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Short communication

Regulation of glutamic acid decarboxylase mRNA expression in rat brain after sertraline treatment

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Abstract

We now investigated the effect of chronic treatment with sertraline on glutamic acid decarboxylase mRNA expression in different rat brain areas by means of in situ hybridization. We found a reduced glutamic acid decarboxylase mRNA expression in the prefrontal cortex, accumbens nucleus, olfactory tubercle and reticular nucleus of the thalamus. The involvement of presynaptic modulation of γ-aminobutyric acid transmission in the anxiolytic effect of sertraline is discussed.

Keywords: Sertraline; Glutamic acid decarboxylase mRNA; Hybridization, in situ; Brain

1. Introduction

Studies on the neurobiological mechanism of anxiety have indicated that γ-aminobutyric acid (GABA) and serotonin (5-HT) are transmitters involved in this mood disorder. Since their discovery, benzodiazepines have been leaders in anxiolytic medication, but over the past 10 years, also in view of the major side-effects observed during benzodiazepine administration, such as risk of dependence and rebound or discontinuation, even after short term treatment, attention has been mainly directed towards 5-HT-ergic anxiolytic drugs (Barrett and Vanover, 1993). 5-HT involvement in anxiety is rather complex, also considering the continuous updating of information regarding 5-HT receptor genes and isoforms (Boess and Martin, 1994). The non-benzodiazepine drug, buspirone, and a number of analogs produce anxiolytic effects probably by acting through highly selective binding to the 5-HT_{1A} receptor subtype. The down-regulation of 5-HT_2 and perhaps 5-HT_3 receptors also seems to be crucial for the anxiolytic-antidepressant effect of these 5-HT_{1A} drugs (see Barrett and Vanover, 1993).

The role of 5-HT in anxiety is confirmed by the clinical use of selective inhibitors of serotonin reuptake as antidepressant drugs. One of the side-effects described in the use of selective inhibitors of serotonin reuptake in the treatment of depression is that the anxiety control has the same delay of onset as was shown for antidepressant action (Nutt, 1995). This is a desirable effect since depression and anxiety are frequently present in the same patient. Moreover, the latter seems to be produced through the inhibition of 5-HT-ergic neural activity. The administration of selective inhibitors of serotonin reuptake drugs in fact induces an early increase in 5-HT synaptic availability, but the extracellular 5-HT elicits activation of autoreceptors located at nerve endings and cell body levels, resulting in decreased 5-HT neural firing and release (see Feighner and Boyer, 1991).

We recently indicated that chronic administration of the newly introduced selective inhibitor of serotonin reuptake drug, sertraline, increased the number and the affinity of benzodiazepine receptors in selected areas of the rat brain (Giardino et al., 1993). This result raises the question of modulation of GABA-ergic transmission after chronic

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treatment with this antidepressant drug. To further investigate this point, we have studied the effect of chronic sertraline treatment on the expression of the glutamic acid decarboxylase mRNA, the limiting enzyme of the GABA synthesis, by means of in situ hybridization in the rat brain.

2. Materials and methods

2.1. Animals and treatments

Male Sprague-Dawley rats (OFA strain), 175–200 g body weight (IffaCredò, Monticello Brianza, Como, Italy) were used. The animals were housed under standard conditions (light on 07.00 a.m., off 7.00 p.m., temperature 20–22°C, humidity 60–70%), 4 animals per each large cage, with food pellets and water ad libitum. Groups of 8 rats each were injected daily i.p. with 10 mg/kg of sertraline hydrochloride for 28 days. Control rats were injected i.p. with the same amount of saline solution. The injections were given at 18:00–19:00 h, and the animals were then killed without washout period.

2.2. In situ hybridization

The rats were decapitated, the brains were promptly removed, immersed in ice-cold saline and frozen on microtome chucks. Sections 14 μm thick were cut in a cryostat (Leitz Kryostat 1720) at −20°C from the areas of interest and thaw-mounted onto precleaned microscope glass slides (ProbeOn, Fisher Scientific, Pittsburgh, USA). Oligonucleotide probes with sequences complementary to mRNAs encoding glutamic acid decarboxylase precursor (nucleotides 1285–1332) (Kobayaschi et al., 1987) were synthesized with a Beckmann Oligo-1000 DNA synthesizer. The oligonucleotide probes were labeled at the 3′ end with α-[^{35}S]dATP (New England Nuclear, Boston, MA, USA)

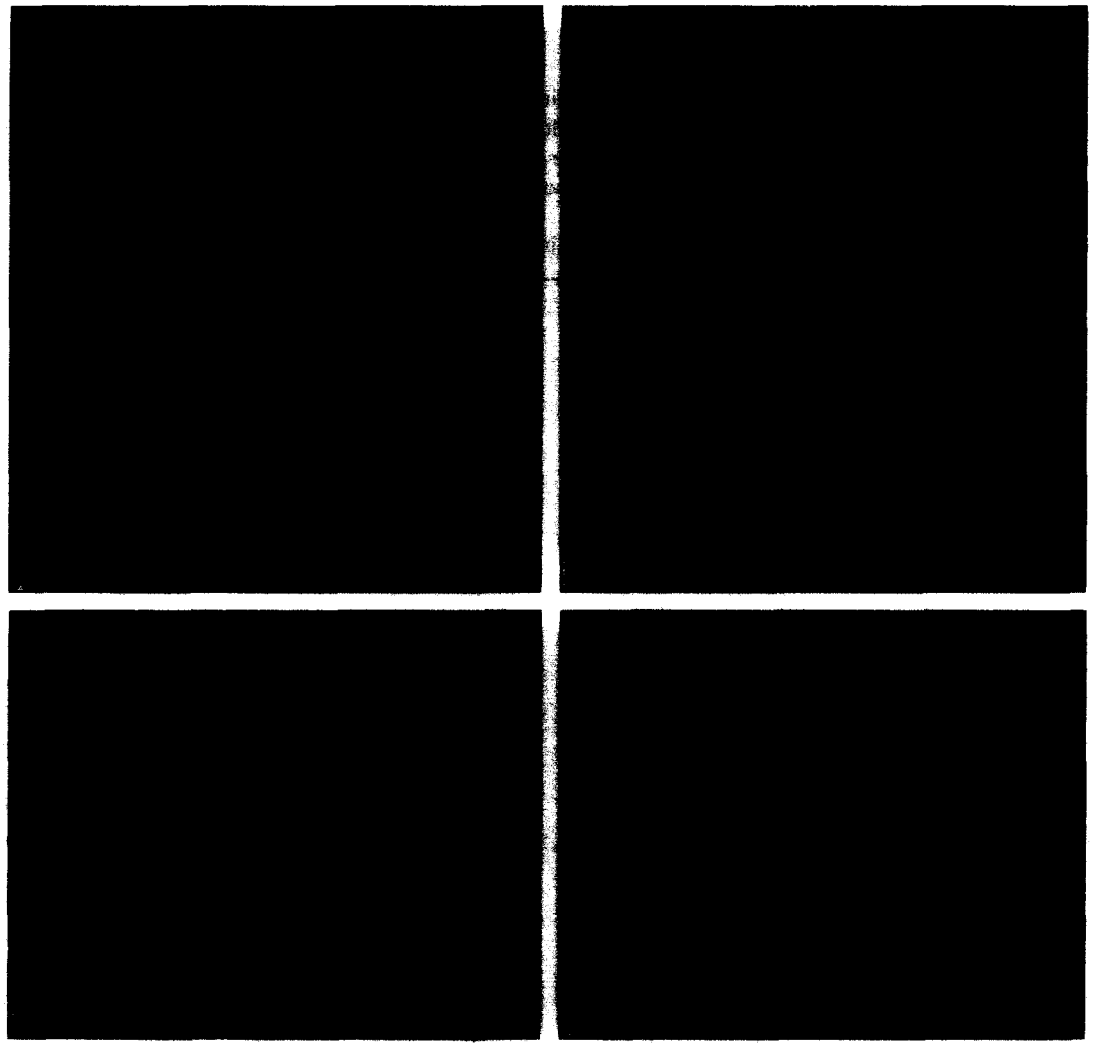

Fig. 1. Autoradiographic visualization of glutamic acid decarboxylase mRNA-expressing neurons in coronal sections from control (A,C) and sertraline-treated (B,D) rats at the rostro-caudal levels +1.6 mm (A,B) and −1.8 mm (C,D) from the bregma according to the Paxinos and Watson (1986) atlas. Acb, accumbens nuc.; Cpu, caudato-putamen nuc.; ret, reticular nuc. of the thalamus; TO, olfactory tubercle.

using terminal deoxynucleotidyltransferase in a buffer containing 10 mM $CoCl_2$, 1 mM dithiothreitol, 300 mM Tris base, and 1.4 M potassium cacodylate (pH 7.2). Afterwards, the labeled probes were purified through Nensorb-20 columns (NEN) and dithiothreitol was added to a final concentration of 10 mM. The specific activity obtained ranged from $1-4 \times 10^6$ dpm/ng oligonucleotide. The tissues were then hybridized. Briefly, the sections were brought to room temperature, air dried, covered with a hybridization buffer containing 50% formamide, 4 × saline sodium solution (SSC) (1 × SSC: 0.15 M NaCl = 0.015 M sodium citrate), 1 × Denhardt's solution (0.02% polyvinyl-pyrrolidone, 0.02% bovine serum albumin and 0.02% Ficoll), 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulphate, 250 μg/ml yeast tRNA, 500 μg/ml heat-denaturated salmon sperm DNA, and 200 mM dithiothreitol and 40 ng/μl of the labeled probes. The slides were placed in a humid chamber and incubated for 15–20 h at 50°C. Afterwards, the sections were rinsed in 1 × SSC at 55°C for 1 h with six changes and washed in the same buffer for 1 h at room temperature. Finally, the slides were rinsed in distilled water and 60% and 95% ethanol (2 min each) and air dried. Amersham β-max films were exposed to the labeled sections for 12 days at −20°C. Films were developed with Kodak AL 4 for 10 min. The tissue was then counterstained with toluidine blue.

2.3. Analysis of the results

The film autoradiograms were quantified by microdensitometry using the Quantimet 520+ image-analyzer (Leica, Witzburg, Germany) equipped with a stabilized illuminator (Leitz) and a CCD black and white TV camera.

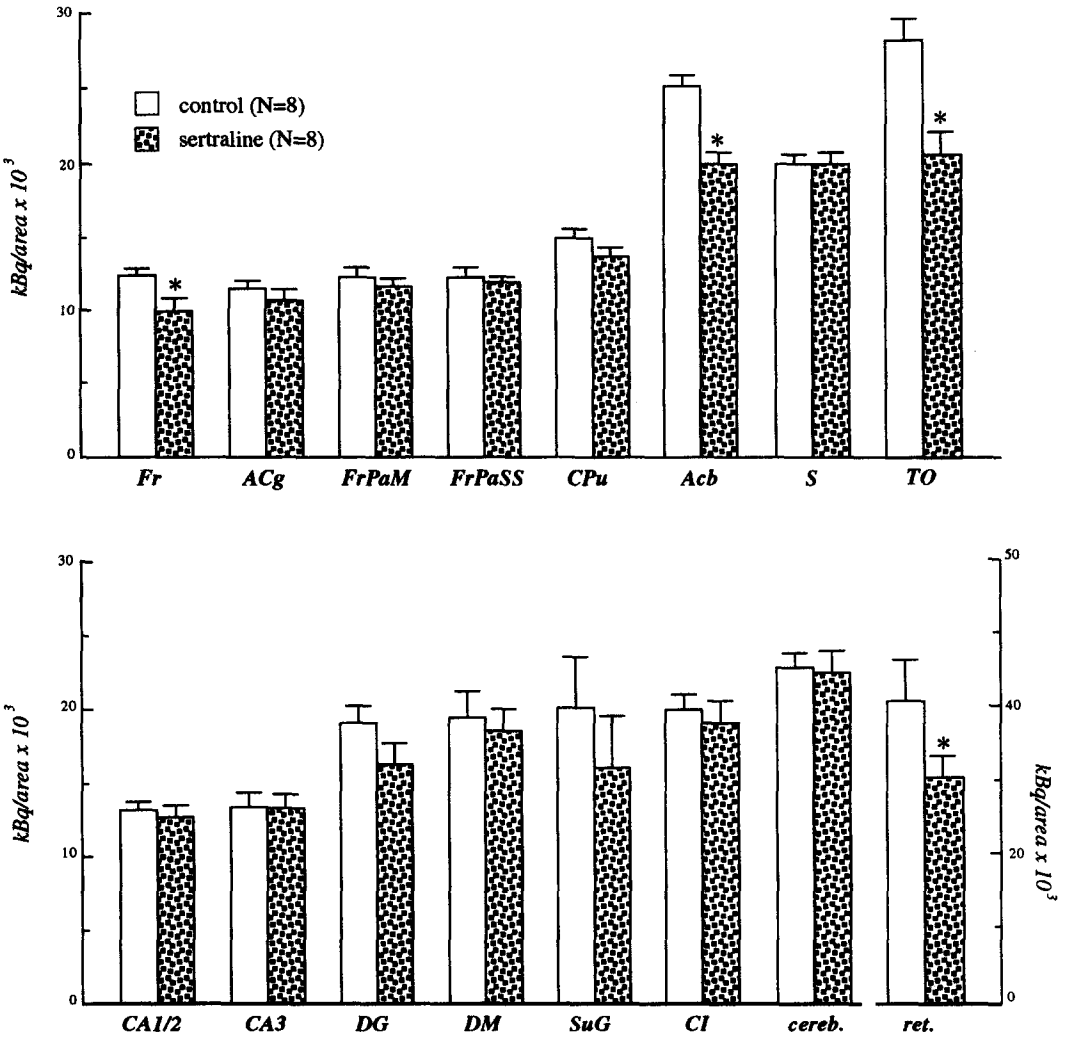

Fig. 2. Effect of chronic treatment with sertraline (28 days) on glutamic acid decarboxylase mRNA expression in different brain areas of rat. Values are presented as rad/area (kBq/area). Each point represents the mean and S.E.M. * $P < 0.05$. Acb, accumbens nuc.; Acg, anterior cingulate cortex; CA1/2, field 1 and 2 of the hippocampus; CA3, field 3 of the hippocampus; cereb, cerebellum; CI, inferior colliculus; CPu, caudato-putamen nuc.; DG, dentate gyrus; DM, dorsomedial nuc. of the hypothalamus; Fr, prefrontal cortex; FrPaM, frontoparietal motor cortex; FrPaSS, frontoparietal somatosensory cortex; ret, reticular nuc. of the thalamus; S, septum; SuG, superficial grey layer of the superior colliculus; TO, olfactory tubercle.

Background shading correction was used to compensate for possible defects of the camera or illumination source. The system was then calibrated with sets of [^{14}C]-plastic standards. A linear relationship was observed between the plastic and brain paste [^{14}C]-standards and [^{35}S]-labeled brain paste standards (Miller, 1991). Each image was digitalized to a 512×512 matrix with 256 grey levels and the unspecific labeling obtained from the tissue background was subtracted from each image. The areas of interest identified by toluidine blue staining were then sampled using an adjustable window. Three consecutive sections were measured from each area. The mean density value obtained from these measurements represented the average radioactivity due to glutamic acid decarboxylase mRNA concentration in the sampled areas. The Student's *t*-test was used for statistical analysis.

3. Results

Glutamic acid decarboxylase mRNA-expressing neurons are found in all areas of the cerebral cortex, in the caudato-putamen nucleus, in the accumbens nucleus particularly regarding the shell portion, in the olfactory tubercle and in the septal nucleus (Fig. 1). The reticular nucleus of the thalamus is strongly labeled, and moderate labeling is also present in the lateral geniculate body and in the dorso-medial nucleus of the hypothalamus. The pyramidal neurons of the hippocampus and the granule cells of the dentate gyrus also contain glutamic acid decarboxylase mRNA-expressing neurons. In the mesencephalon, diffuse labeling was seen in the superior and inferior colliculi. In the cerebellum, all classes of cells were labeled, including the Purkinje layer, Golgi granules and numerous stellate cells.

Rats chronically treated with sertraline (28 days) had a body weight lower than that of the control group (mean ± S.E.M.: control 426.2 g ± 5.0; sertraline 400.0 g ± 3.7, $P < 0.05$), probably due to the decrease in feeding induced by sertraline treatment. Chronic sertraline treatment induced a decreased glutamic acid decarboxylase mRNA expression in the prefrontal cortex, accumbens nucleus, olfactory tubercle and reticular nucleus of the thalamus (Fig. 2).

4. Discussion

We have demonstrated that chronic treatment with sertraline affects GABA-ergic neurons by inducing a decrease in glutamic acid decarboxylase mRNA expression in selected brain areas, i.e. the prefrontal cortex, the accumbens nucleus, the olfactory tubercle and the reticular nucleus of the thalamus. We suggest that this regulation of GABA-ergic systems could participate to the anxiolytic effect of sertraline. In the past, modifications in GABA transmission on the postsynaptic side after tricyclic antidepressant treatment have been reported, however, with contrasting results. The decrease in benzodiazepine receptors found by Suranyi-Cadotte et al. (1985) in whole-brain homogenate was not confirmed by others (Kimber et al., 1987), whereas we found an increase in benzodiazepine binding sites in the anterior cingolate cortex after chronic treatment with sertraline (Giardino et al., 1993). Peripheral-type benzodiazepine receptors can be up-regulated (liver), down-regulated (adrenal) or non-regulated (kidney) after chronic antidepressant treatment (Weizman et al., 1993). Moreover, repeated antidepressant treatment such as electroconvulsive shock increases $GABA_B$ receptor function in the frontal cortex of mice (Gray and Green, 1987), whereas $GABA_B$ binding is not modified in the frontal cortex of rats after chronic desmethylimipranine or zimelidine treatment (Cross and Horton, 1987).

The regulation of GABA-ergic transmission at the presynaptic level also now suggests another likely site of action for chronic selective inhibitors of serotonin reuptake, yet our experiment provides no hypothesis regarding the mechanism of action of selective inhibitors of serotonin reuptake on GABA-ergic neurons. We can, however, presume that the down-regulation in glutamic acid decarboxylase mRNA expression is produced through modification of the serotoninergic input to GABA-ergic elements. The interaction between GABA-ergic and 5-HT-ergic neurons is rather complex and involves both the pre- and postsynaptic side. GABA and 5-HT coexist in the dorsal nucleus of the raphe (Harandi et al., 1987), which is a primary site of action of chronic treatment with selective inhibitors of serotonin reuptake (Feighner and Boyer, 1991). Cortical GABA-ergic interneurons are also postsynaptic to 5-HT nerve endings. The effect of chronic selective inhibitors of serotonin reuptake on serotoninergic systems especially on 5-HT extracellular concentration, could be caused by different mechanisms at the cell body (i.e. raphe dorsalis) or nerve terminal (frontal cortex) levels. These mechanisms are probably related to the distribution and the concentration of autoreceptor types (Boess and Martin, 1994) and also to the duration of treatment. Presynaptically, decreased firing of raphe neurons and decreased synthesis of 5-HT occur with selective and non-selective inhibitors of 5-HT uptake. Extracellular 5-HT concentrations are nonetheless higher in all brain regions with the exception of the cortex, where some investigators have found increases whereas others have not (Fuller, 1993). Fluoxetine has been said to significantly increase the electrical activity of the fronto-cortical neurons (Ceci et al., 1993), moreover, chronic antidepressants (including sertraline) down-regulate the induction of *c*-fos mRNA in response to acute stress in rat frontal cortex (Morinobu et al., 1995). In view of the role of GABA-ergic interneurons in regulating cortical electrical activity under resting conditions, the decrease in glutamic acid decarboxylase mRNA expression could account for these effects.

It is interesting to note that apart from the reticular nucleus of the thalamus, which is a crucial relay nucleus in cortico-thalamus-cortical loops, the brain areas (prefrontal cortex, accumbens nucleus, olfactory tubercle) in which chronic sertraline down-regulates glutamic acid decarboxylase mRNA receive dopaminergic inputs from the A10 mesencephalic area which is mainly involved in anticipatory anxiety. It has been reported that repeated administration of fluoxetine significantly reduces dihydroxy-phenylalanine accumulation in the basal ganglia (Baldessarini and Marsh, 1990), and furthermore, glutamic acid decarboxylase mRNA itself is regulated by dopaminergic activity in the rat basal ganglia (Lindefors et al., 1989). GABA-ergic medium spiny neurons are considered the main source of striatal efferent axons terminating in the globus pallidus and on dopamine cells in the mesencephalon (Paxinos, 1995). Thus, considering this close anatomical and functional relationship between dopamine and GABA in the striatum we can suppose that glutamic acid decarboxylase mRNA regulation in these areas could be involved in the extrapyramidal effects observed during sertraline treatment.

In conclusion, from a neurochemical point of view, our data support the anxiolytic effect of sertraline treatment which also involves modulation of the GABA-ergic system. Considering the role played by the GABA-ergic system in multiple neurochemical regulation, this effect could also involve other transmitter-identified pathways.

References

Baldessarini, R.J. and E. Marsh, 1990, Fluoxetine and side effects, Arch. Gen. Psychiatry 47, 191.

Barrett, J.E. and K.E. Vanover, 1993, 5HT receptors as targets for the development of novel anxiolytic drugs: models, mechanisms and future directions, Psychopharmacology 112, 1.

Boess, F.G. and I.L. Martin, 1994, Molecular biology of 5-HT receptors, Neuropharmacology 33, 275.

Ceci, A., A. Baschirotto and F. Borsini, 1993, Effect of fluoxetine on the spontaneous electrical activity of fronto-cortical neurons, Eur. J. Pharmacol. 250, 461.

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

Feighner, J.P. and W.F. Boyer, 1991, Selective serotonin re-uptake inhibitors (John Wiley and Sons, Chichester).

Fuller, R.W., 1993, Microdialysis studies with 5-HT reuptake inhibitors, TiPS 14, 397.

Giardino, L., M. Zanni, A. Velardo, G. Amato and L. Calzà, 1993, Effect of sertraline treatment on benzodiazepine receptors in the rat brain, J. Neural Transm. 94, 31.

Gray, J.A. and A.R. Green, 1987, Increased $GABA_B$ receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks, Br. J. Pharmacol. 92, 357.

Harandi, M., M. Aguera, H. Gamrani, M. Didier, M. Maitre, A. Calas and M.F. Belin, 1987, γ-Aminobutyric acid and 5-hydroxytryptamine interrelationship in the rat nucleus raphe dorsalis: combination of radioautographic and immunocytochemical techniques at light and electron microscopic levels, Neuroscience 21, 237.

Kimber, J.R., J.A. Cross and R.W. Horton, 1987, Benzodiazepine and GABAA receptors in the rat brain following chronic antidepressant drug administration, Biochem. Pharmacol. 36, 4173.

Kobayaschi, Y., D.L. Kaufman and A.J. Tobin, 1987, Glutamic acid decarboxylase cDNA: nucleotide sequence encoding an enzimatically active fusion protein, J. Neurosci. 7, 2768.

Lindefors, N., S. Brebe, M. Herrera-Marschitz and H. Persson, 1989, Region specific regulation of glutamic acid decarboxylase mRNA expression by dopamine neurons in rat brain, Exp. Brain Res. 77, 611.

Miller, J.A., 1991, The calibration of 35S or 32P with 14C-labeled brain paste or 14C-plastic standards for quantitative autoradiography using LKB Ultrofilm or Amerhsam Hyperfilm, Neurosci. Lett. 121, 211.

Morinobu, S., M. Nibuya and R.S. Duman, 1995, Chronic antidepressant treatment down-regulates the induction of c-fos mRNA in response to acute stress in rat frontal cortex, Neuropsychopharmacology 12, 221.

Nutt, D., 1995, The anxiety factor in depression, J. Psychopharmacol. 9, 185.

Paxinos, G., 1995, The Rat Nervous System (Academic Press, New York).

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Suranyi-Cadotte, B.E., T.V. Dam and R. Quirion, 1985, Antidepressant-anxiolytic interaction: decreased density of benzodiazepine receptors in rat brain following chronic administration of antidepressants, Eur. J. Pharmacol. 106, 673.

Weizman, R., R. Burgin and M. Gavish, 1993, Modulatory effect of antidepressant on peripheral-type benzodiazepine receptors, Eur. J. Pharmacol. 250, 289.