Changes in peripheral benzodiazepine receptors in patients with bipolar disorder

Donatella Marazziti
Bernardo Dell'Osso
Stefano Baroni
Irene Masala
Elena Di Nasso
Gino Giannaccini
Liliana Dell'Osso

Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie University of Pisa, Pisa, Italy

Abstract: Peripheral benzodiazepine (BDZ) receptors were investigated by means of the binding of the specific ligand ^{3}H-PK 11195 to platelet membranes in patients suffering from bipolar disorder and in healthy controls. The results showed that the density (Bmax) of peripheral BDZ receptors was significantly higher in patients than in control subjects, with no change in the dissociation constant. No correlation with demographic or clinical features was observed. These findings would suggest that alterations of peripheral BDZ receptors are present in patients suffering from bipolar disorder, but it is premature to conclude whether they may be related to the pathophysiology of the disorder, or are secondary to changes occurring in other systems, such as those regulating the stress response.

Keywords: benzodiazepine receptors, peripheral benzodiazepine receptors, mitochondria, ^{3}H-PK 11195, bipolar disorder

Introduction

Several types of high-affinity binding sites for benzodiazepines (BDZ) have been described in the central nervous system (CNS) and in peripheral organs on the basis of their different affinity for radiolabeled ligands (Mohler and Okada 1977; Olsen and Venter 1986). The central receptors, almost all of which are found in the cortex, limbic areas, and cerebellum (Young and Kuhar 1979), are mainly labeled by flumazenil, whereas the peripheral type of BDZ receptors are identified specifically by Ro 5-4864 and PK 11195 (Marangos et al 1982). The most abundant sources of peripheral BDZ receptors are kidneys, lungs, ovaries, testes, and adrenal glands, but they are also present in the CNS, particularly in glial cells (Anholt et al 1984).

From a functional point of view, while the salient feature of the central BDZ receptor is coupling with the GABA-A receptor complex and facilitation of GABA transmission (Costa et al 1978), the peripheral type lacks this association and is localized to the mitochondrial outer membrane (Anholt et al 1986; Sprengel et al 1989). The most exhaustive hypothesis on the role of this receptor is the one suggesting a role in steroidogenesis, since in adrenals it has been demonstrated that it mediates the translocation of cholesterol from the outer to the inner mitochondrial membrane and is involved in the chain cleavage of cholesterol (Muhkin et al 1989; Papadopoulos et al 1990). Therefore, it is supposed that peripheral BDZ receptors subserve the same function in nonsteroidogenic tissues; particularly in glial cells, which have been shown to synthetize steroids, such as pregnenolone and progesterone and its derivatives called neurosteroids, which are able to alter the electrical properties of neuronal membranes and thus the firing patterns of neurons (Anholt et al 1986; Hu et al 1989; Jung-Testas et al 1989; Wodarz et al 1998). Such compounds, in turn, strongly modulate the GABA-A receptor complex and thereby the stress response

Correspondence: Donatella Marazziti
Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, University of Pisa, via Roma, 67, I-56100 Pisa, Italy
Tel +39 050 835 412
Fax +39 050 21581
Email dmarazzi@psico.med.unipi.it

(Purdy et al 1990). It is noteworthy that different stress paradigms in animals produce changes in peripheral BDZ receptors (Drugan et al 1986, 1987; Okun et al 1988).

Furthermore, the peripheral BDZ receptor is involved in a variety of other actions, such as control of cell proliferation, chemiotaxis, and contractility of the myocardium (Ruff et al 1985; Lenfant 1989; Taupin et al 1991; Ferrarese et al 1993).

The presence of these receptors in blood cells, such as platelets or lymphocytes, which undergo the same regulation and changes as in other tissues, particularly in the brain (Drugan et al 1986), has offered a peripheral tool for studies in humans. Stressful situations, such as sitting an exam (Karp et al 1989) or a parachute-training course (Dar et al 1991), influence the density of receptors as an acute stress seems to provoke an up-regulation, while a repeated stress provokes a down-regulation (Drugan et al 1987; Okun et al 1988; Hu et al 1989; Jung-Testas et al 1989; Purdy et al 1990).

The literature is also reporting changes of peripheral BDZ receptors in different psychiatric disorders, such as generalized anxiety disorder (GAD), social phobia, panic disorder, obsessive-compulsive disorder (OCD), Alzheimer's disease, and schizophrenia (Gavish et al 1986; Bonuccelli et al 1991; Rocca et al 1991; Marazziti, Rotondo, et al 1994; Bidder et al 1997), while no information is available in patients with mood disorders, in spite of evidence that stress systems are altered in these diseases (for review, see Claes 2004), and there is frequent comorbidity with various anxiety disorders (Chen and Dilsaver 1995; Goodwin and Hoven 2002; Simon et al 2004).

To further clarify this issue, we investigated peripheral BDZ receptors by the specific binding of ^{3}H-PK 11195 in platelets of patients suffering from bipolar disorder, according to strict DSM-IV criteria, and in a similar group of healthy control subjects.

Subjects and methods

Subjects

Twenty outpatients (12 male and 8 female, between 23 and 55 years of age, mean ± SD: 35.7 ± 11.4) affected by bipolar disorder were included in the study. Diagnoses were made by trained psychiatrists according to the Structured Clinical Interview (SCID) for DSM-IV (APA 1994; First et al 1997). Twelve patients were bipolar II (5 were depressed, 7 euthymic) and 8 were bipolar I (all in the euthymic phase). Five patients out of the total 20 were drug-free and fifteen were taking mood stabilizers (5 lithium salts, 8 valproate, 1 gabapentin, 1 lamotrigine). Of the total 5 depressed patients, 3 were taking antidepressants (1 clomipramine, 1 sertraline, 2 citalopram, 1 fluvoxamine), drugs that do not interfere with ^{3}H-PK 11195 binding parameters. No patient was taking BDZs.

The controls included 20 healthy drug-free subjects (10 male and 10 female, between 25 and 45 years of age, mean ± SD: 28.6 ± 5.4), with neither family nor personal history of any major psychiatric disorder, as recorded by the SCID.

In addition, both patients and controls had no physical illness, as documented by a physical exam and blood and urine tests. Informed consent was obtained from all subjects of the study, which was approved by the Ethics Committee at Pisa University.

Methods

Thirty milliliters of venous blood was drawn from fasting subjects between 8 am and 10 am, collected into plastic tubes containing 5 mL of anticoagulant (2.2% sodium citrate and 1.2% citric acid), and centrifuged at 150 × *g* for 15 min at 23 °C. Platelet-rich-plasma was collected and centrifuged at 1500 × *g* for 15 min at 23 °C. The drained platelet pellet was frozen at –80 °C until the binding assay, which was carried out within 2 weeks. Prior to the binding assay, the platelets were lysed and homogenized in 12 mL buffer (50 mmol/L Tris HCl, pH 7.4) using an Ultra Thurrax homogenizer for 5 s at 2/3 full speed and then centrifuged at 49 000 × *g* for 15 min at 4 °C. This procedure was immediately repeated. The membranes were resuspended in 12 mL of 50 mmol/L tris HCl and used for binding studies.

The binding of ^{3}H-PK 11195 to platelet membranes was performed according to a slight modification of the method of Gavish et al (1986). Four hundred microliters of membrane suspension (50 µg protein) was incubated with 20 µL of PK 11195 (specific activity: 82.4 Ci/mmol NEN, England,) at final concentrations ranging between 1 and 8 nmol/L, and 50 mmol/L Tris HCl buffer, pH 7.4, in a total volume of 500 µL. Non-specific binding was determined in the presence of 10 µmol/L unlabelled PK 11195 (Sigma, Milan, Italy). The incubation lasted for 90 min at 0 °C and was stopped by the addition of 5 mL of ice-cold incubation buffer and filtration under vacuum over Whatman GF/B filters. The filters were washed three times with 5 mL of the same buffer, placed in vials and counted in 4 mL of scintillation cocktail in a liquid scintillation counter.

The data were evaluated by Scatchard analysis to obtain the maximal number of binding sites (Bmax, fmol/mg protein) and their affinity to ^{3}H-PK 11195 as expressed by the equilibrium dissociation constant (Kd, nmol/L), by means of microcomputer programs (EBDA and LIGAND) (McPhearson 1985).

Protein concentration was determined according to the method of Peterson (1977). The difference between the groups was measured by means of Student's test (un-paired, two-tailed). The effect of age and sex on biological parameters was assessed by means of covariance analysis, and the possible correlations between biological findings and psychopathological data were analyzed according to Pearson's method, with Personal Computer programs (StatView V, SSPS) (Nie et al 1998).

Results

The results showed that the Bmax (mean ± SD, fmol/mg protein) was 6263 ± 1960 in bipolar disorder patients and 4855 ± 1621 in the control subjects, statistically higher in the first than in the other group (t-test: $p < 0.01$). On the contrary, the Kd (mean ± SD, nmol/L) did not show any difference, with the values 5.43 ± 1.53 in the patients and 5.33 ± 1.92 in the control subjects. No difference between patients with bipolar I and bipolar II disorder was detected.

No age or sex effect was observed, and no relationship between biological parameters and clinical features was noticed.

Discussion

The major finding of this study is the presence of an increased density (Bmax) of peripheral BDZ receptors in platelets of patients with bipolar disorder, as compared with healthy controls, with no associate change in the dissociation constant. No difference between bipolar I and bipolar II patients was detected. Since a significant increase in Kd values has been reported in subjects over 70 years of age (Marazziti, Pancioli, et al 1994), we controlled this variable in the groups who were selected on the basis of a limited age range, and we observed no effect. Similarly, no sex-related influence on the binding of ^{3}H-PK-11195 was noticed. Although no seasonal change in the binding has been observed to date, we performed all the blood sample analysis in a limited period of the year (from March to May).

Our results in patients with bipolar disorder are the first report in this group of patients; however, it must be mentioned that due to the small sample size it was not possible to perform reliable statistical analyses or to ascertain the real impact, if any, of the different phases of the illness on ^{3}H-PK 11195 binding.

Previously, patients with panic disorder, social phobia, or GAD have been shown to have a low density of PK11195 binding sites (Weizman et al 1987; Ferrarese et al 1990; Johnson et al 1998). In addition, in patients with GAD, the density of peripheral BDZ receptors would return towards the values found in healthy controls after treatments with BDZs (Ferrarese et al 1990). With regard to patients with OCD, the ensuing findings are quite controversial: patients with a chronic course seem to be characterized by no change in the binding of ^{3}H-PK11195 (Marazziti, Rotondo, et al 1994), while patients with an episodic course would show a decreased density of ^{3}H-PK11195 binding sites (Rocca et al 1991). In some cases, decreased peripheral BDZ receptor density in lymphocytes has been showed to be paralleled by similar changes in mRNA levels (Rocca et al 1991; Nudmamud et al 2000).

The modifications observed in platelets should reflect similar changes in other organs rich in peripheral BDZ receptors, such as glial cells and pituitary at the level of the CNS, heart, and kidney, as it has been demonstrated in rats that underwent inescapable tail shock (Drugan et al 1986). Such changes might be linked with a general membrane abnormality, as it has been proposed for Alzheimer's dementia (Zubenko et al 1987), or with abnormalities in endogenous ligands acting at their level, or represent a non-specific sign of stress, as it has been demonstrated that peripheral BDZ receptor density is significantly correlated with changes in corticosteroid levels (Nudmamud et al 2000).

In any case, it is puzzling that bipolar patients with no comorbid disorder have an increased density of peripheral BDZ receptors, at variance with patients with other psychiatric conditions, mainly of the anxiety spectrum. It is not possible as yet to conclude whether the changes in peripheral BDZ receptors represent a primary phenomenon related to the etiology or pathophysiology of the disease, or alternatively are secondary to changes in other systems, such as those regulating the stress response.

Future studies should aim to explore peripheral BDZ receptors in patients with other mood disorders with and without comorbid conditions in order to clarify whether eventual changes at their level might be helpful in confirming diagnostic categories or in characterizing different comorbidity patterns.

References

Anholt RRH, Aebi U, Pedersen PL, et al. 1986. Solubilization and reassembly of the mitochondrial benzodiazepine receptors. *Biochemistry*, 25:2120–5.

Anholt RRH, Murphy KMM, Mack GE, et al. 1984. Peripheral-type benzodiazepine receptors in the central nervous system: localization to olfactory nerves. *J Neurosci*, 4:593–603.

[APA] American Psychiatric Association. Diagnostic and statistical manual for mental disorders. 4th ed (DSM-IV). Washington: APA.

Bidder M, Ratzoni G, Weizman A, et al. 1987. Platelet benzodiazepine binding in Alzheimer's disease. *Biol Psychiatry*, 28:641–3.

Bonuccelli U, Nuti A, Del Dotto P, et al. 1991. Platelet peripheral benzodiazepine receptors are decreased in Parkinson's disease. *Life Sci*, 48:1185–90.

Chen YW, Dilsaver SC. 1995. Comorbidity of panic disorder in bipolar illness: evidence from the Epidemiological Catchment Area survey. *Am J Psychiatry*, 152:280–2.

Claes SJ. 2004. CRH, stress, and major depression: a psychobiological interplay. *Vitam Horm*, 69:117–50.

Costa E, Guidotti A, Toffano G. 1978. Molecular mechanisms mediating the action of diazepam on GABA receptors. *Br J Psychiatry*, 133: 239–48.

Dar DE, Weizman A, Karp L, et al. 1991. Platelet peripheral benzodiazepine receptors in repeated stress. *Life Sci*, 48:341–6.

Drugan RC, Basile AS, Crawley JN, et al. 1986. Inescapable shock reduces (^{3}H)-Ro 5-4864 binding to peripheral-type benzodiazepine receptor in the rat. *Pharmacol Biochem Behav*, 24:1673–8.

Drugan RC, Basile AS, Crawley JN, et al. 1987. Peripheral benzodiazepine binding sites in Maudsley rats: selective decrease confined to peripheral tissues. *Brain Res Bull*, 18:143–5.

Ferrarese C, Apollonio I, Bianchi G, et al. 1993. Benzodiazepine receptors and diazepam binding inhibitor: a possible link between stress, anxiety and the immune system. *Psychoneuroendocrinology*, 18:3–22.

Ferrarese C, Apollonio I, Frigo M, et al. 1990. Decreased density of benzodiazepine receptors in lymphocytes of anxious patients: reversal after chronic diazepam treatment. *Acta Psychiatr Scand*, 82:169–73.

First MB, Spitzer RL, Gibbon M, et al. 1997. Structured clinical interview for DSM-IV axis I disorders. Patient edition (SCID-I/P, version 2.0, 4/97 revision). New York: New York State Psychiatric Institute.

Gavish M, Weizman A, Karp L, et al. 1986. Decreased peripheral benzodiazepine sites in platelets of neuroleptic treated schizophrenics. *Eur J Pharmacol*, 121:275–9.

Goodwin RD, Hoven CW. 2002. Bipolar-panic comorbidity in the general population: prevalence and associated morbidity. *J Affect Dis*, 55: 51–4.

Hu ZY, Jung-Testas I, Robel P, et al. 1989. Neurosteroids: steroidogenesis in primary cultures of rat glial cells after release of aminoglutethimide blockade. *Biochem Biophys Res Commun*, 161:917–22.

Johnson MR, Marazziti D, Brawman-Mintzer O, et al. 1998. Abnormal peripheral benzodiazepine receptor density associated with generalized social phobia. *Biol Psychiatry*, 43:306–9.

Jung-Testas I, Hu ZY, Baulieu EE, et al. 1989. Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology*, 125:2083–91.

Karp L, Weizman A, Tyano S, et al. 1989. Examination stress, platelet peripheral benzodiazepine binding sites and plasma hormone levels. *Life Sci*, 44:1077–82.

Lenfant M. 1989. Neuroimmunomodulation: a search for the target of PK11195 a structurally unrelated ligand for peripheral benzodiazepine binding sites. In Hadden JW, Masek K, Nisticò G (eds). Interactions among central nervous system, neuroendocrine and immune systems. Rome: Pythagora Pr. p 283–92.

Marangos PJ, Patel J, Boulenger JP. 1982. Characterization of peripheral type benzodiazepine binding sites in brain using 3H-Ro-5-4864. *Mol Pharmacol*, 22:26–32.

Marazziti D, Pancioli Guadagnucci DL, Rotondo A, et al. 1994. Age-related changes in peripheral benzodiazepine receptors of human platelets. *J Psychiatry Neurosci*, 19:136–9.

Marazziti D, Rotondo D, Martini C, et al. 1994. Changes in peripheral benzodiazepine receptors in patients with panic disorder and obsessive-compulsive disorder. *Neuropsychoiology*, 29:8–11.

McPhearson GA. 1985. Kinetic, EBDA, Ligand, Lowry: a collection of radio ligand binding analysis programs. Amsterdam: Elsevier Science Publ.

Mohler H, Okada T. 1977. Benzodiazepine receptor: demonstration in the central nervous system. *Science*, 198:849–51.

Muhkin AG, Papadopoulos V, Costa E, et al. 1989. Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proc Natl Acad Sci U S A*, 86:9813–16.

Nie NH, Hull CH, Steinbrenner K, et al. 1998. Statistical package for the social science (SPSS). 4th ed. New York: McGraw-Hill.

Nudmamud S, Siripukpong P, Chindaduangratana C, et al. 2000. Stress, anxiety and peripheral benzodiazepine receptor mRNA levels in human lymphocytes. *Life Sci*, 67:2221–31.

Okun F, Weizman R, Katz Y, et al. 1988. Increase in central and peripheral benzodiazepine receptor following surgery. *Brain Res*, 458:31–6.

Olsen R, Venter C. 1986. Benzodiazepine/GABA receptor and chloride channels: structural and functional properties. New York: Liss Pr.

Papadopoulos V, Muhkin AG, Costa E, et al. 1990. The peripheral-type benzodiazepine receptor is functionally linked to Leydig-cell steroidogenesis. *J Biol Chem*, 265:3772–9.

Peterson GL. 1977. A simplification of the protein assay method which is more generally applicable. *Ann Biochemistry*, 83:346–56.

Purdy RH, Morrow AL, Moore PH, et al. 1990. Stress-induced elevation of GABA-A receptor-active 3 alpha-hydroxysteroids in the rat brain. *Soc Neurosci Abstract*, 16:289–93.

Rocca P, Ferrero P, Gualerzi A, et al. 1991. Peripheral-type benzodiazepine receptors in anxiety disorders. *Acta Psychiatr Scand*, 84:537–44.

Ruff MR, Pert CB, Weber RJ, et al. 1985. Benzodiazepine receptor mediated chemiotaxis of human monocytes. *Science*, 229:1281–3.

Simon NM, Smoller JW, Fava M, et al. 2004. Comparing anxiety disorder and anxiety-related traits in bipolar disorder and unipolar depression. *J Psychiatr Res*, 37:187–92.

Sprengel R, Werner P, Seeburg PH, et al. 1989. Molecular cloning and expression of cDNA encoding a peripheral type benzodiazepine receptor. *J Biol Chem*, 264:20415–21.

Taupin V, Herbelin A, Descamps-Latscha B, et al. 1991. Endogenous anxiogenic peptide, ODN-diazepam binding inhibitor and benzodiazepine enhance the production of interleukin-1 and tumor necrosis factor by human monocytes. *Lymph Cytok Res*, 10:7–13.

Weizman R, Tanne Z, Granek M, et al. 1987. Peripheral benzodiazepine binding sites on platelet membranes are increased during diazepam treatment of anxious patients. *Eur J Pharmacol*, 138:289–92.

Wodarz N, Rothenhofer C, Fisher R, et al. 1998. Peripheral type benzodiazepine receptors in diagnostic subtypes of schizophrenic patients. *Psychiatry Res*, 81:363–9.

Young WS, Kuhar M. 1979. Autoradiographic localization of benzodiazepine receptors in the brains of human and animals. *Nature*, 280:393–5.

Zubenko GS, Wusilko M, Boller F, et al. 1987. Family study of platelet membrane fluidity in Alzheimer's disease. *Science*, 238:539–42.