Eur Arch Psychiatry Clin Neurosci (2001) 251 : 136–140

ORIGINAL PAPER

Simon Chiu · Amarendra N. Singh · Pauline Chiu · Ram K. Mishra

Inverse agonist binding of peripheral benzodiazepine receptors in anxiety disorder

Received: 15 November 2000 / Accepted: 16 May 2001

Abstract The binding characteristics of the pBR (peripheral benzodiazepine receptor) inverse agonist, [^{3}H]-Ro 5-4864, were examined in patients diagnosed as generalized anxiety disorder. As compared to normal healthy controls, the anxious subjects demonstrated a statistically significant ($p < 0.001$) increase in the density of pBR in platelets. The enhanced pBR binding correlated significantly with the severity of global anxiety symptom of the Hamilton Anxiety Rating Scale (HAR-S, $p < 0.001$). The Psychic component, but not the Somatic component, of the HAR-S, correlated significantly ($p < 0.001$) with the enhanced pBR binding in platelets. The results provide evidence for the hypothesis of dysregulation of peripheral benzodiazepine receptors in generalized anxiety disorder.

Key words Anxiety · Inverse agonist · Peripheral benzodiazepine receptors

Facility where study was completed:
Dr. Ram K. Mishra Ph D
Neuropharmacology Laboratory
Department of Psychiatry/Behavioural Neurosciences
McMaster University
Hamilton, Ontario L8N 3Z5 Canada

Current address:
Dr. Amarendra N. Singh MD, FRCP(c), FRCPsy
Department of Psychiatry
Queen's University
Kingston, Ontario

Dr. Pauline Chiu MD, PhD
Family Practice Associates
Madisonville, TN, USA.

Corresponding author:
Dr. Simon Chiu, MD, PhD, FRCP(c)
Regional Mental Health Care, ARU
St. Thomas site
467 Sunset Drive
St. Thomas, Ontario, N5P 3V9, Canada
Tel.: 519-631-8510 Ext. 2510
Fax: 519-633-0852

EAPCN 324

Introduction

The symptom cluster of generalized anxiety disorder consists of autonomic hyperactivity, motor tension and hyper-vigilance. Although the anxiolytic action of benzodiazepines has been well established [11], the specificity of the types/ subtypes of benzodiazepine receptors in mediating the pharmacological effects remains inconclusive. Converging evidence suggest that the efficacy of benzodiazepines in the treatment of the spectrum of anxiety disorders is related, either directly or indirectly, to the allosteric interaction with central benzodiazepine receptors (cBR) as coupled to Type A of Gamma-Amino-Butyric acid (GABAa) receptors-chloride ionophore macro-molecular complex [3]. Once occupied with cBR agonists, cBR interact synergistically with GABAa receptors, hence facilitating the influx of chloride and resulting in hyperpolarization of postsynaptic membranes. However, benzodiazepines may not interact exclusively with cBR as the therapeutic target. Recently, a novel class of benzodiazepine receptors named "peripheral benzodiazepine receptors", has been found and expressed in platelets, lymphocytes, steroid-producing tissues and glial cells of the mammalian brain: the so-called peripheral benzodiazepine receptors (pBR) [23]. In contrast to the association of cBR with neuronal elements, the pBR are found in astro-glia cells. The differential central nervous system (CNS) localization of the two main subtypes draws attention to certain intriguing questions as to: 1)whether cross-talk occurs between pBR and cBR; 2) whether such presumed cBR-pBR interaction reflects glial-neuron coupling; 3) whether the putative pBR and cBR contribute towards the phenomenology of generalized anxiety disorder.

The complementary DNA (cDNA) of pBR, a protein with molecular weight of 18 kDa, has been cloned in three mammalian species: human, rodent and bovine species [5, 8, 20]. Two subtypes of pBR have been characterized: a) the "benzodiazepine" site labelled with

[^{3}H]-Ro 5-4864 (4′chlorodiazepam), the putative pBR inverse agonist; b) the "isoquinoline" site labelled with [^{3}H]PK 11195 (isoquinoline carboxamide derivative), the prototypal pBR antagonist [17]. In the pro-conflict and seizure behavioural paradigm in rodents, the pure inverse agonist, Ro 5-4864 devoid of any anxiolytic activity, has been demonstrated to display intrinsic anxiogenic activity which can be antagonized by PK 11195 [13]. Interestingly enough, the prototypal pBR antagonist, PK 11195 has been shown to antagonize the behavioural effects of the pBR inverse agonist such as Ro 5–4864, as well as the action of pBR agonists and cBR agonists eg lorazapam [12]. The therapeutic efficacy of flumazenil in reversing the toxicity of commonly prescribed benzodiazepines in overdose, is thought to be related to its antagonism at the cBR site.

Recently, a series clinical studies provide empirical evidence in support of the hypothesis that dysregulation of pBR plays a pivotal role in anxiety disorder. The density of pBR in platelets as labelled by the pBR antagonist, PK 11195, has been found to be decreased in both generalized anxiety disorder and social phobia [6, 9]. Furthermore, in the lymphocyte model, both the pBR and mRNA pBR were significantly decreased in medication naive patients diagnosed as generalized anxiety disorder, suggesting that the reduced turnover of pBR occurs at the transcriptional level [9]. However, none of the previous studies have been conducted to examine possible changes in the binding characteristics of the *inverse agonist ligand*, Ro 5–4864 in generalized anxiety disorder. The pharmacological profile of the pBR inverse agonist, Ro 5-4864 distinguishes from that of the pBR antagonist, PK 11195 in binding domains and differential mode of regulation by GABA. At the molecular level, Farges et al. [5] reported that the recombinant human and bovine pBR produced in pBR -lacking yeast, can be labelled with [^{3}H]PK 11195, but only the human pBR can be labelled with [^{3}H]Ro 5-4864. Analysis of human-bovine chimaeric receptors revealed that the region near the C-terminal end of pBR, with only five non-conserved amino acids between human and bovine sequences, possesses essential determinants accounting for differences in high-affinity binding of Ro 5-4864 to the two receptor subtypes.

In the present study, we investigated the possible changes in the binding of the inverse agonist, Ro 5-4864, in platelets from patients diagnosed as generalized anxiety disorder. In the model of dysregulation of pBR in anxiety, we hypothesize that: 1) the dysregulated system of pBR will be manifested in alteration in the receptor density and 2) the changes in the binding profile of Ro 5–4864 parallel the severity of global anxiety syndrome, in anxious subjects.

Materials and methods

The patients selected for the present study were diagnosed as generalized anxiety disorder according to the criteria of Diagnostic and Statistical Manual (DSM IV) [4]. The Anxiety was measured with the Hamilton Anxiety Rating Scale (HAR-S) [8] with scores of 14 or greater. Their ages ranged from 28 to 46 years of both sexes. None of the patients has had any lifetime or current history of substance abuse, episodes of psychosis, cognitive deficits suggestive of organic brain syndrome or any concurrent primary or secondary categorical psychiatric diagnosis. In addition, their medical history was non-contributory. They did not use any benzodiazepines, analgesics, antibiotics or over-the-counter drugs for more than two weeks prior to the study. Caffeine abusers were excluded from the study. They were required to sign informed consent for the clinical study, and were made to understand that the data obtained would be treated in a strictly confidential manner and anonymity would be vigorously preserved. The protocol of the study was approved by the Ethics Committee of the hospital concerned. The anxious subjects were recruited from the community mental health clinics affiliated with the University. No monetary reinforcement was utilized for recruiting the subjects into the study. The patients were assessed and received regular psychiatric treatment from an experienced research psychiatrist for various time periods. The anxious subjects were medication naive upon entry to the study.

As the experimental control, human subjects were recruited from the hospital and university community and had negative psychiatric history and non-contributory medical history. They were informed as to the protocol of the study and required to refrain from any oral contraceptives, over-the-counter medications and alcohol for at least 48 hours prior to the study.

The blood was taken by venipunctures from the forearm during the day and platelets were isolated and assayed for [^{3}H]RO-5-4864 on the same day. The optimal conditions for pBR assay with the ligand [^{3}H]RO-5-4864 were established in our laboratory prior to the start of the study. Close monitoring of inter-assay variations was maintained throughout the study period. All the platelet samples from the anxious and control subjects were included in the results and no blood sample was discarded.

The subjects for the study included 20 individuals with generalized anxiety disorder and 14 normal controls. All the subjects were of Caucasian descent. The gender ratio (male/female) was comparable for the anxious and control groups: 6/8 for the control group and 9/11 for the anxious group. For the anxious group, the mean age was 34 ± 2 years and for the control group, the mean age was 36 ± 3 years. The anxious subjects had mean total HAR-S scores 34 ± 1 with range from 27 to 40. All the control subjects upon psychiatric screening did not have any score on the HAR-S.

Platelet isolation and preparation

The method for platelet membrane preparation was adopted from Moingeon et al. [14]. The blood was collected in venipunctures glass tubes containing 2.2 % sodium citrate, 1.2 % citric acid and heparin in the ratio of 8.5 parts (by volume) to 1.5 parts of blood. The platelet count was performed with the automated Coulter counter.

Benzodiazepine receptor binding assay

[^{3}H-N-methyl]RO-5-4864 (specific activity 74.9 Ci/mmol) was purchased from New England Nuclear, Montreal, P.Q,Canada. β-carboline, clonazepam, flunitrazepam, nitrazepam, diazepam and chlordiazepoxide were obtained as gifts from Hoffmann-La Roche Lab.

For the receptor binding assay, the method described by Moingeon et al. [14] was adopted. In all the binding assays, the specific binding of [^{3}H]RO-5-4864 was defined as the difference in total binding and nonspecific binding in the presence of 10 μM of diazepam.

Data analysis

The binding data were analysed by Scatchard plot using the Prism computer software program to determine the line of best fit from which the binding parameters, Bmax and KD were derived. The dif-

ference in pBR binding parameters (Kd and Bmax) between the control and the anxious subjects was analysed with the unpaired two-tailed Student's t-test. Linear regression analysis was used to determine the relationship between global anxiety symptom as measured by the HAR-S total score, the Psychic and Somatic components of the global anxiety syndrome, and the pBR density. For both the Student's t-test and the Pearson's correlation coefficient, the level of significance was set to be at the 0.05 level.

Results

The data indicated that [^{3}H]RO-5-4864 displays saturable and specific binding in intact human platelets. Scatchard analysis showed that [^{3}H]RO-5-4864 binding to the blood platelets is best described as a single class of noninteracting binding sides with high affinity to the putative benzodiazepine receptor sides (Bmax = 183.5 fmol/10^6 cells, Kd = 15.48 nM) (Table 1). The results of our present study on intact platelets compare favourably with those reported by Moingeon et al. [14] on membrane preparations of platelets.

In the patients diagnosed as generalized anxiety disorder, the cluster of the anxiety symptoms were ranked with the Hamilton Anxiety Rating Scale (HAR-S) and the mean global HARS scale was tabulated in Table 1. Blood platelets obtained from them demonstrated a significant ($p < 0.001$) increase in the density of [^{3}H]RO-5-4864 binding sites with no apparent change in the affinity of [^{3}H]RO–5–4864 for the putative benzodiazepine receptor, when compared with normal human subjects with no previous history of any psychiatric disorder. In the anxious group, the relative enhancement in benzodiazepine receptor occupancy most likely is associated with their pathological anxiety state, since none of the patients exhibited rebound insomnia, behavioural excitation, psychomotor agitation or depressive symptoms. In the control group, the basal level of pBR receptor density in the *absence* of any demonstrable anxiety symptoms, probably indicates optimal occupancy by endogenous pBR ligands to maintain homeostasis in brain-behaviour relationship.

The overall symptom severity of generalized anxiety disorder (total HAR-S score) is positively correlated with changes in peripheral benzodiazepine receptor, as shown in Figure 1 by the linear relationship between the global scores of the HAR-S and the absolute increase in the receptor density (Bmax) as compared to the control ($r = 0.86$). The relationship reached a high level of statistical significance ($p < 0.001$).

We further analysed the 4-item global HAR-S score changes into two principal components:1)6-item Psychic component; 2) 8-item Somatic component. The 6-item Psychic component consisted of anxious mood, tension, fears, insomnia, intellectual cognitive change and depressed mood. The 8-item Somatic component comprised the following symptoms: somatic muscular symptom, sensory symptom, cardiovascular symptom, respiratory symptoms, gastrointestinal symptoms, genital-urinary symptoms, autonomic symptoms and behaviour at interview. Interestingly enough, none of the anxious subjects displayed any depressed mood. Linear regression analysis demonstrated that for patients diagnosed as generalized anxiety disorder, a linear relationship between the changes of the Psychic component score and the changes of Bmax of the peripheral benzodiazepine binding reached statistical significance ($r = 0.78$, $p < 0.001$). (Fig. 2) On the other hand, no statistical significant relationship was found between the Somatic component score and the changes of Bmax of peripheral benzodiazepine binding ($r = 0.12$, $p = 0.66$). (Fig. 3). Given the small sample size, no further statistical analysis was conducted to examine whether any gender-specific changes in pBR density occurs in male vs female anxious subjects when compared with sex-matched controls.

Discussion

The major findings of our study are as follows: 1) In generalized anxiety disorder, there was a significant increase in the density of inverse agonist binding of Ro 5-4864, to peripheral benzodiazepine binding (pBR) in

Table 1 Platelet benzodiazepine receptors in anxious patients

	Control (N = 14)	Patients with generalized anxiety disorder (N =20)
Age	34 ± 2 years	36 ± 3 years
Sex (male/female)	6/8	9/11
Hamilton anxiety rating scale (HAR-S)	–	34 ± 1**
[^{3}]Ro-5-4964 binding: *Receptor Density (B_{max}:fmoles/10^6 cells)	53.4 ± 11.1	202.6 ± 37.9***
Binding Affinity (K_D = nM)	8.1 ± 3.2	10.5 ± 2.2

* Mean ± S. E. M.
** Mean score ± S. E. M.
*** $P < 0.001$ (unpaired Two-tailed Student's T-test, as compared with controls)

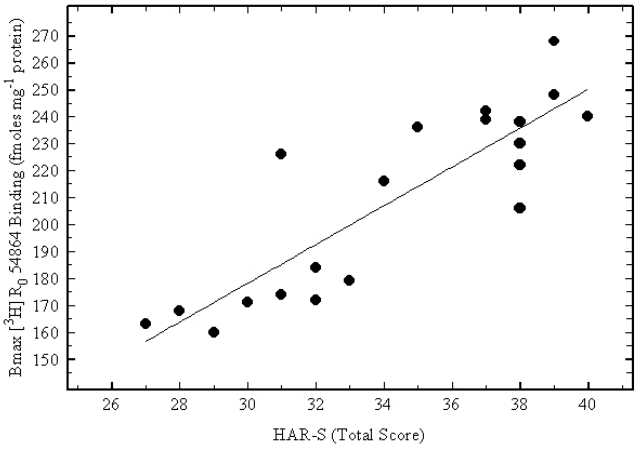

Fig. 1 Total HAR-S score vs pBR [^{3}H] RO-5-4864 binding

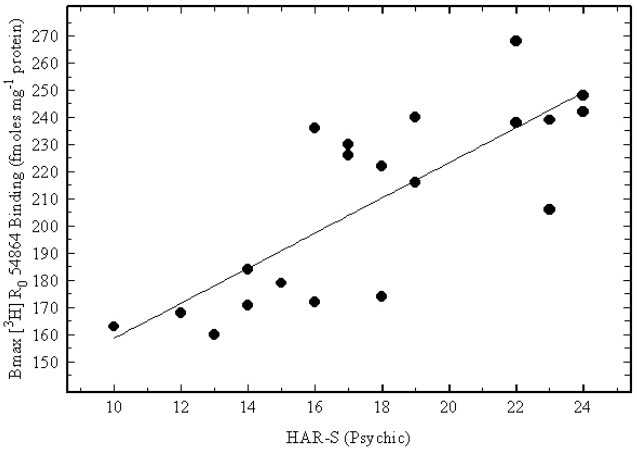

Fig. 2 HAR-S (Psychic Component) vs pBR [^{3}H] RO-5-4864 binding

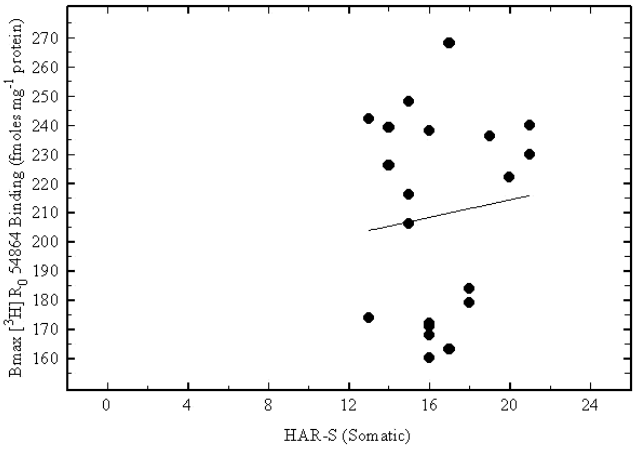

Fig. 3 HAR-S (Somatic Component) vs pBR [^{3}H] RO-5-4864 binding

platelets: 2) the change in the binding density correlated positively with the global severity of anxiety symptom cluster and the psychic component of the anxiety syndrome. Earlier studies with the pBR antagonist, PK-11195 have shown *reduced* receptor density of pBR in generalized anxiety and social phobia [6, 9, 19]. The decline in Bmax of pBR with the pBR antagonist, PK-11195, is consistent with the construct of down-regulation of pBR in anxiety disorder. The *increase* in the pBR binding with the inverse agonist, pBR may also be explained in the context of *up-regulation* of pBR. The reciprocal changes of inverse agonist binding and antagonist binding in generalized anxiety disorder is likely to reflect the differential pharmacological activities and functional and structural requirements of the binding domains of the two subtypes of pBR may determine via complex mechanisms Regardless of the molecular mechanisms, our data, when considered with previous findings by other investigators [6, 9, 19], provide empirical evidence to support the model of dysregulation of pBR in generalized anxiety disorder: pBR *up-regulation* with the *inverse agonist binding* of pBR and pBR *down-regulation* with the *antagonist binding* to pBR Since inverse agonists and antagonists bind to different sites or subtypes of pBR, the empirical findings of opposite changes in inverse agonist binding and antagonist binding in anxiety are explicable in the context of dysregulation of pBR.

The robust finding that pBR changes correlate selectively to the Psychic, but not to the Somatic components of the generalized anxiety syndrome emphasizes the multiplicity and complexity of signalling pathways mediating pBR occcupancy and activation. Apparently the affective overlay of the anxiety syndrome is more closely associated with pBR interaction, whereas the Somatic manifestations of generalized anxiety disorder are driven by cascade of neurochemical events distal to the initial pBR activation. This explains why the observed relationship between the Somatic component of HAR-S and pBR binding is extremely poor.

Taken together, the present results raise the question as to delineate the specificity of the neurobiological mechanisms underlying the dysregulation of pBR in anxiety disorder. Putative endogenous inverse agonist ligands for pBR have been identified in the human brain: the polypeptide diazepam binding protein, benzodiazepine-like compounds and prophyrins (1,15). It may be speculated that the enhancement in the observed inverse agonist binding of pBR may be due to an increase in the turnover and expression of the endogenous ligand. It remains to define the nature of the aberration of the signaling pathway linking pBR and cBR in anxiety disorder. In this respect, *in vivo* neuroimaging affords a highly promising approach in unraveling the mechanisms involved. specific imaging ligands of cBR are available for SPECT (Single photon emission tomography). A recent study by Tilhonen et al. [22] using [^{123}I]-NNC 13-8241 demonstrated that in generalized anxiety disorder, subjects had decreased binding of cBR in the left temporal pole of the cerebral cortex. However, no SPECT radioligands have yet been developed for probing the kinetics of pBR binding in the astro-glial cells of the mammalian central nervous system

Perhaps the missing link may be found in the novel class of *neuroactive steroid*s. pBR has been shown to play a significant role in regulating the biosynthesis of steroids not only in the peripheral tissues, but in the nervous system (central and peripheral) [10]. There is ample evidence in support of the putative role of neurosteroids synthesized in the central nervous system as modulators of synaptic activity. In general, two classes of neurosteroids are recognized: 1) the group exerting direct effect on neuronal excitability as represented by Allo-3-α-5-α-tetrahydroprogesterone (ALLO) and DHEA (dehydroepiandrosterone); 2) the group interacting with gene expression and modulating neuronal plasticity eg 5α-dihydroprogesterone (DHP) [7]. The prototypal neurosteroid ALLO has shown to amplify the GABA gating of chloride channels in the mammalian brain. We propose a heuristic model of stress supersensitivity for anxiety disorder. The model postulates that when environmental stimuli reaches the presumably ge-

netic determined threshold of adaptive coping in individuals, different subsets of pBR are activated and occupied by various endogenous ligands. The enhanced turnover of pBR will stimulate the biosynthesis of neurosteroids which bind preferentially to GABAa-cBR-chloride ionophore complex in the brain in a dose-dependent and region specific manner. Conceivably, repetitive cumulative stressors convert the adaptive stress response to maladaptive behavioral repertoire characterized as the generalized anxiety syndrome. Hence the generalized anxiety disorder can be considered as the pathological paradigm of stress supersensitivity. In all likelihood the perturbations in dysregulated benzodiazepine receptor system contribute significantly to stress supersensitivity. Whether anxiolytic agents reverse or interfere with the cascade events remains to be investigated. There is preliminary evidence showing that the the anxiolytic action of serotonin reuptake inhibitor, fluoxetine, is related to the increase in the content of ALLO in the cerebrospinal fluid [7].

The significance of the correlation of pBR with the severity of anxiety symptom is uncertain, but the changes of the inverse agonist/antagonist binding of pBR merit more serious consideration as a putative biochemical marker for anxiety state. Both alcohol and cocaine are well known to induce anxiety syndrome and hence their behavioural effects may explain the reported increase in Ro 5-4864 binding in cocaine and alcohol abusers [2, 21].

Conclusion

In summary, our results with the inverse agonist binding of pBR in generalized anxiety extend earlier studies with the antagonist binding in support of the hypothesis of dysregulation of pBR in anxiety disorder. It remains to clarify further the various components of the dysregulated pBR system in anxiety disorder: 1) the relationship of inverse and antagonist pBR binding; 2) the specificity of the disturbances in the pBR-neurosteroid-cBR-chloride ionophore axis, in anxiety disorder. Tissue culture, and in vivo human and animal paradigms will be productive areas of research in the future.

Acknowledgments The study was financially supported by the Medical Research Council of Canada. The expert editorial assistance and skillful graphic design of Ms. Liz Goble, BA, and the expert statistical data analysis of Larry Lalone, MA, in the preparation of the manuscript were thankfully appreciated.

References

1. Alho H, Varga V, Krueger KE (1994) Expression of mitochondrial benzodiazepine receptor and its putative endogenous ligand diazepam binding inhibitor in cultured primary astrocytes and C-6 cells: Relation to cell growth. Cell Growth Differ 5:1005–1014
2. Chesley SF, Schatzki AD, DeVrrutia J, Greenblat DJ, Shader RI, Miller LG (1990) Cocaine augments peripheral benzodiazepine binding in humans. J Clin Psychiatry 5:10–12
3. Costa E (1998) From GABA receptor diversity emerges a unified vision of GABAergic inhibition. Ann. Rev. of Phamacol Toxicol 38:321–350
4. Diagnostic and Statistical Manual (DSM IV). 4th Edt. (1994) Am. Psychiat. Associat. USA, APA Press
5. Farges, R, Joseph-Liauzun E, Shire D, Caput D, LeFur G, Loison G, Ferrara P (1993) Molecular basis for the different binding properties of benzodiazepines to human and bovine peripheral-type benzodiazepine receptors. Fed Europ Biochem Societ 335(3): 305–308
6. Ferrarese C, Appolonio I, Frigo M, et al. (1990) Decreased density of benzodiazepine receptors in lymphocytes of anxious subjects: Reversal after chronic diazepam treatment. Acta Psychiat Scand 82:169–173
7. Guidotti A, Costa E (1998) Can the antidysphoric and anxiolytic profiles of selective serotonin reuptake inhibitors be related to their ability to increase brain 3%–5% Tetrahydroprogesterone (Allo prognanolone) availability? J Biol Psychiat 44:865–873
8. Hamilton M (1959) The assessment of anxiety by rating. Brit J Med Psy 32:50–62
9. Johonson M, Marazziti D, Brawman-Mintzer O, Emmanuel N, Ware MR, Morton WA, Rossi A, Carsano GB, Lydiard RB (1998) Abnormal peripheral benzodiazepine receptor density associated with generalized social phobia. Biol Psychiat 43:306–309
10. Krueger KE, Papadopoulos V (1992) Mitochondrial benzodiazepine receptors and the regulation of steroid biosynthesis. Ann Rev Pharmacol Toxicol 32:211–237
11. Malizia AL, Coupland NJ, Nutt DJ (1995) GABAa receptors and anxiety: from Neurobiology to treatment. Biggio G, Sanna E, Costa E (eds) New York, Raven Press
12. Miller LG, Galpern WR, Byrnes J, Greenblat DJ and Shader R (1992) Chronic benzodiazepine administration. Concurrent administration of the peripheral-type benzodiazepine ligand PK11195 attenuates chronic effects of lorazepam. J Pharmacol Exp Therap 261(1):285–289
13. Mizoule J, Gauthier A, Uzan A (1985) Opposite effects of two ligand for the peripheral-type benzodiazepine binding sites PK 11195 and Ro 5-4864 in a conflict situation in the rat. Life Sci 36:1059–1068
14. Moingeon P, Dessaux JJ, Fellow R, Alberici GF, Bidhart JM, Motte PH, Bohvon C (1984) Benzodiazepine receptors on human blood platelets. Life Sci 35:2003–2009
15. Papadopoulos V (1993) Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: Biological role in steroidogenic cell function. Endocr Review 14:222–240
16. Parola AL, Stump DG, Pepperi DJ, Krueger KE, Regan JW, Laird HE II (1991) Cloning and expression of a pharmacologically unique bovine peripheral-type benzodiazepine receptor isoquinoline binding protein. J Biol Chem 266:14082–14087
17. Parola AL, Yamamura HI (1993) Molecular properties of mitochondrial benzodiazepine receptors. E. Gieser-Crouse (ed) Peripheral Benzodiazepine Receptors. London, Academic Press
18. Riond J, Mattei MG, Kaghad M, Dumont X, Guillemot JC, LeFur G, et al. (1991) Molecular cloning and chromosomal localization of a human peripheral-type benzodiazepine receptor. Eur J Biochem 195:305–311
19. Rocca P, Beoni AM, Eva C, et al. (1998) Peripheral benzodiazepine receptor messenger RNA is decreased in lymphocytes of generalized anxiety disorder patients. Biol Psychiat 43:767–773
20. Sprengel R, Werner P, Seeburg PH, Mukhin AG, Santi MR, Grayson DR, et al. (1989) Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. J Biol Chem 264:20414–20421
21. Suranyl-Cadotte R, Rafaille F, Dongier M, Dumas M, Quirion R (1988) Decreased density of peripheral benzodiazepine binding sites on platelets of currently drinking but not abstinent alcoholics. Neuropharmacol 44:443–444
22. Tiihonen J, Kuikka J, Rasanen P, Lepola U, et al. (1997) Cerebral benzodiazepine receptor binding and distribution in generalized anxiety disorder: A fractal analysis. Molecular Psychiat 2(6):463–471
23. Verma A, Snyder SH (1989) Peripheral-type benzodiazepine receptors. Ann Rev Pharacol Toxicol 29:307