological responses to $GABA_B$ receptor agonists, and investigating whether $GABA_B$ receptor ligands might differ in their ability to influence the plasticity of these receptors, possibly disclosing compounds with less propensity to produce tolerance or sensitisation.

References

Bettler, B., Kaupmann, K., Mosbacher, J., Gassmann, M., 2004. Molecular structure and physiological functions of GABAB receptors. Physiol. Rev. 84, 835–867.

Bittiger, H., Remann, N., Froestl, W., Mickel, S.J., 1992. ^{3}HCGP 54626: a potent antagonist radioligand for $GABA_B$ receptors. Pharmacol. Commun. 2, 23.

Bowery, N.G., Enna, S.J., 2000. Gamma-aminobutyric acid (B) receptors: first of the functional metabotropic heterodimers. J. Pharmacol. Exp. Ther. 292, 2–7.

Bowery, N.G., Bettler, B., Froestl, W., Gallagher, J.P., Marshall, F., Raiteri, M., Bonner, T.I., Enna, S.J., 2002. International Union of Pharmacology: XXXIII. Mammalian gamma-aminobutyric acid (B) receptors: structure and function. Pharmacol. Rev. 54, 247–264.

Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle protein-dye binding. Anal. Biochem. 72, 248–254.

Castelli, M.P., Ferraro, L., Mocci, I., Carta, F., Carai, M.A.M., Antonelli, T., Tanganelli, S., Cignarella, G., Gessa, G.L., 2003. Selective γ-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the sedative/hypnotic effect of γ-hydroxybutyric acid. J. Neurochem. 87, 722–723.

Cross, J.A., Horton, R.W., 1987. Are increases in $GABA_B$ receptors consistent findings following chronic antidepressant administration? Eur. J. Pharmacol. 141, 159–162.

Enna, S.J., Bowery, N.G., 2004. $GABA_B$ alterations as indicators of physiological and pharmacological function. Biochem. Pharmacol. 68, 1541–1548.

Enna, S.J., Harstad, E.B., McCarson, K.E., 1998. Regulation of neurokinin-1 receptor expression by $GABA_B$ receptor agonists. Life Sci. 62, 1525–1530.

Green, A., Walls, S., Wise, A., Green, R.H., Martin, A.K., Marshall, F.H., 2001. Characterization of [^{3}H]CGP 54626 binding to heterodimeric $GABA_B$ receptors stably expressed in mammalian cells. Br. J. Pharmacol. 131, 1766–1774.

Lehmann, A., Mattsson, J.P., Edlund, A., Johansson, T., Ekstrand, A.J., 2003. Effects of repeated administration of baclofen to rats on $GABA_B$ receptors binding sites and subunit expression in the brain. Neurochem. Res. 2, 385–391.

Lloyd, K.G., Thuret, F., Pile, A., 1985. 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. Ther. 235, 191–199.

Malcangio, M., Da Silva, H., Bowery, N.G., 1993. Plasticity of $GABA_B$ receptors in rat spinal cord detected by autoradiography. Eur. J. Pharmacol. 250, 153–156.

McManus, D.J., Greenshaw, A.J., 1991a. Differential effects of chronic antidepressants in behavioural tests of beta-adrenergic and GABA (B) receptor function. Psychopharmacology 103, 204–208.

McManus, D.J., Greenshaw, A.J., 1991b. Differential effects of antidepressants on $GABA_B$ and beta-adrenergic receptors in rat cerebral cortex. Biochem. Pharmacol. 42, 1525–1528.

Motohashi, N., 1992. GABA receptor alterations after chronic lithium administration. Comparison with carbamazepine and sodium valproate. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 16, 571–579.

Motohashi, N., Ikawa, K., Kariya, T., 1989. $GABA_B$ receptors are up-regulated by chronic treatment with lithium or carbamazepine. GABA hypothesis of affective disorders? Eur. J. Pharmacol. 166, 95–99.

Pratt, G.D., Bowery, N.G., 1993. Repeated administration of desipramine and a $GABA_B$ receptor antagonist, CGP 36742, discretely up-regulates $GABA_B$ receptor binding sites in rat frontal cortex. Br. J. Pharmacol. 110, 724–735.

Sands, S.A., MCCarson, K.E., Enna, S.J., 2003. Differential regulation of $GABA_B$ receptor subunit expression and function. J. Pharmacol. Exp. Ther. 305, 191–196.

Sim, L.J., Selley, D.E., Childers, S.R., 1995. In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5′-[γ-[^{35}S]thio]-triphospate binding. Proc. Natl. Acad. Sci. 92, 7242–7246.

Szekely, A.M., Barbaccia, M.L., Costa, E., 1987. Effect of a protracted antidepressant treatment on signal transduction and [^{3}H](−)-baclofen binding at $GABA_B$ receptors. J. Pharmacol. Exp. Ther. 243, 155–159.