ELSEVIER

European Journal of Pharmacology 321 (1997) 13–17

ejp

Short communication

In vivo modulation of benzodiazepine receptor function after inhibition of endogenous γ-aminobutyric acid synthesis

Michel Bottlaender [b,*], Lorenz Schmid [b], Chantal Fuseau [a], Denis Fournier [a], Emmanuel Brouillet [b], Mariannick Mazière [b]

[a] *CEA, DRM, DSV, Service Hospitalier Frédéric Joliot, 4, Place du Général Leclerc, 91400 Orsay Cedex, France*
[b] *URA CNRS 2210, Service Hospitalier Frédéric Joliot, 4, Place du Général Leclerc, 91400 Orsay Cedex, France*

Received 11 November 1996; accepted 24 December 1996

Abstract

The influence of decreased endogenous γ-aminobutyric acid (GABA) concentration on benzodiazepine receptor function was studied in the brain of living baboons. Positron emission tomography and the radiotracer [^{11}C]flumazenil combined with electroencephalography were used to determine the pharmacological properties of two benzodiazepine receptors agonists, diazepam and bretazenil, in baboons pre-treated or not with DL-allylglycine (an inhibitor of GABA synthesis). Our results show that, in vivo, DL-allylglycine reduces the affinity of benzodiazepine receptors for their agonists without altering the intrinsic capability of agonists to allosterically modulate GABAergic transmission.

Keywords: GABA (γ-aminobutyric acid); Benzodiazepine receptor, in vivo; Efficacy, intrinsic; (Non-human primate); DL-Allylglycine; Brain

1. Introduction

The $GABA_A$ receptor is a multimeric protein complex which, in addition to binding γ-aminobutyric acid (GABA), displays several allosteric modulatory binding sites including the benzodiazepine receptor (for review, see Sieghart, 1992). Although GABA and benzodiazepine binding sites are located on different subunits (Sigel et al., 1990), they are functionally coupled one to another. Benzodiazepine receptor ligands exert their biological activity by modulating GABAergic transmission. The type and the magnitude of the modulation are specific features of the ligand and have been referred to as its intrinsic efficacy (Haefely and Polc, 1986). In vitro, the binding of a benzodiazepine receptor agonist allosterically increases the binding of GABA, while GABA has been shown to alter binding properties of benzodiazepine receptor (Olsen, 1982; Sieghart, 1995). In ex vivo binding studies, however, the results are less clear and depending on the tracer and the method used to modify GABA concentration, GABA may or may not modulate the binding of benzodiazepine receptor ligands (for review see Miller et al., 1988). Thus far, no data are available concerning such modulation in vivo, its consequences on benzodiazepine activity and the putative modifications of the intrinsic efficacy of benzodiazepine receptor ligands which is the ability of the agonist to activate the benzodiazepine receptor.

Positron emission tomography (PET) using [^{11}C]flumazenil is an appropriate tool to address these questions. This technique allows the non-invasive study of the molecular mechanisms of benzodiazepine receptors both in non-human primate (Brouillet et al., 1991) and in humans (Samson et al., 1987). We recently showed that PET in combination with electroencephalographic (EEG) recording can be used to measure in vivo pharmacological characteristics of benzodiazepine receptor ligands, in particular their relative affinity (determined by their potency to displace the radiotracer) and their in vivo intrinsic efficacy (Bottlaender et al., 1994; Brouillet et al., 1991).

The main objective of the present PET and EEG study was to investigate in vivo the effect of a GABA synthesis inhibitor on the activity of benzodiazepine receptor agonists. We determined the intrinsic efficacy and potency of two benzodiazepine receptor agonists: a full agonist, diazepam, and a partial agonist, bretazenil after treatment with DL-allylglycine which is an inhibitor of the primary enzyme for GABA synthesis, glutamic acid decarboxylase (EC 4.1.1.15) (Horton et al., 1978).

* Corresponding author. Tel.: (33-1) 6986-7712; Fax: (33-1) 6986-7768; e-mail: mbottlaender@cea.fr

0014-2999/97/$17.00
PII S0014-2999(97)00004-6

2. Material and methods

2.1. Biological model

PET experiments were carried out on 5 male *Papio papio* baboons weighting 19.3 ± 2.4 kg (mean ± S.D.). Each animal was allowed a rest period of at least 4 weeks between PET studies. All animal procedures were in strict accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (Décret 87/848) for the care and use of laboratory animals.

2.2. Drugs

[^{11}C]Flumazenil ([^{11}C]Ro15-1788; ethyl 8-fluoro-5,6-dihydro-5-[^{11}C]methyl-6-oxo-4*H*-imidazo[1,5-*a*] [1,4]benzodiazepine-3-carboxylate) was synthesised in our laboratory as described previously (Mazière et al., 1984). Diazepam (galenic preparation, Hoffmann-La Roche, Basel, Switzerland) and bretazenil (dissolved in propyleneglycol and diluted in saline) were injected intravenously. DL-Allylglycine (210 mg/kg) (2-keto-4-pentenoic acid; Sigma, St. Louis, MO, USA) was diluted in 30 ml of saline, and injected intravenously. In *Papio papio* baboons, DL-allylglycine administration was shown to induce a proconvulsant effect associated with a reduction of the GABA concentration in the cerebrospinal fluid (Chavoix et al., 1991; Valin et al., 1991). For the pharmacological convulsant test 140 mg/kg pentylenetetrazol (Richard Laboratory, Sauzet, France), dissolved in 10 ml, was intravenously infused.

2.3. PET procedures

All experimental procedures used in this work (except DL-allylglycine treatment) were carried out according to previously published procedures (Bottlaender et al., 1994; Brouillet et al., 1991). The in vivo [^{11}C]flumazenil brain kinetics studies were performed with a time-of-flight TTV03 (LETI, Grenoble, France) positron tomograph. DL-Allylglycine (210 mg/kg) was administered i.v. 4 h before the PET experiment. At this dose, DL-allylglycine has been shown to decrease GABAergic transmission 4 h after its administration in *Papio papio* baboons (Chavoix et al., 1991; Valin et al., 1991). After the animal was anaesthetised (ketamine 5 mg/kg, i.m.), subcutaneous electrodes were placed on the baboon's skull and the head was positioned inside the positron camera using the orbitomeatal line as anatomical reference and the EEG recording was started. The PET study began, at time zero (T_0), with the intravenous injection of [^{11}C]flumazenil (14.9 ± 2.4 mCi, i.e., 552 ± 97 MBq, equivalent to 22.7 ± 9.9 nmol, i.e., 6.8 ± 3 μg). [^{11}C]Flumazenil kinetics were determined on several cortical regions and expressed as percent of the injected dose per 100 ml of tissue (%ID/100 ml). Three control studies and three full saturation studies were performed to determine the regional specific binding of the [^{11}C]flumazenil in treated and untreated animals. Displacement studies were performed by intravenous injection of the unlabeled agonist at T_{0+20}, to provoke a competitive displacement of the [^{11}C]flumazenil from its specific binding sites. The [^{11}C]flumazenil displacement, representing the fractional benzodiazepine receptor occupancy by a given agonist, was evaluated between T_{0+38} and T_{0+40}. Several displacement experiments were carried out in DL-allylglycine-treated animals and in untreated animals using increasing doses of diazepam (0.23; 0.5; 1; 1.8 and 0.1; 0.25; 0.5; 1; 2 mg/kg, respectively) or bretazenil (0.005; 0.01; 0.015; 0.02; 0.03; 0.8 and 0.002; 0.005; 0.02; 0.03; 0.2; 1 mg/kg, respectively).

At T_{0+40} a pharmacological test was begun to measure the anticonvulsant activity of the unlabeled ligands. The test consisted of a slow intravenous infusion of pentylenetetrazol (20 mg/kg per min) which was stopped at the EEG seizure onset. At this time, the pentylenetetrazol administered did not interfere with the pharmacological status of the animal since the only alterations it causes are rather the consequence of the induced seizure (Hantraye et al., 1987). The total dose (mg/kg) of infused pentylenetetrazol needed to trigger paroxysmal activity was determined. The increased convulsant threshold dose of pentylenetetrazol after ligand injection indicated the magnitude of the ligand's anticonvulsant effect and its capability to increase GABAergic transmission (Brouillet et al., 1991). As discussed previously (Bottlaender et al., 1994; Brouillet et al., 1991), the relationship between the estimated fractional receptor occupancy and the simultaneously measured pharmacological effect gives an index of the intrinsic efficacy of the ligand. Absolute values of the convulsant threshold dose of pentylenetetrazol can be affected slightly by anaesthesia. However, the increase in this threshold dose is calculated relative to the appropriate control animal (i.e., no diazepam or bretazenil injection and equal anaesthetic dose) and is likely to be independent of our anaesthetic procedure.

The log dose-displacement curves were fitted using a four-parameter, non-linear, least-square analysis. ID_{50} and 'in vivo Hill coefficients' were calculated after linearisation of the dose-displacement curves using a logistic model.

Results are expressed as mean ± S.D. Statistical methods include linear regression analysis and the slopes of the Hill plots were compared to a value of one using a Student *t*-test with $n - 2$ degrees of freedom.

3. Results

The convulsant threshold dose of pentylenetetrazol determined in untreated animals and without administration of any benzodiazepine receptor ligand was 23.3 ± 1.8 mg/kg ($n = 3$) and represents the physiological state of

the GABAergic transmission. After treatment with DL-allylglycine, the dose of pentylenetetrazol needed to trigger an EEG seizure was significantly reduced by almost 50% (12.9 ± 2.8 mg/kg, $n = 3$; $P < 0.01$), indicating a substantial decrease in the efficiency of GABA transmission.

The brain kinetics of [^{11}C]flumazenil, after its i.v. administration, were similar in both experimental conditions. The maximal cerebral uptake, obtained at about T_{0+20} min, was not different (e.g., in frontal cortex: 2.87 ± 0.70 vs. 2.70 ± 0.25% ID/100 ml of tissue in untreated vs. DL-allylglycine-treated animals, respectively).

The administration of diazepam or bretazenil at T_{0+20} initiated a rapid washout of the cerebral radioactivity in all brain regions studied. Increasing doses produced dose-dependent displacements of [^{11}C]flumazenil. In all cortical structures, the sigmoidal dose-displacement curves of diazepam and bretazenil trend to be shifted to the right in DL-allylglycine-treated animals as compared with that obtained in untreated animals (Fig. 1). ID_{50} values of diazepam (mean in cortex = 0.62 ± 0.02 mg/kg) and bretazenil (0.006 ± 0.001 mg/kg) in untreated animals were found to be increased in animals treated with DL-allylglycine (diazepam: 0.84 ± 0.12 mg/kg, $P < 0.005$; bretazenil: 0.012 ± 0.004 mg/kg, $P < 0.005$).

In vivo Hill coefficients, determined as the slope of the Hill curves, were close to unity for both drugs in untreated (1.07 ± 0.05 and 0.90 ± 0.26 for diazepam and bretazenil, respectively), as well as in treated animals (1.15 ± 0.13 and 1.24 ± 0.29, respectively). This indicates uniform binding of diazepam and bretazenil whatever the experimental conditions.

The convulsant threshold dose of pentylenetetrazol also increased with increasing doses of diazepam or bretazenil in both experimental conditions (i.e., with or without DL-allylglycine), indicating a dose-dependent enhanced anticonvulsant effect. For every given dose of agonists, the corresponding pentylenetetrazol threshold dose was always lower in animal treated with DL-allylglycine than in non-treated animals. For example, the pentylenetetrazol threshold dose after administration of 0.5 mg/kg of diazepam was 58 mg/kg in untreated animals and only 40.7 mg/kg in DL-allylglycine-treated animals. Similarly, after administration of 0.02 mg/kg of bretazenil, this threshold dose was 49 and 20 mg/kg respectively.

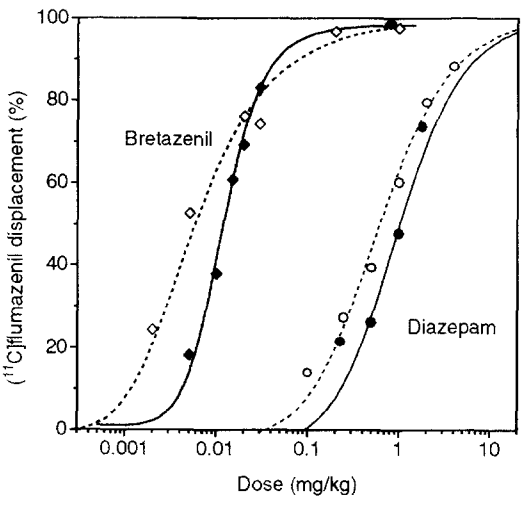

Fig. 1. [^{11}C]Flumazenil displacement curves in occipital cortex 20 min after injection of increasing doses of unlabeled diazepam (circles) and bretazenil (diamonds) in non-treated (open symbols), and DL-allylglycine-treated baboons (filled symbols). Each dose represents a single PET experiment. Lines are obtained after nonlinear least-square fitting of the data. Note that the sigmoidal dose-displacement curves of both agonists are shifted to the right after pre-treatment with DL-allylglycine (solid lines) compared to untreated condition (broken lines).

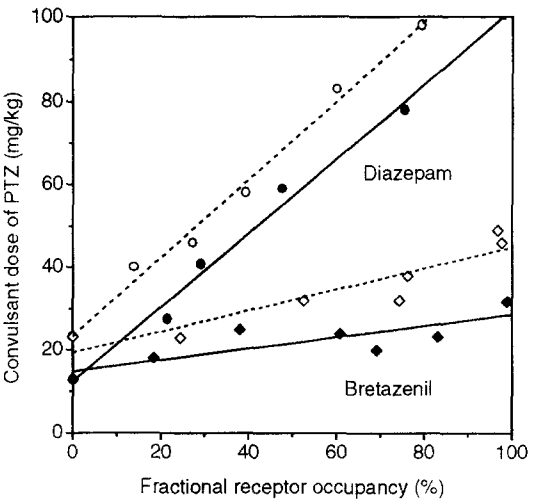

Fig. 2. Pharmacological (anticonvulsant) effect of diazepam and bretazenil as a function of fractional benzodiazepine receptor occupancy estimated in vivo, (broken lines) in non-treated and (solid lines) in DL-allylglycine-treated animals. In non-treated animals (broken lines), the slope of the relationship for diazepam (○, full agonist) is higher than for bretazenil (◇, partial agonist) indicating a stronger positive intrinsic efficacy. Note that after pre-treatment with DL-allylglycine (solid lines), the intrinsic efficacy of both the full agonist (diazepam, ●) and the partial agonist (bretazenil, ◆) is statistically not modified.

Increasing fractional receptor occupancy by diazepam and bretazenil induced an increasing anticonvulsant effect as shown in Fig. 2. The relationship between the benzodiazepine receptor occupancy by the drug and the resulting pharmacological effect reflects the in vivo intrinsic efficacy of the drug (Brouillet et al., 1991). As the least-square regression analysis revealed high coefficients of correlation (see below), the slope of this relationship can be considered as an index of intrinsic efficacy (Fig. 2). In untreated animals, the slope of diazepam (4.05; $r = 0.99$, $P < 0.0001$) was higher than that obtained with bretazenil (1.10: $r = 0.92$, $P < 0.0001$) confirming that in vivo diazepam acts as a full agonist and bretazenil as a partial agonist. In DL-allylglycine-treated animals, the slopes of both drugs (diazepam: 3.84; $r = 0.99$, $P < 0.0001$ and bretazenil: 0.59; $r = 0.83$, $P < 0.005$) were not significantly modified compared to those obtained in untreated animals ($t = 0.62$, df = 7 for diazepam and $t = 1.85$, df = 10 for bretazenil). This indicates that the intrinsic effica-

cies of both the full and the partial agonists are not altered after treatment with DL-allylglycine.

4. Discussion

The GABA and benzodiazepine binding sites are two distinct binding sites within the $GABA_A$ receptor complex and they are functionally coupled. Electrophysiological studies reveal that benzodiazepine receptor agonists facilitate GABA binding. Conversely, in vitro, GABA was shown to facilitate the binding of benzodiazepine receptor agonists. In the present study we show that in vivo DL-allylglycine, which can decrease the endogenous GABA concentration, causes a slight reduction of the benzodiazepine receptor agonists' affinity without altering their intrinsic efficacy. We pre-treated animals with DL-allylglycine, an inhibitor of glutamic acid decarboxylase, the enzyme responsible for the GABA synthesis. The threshold dose of pentylenetetrazol to trigger paroxysmal EEG activity in animals treated with DL-allylglycine is significantly reduced when compared to untreated animals. This is consistent with other studies demonstrating the proconvulsant effect of DL-allylglycine after i.v. administration in baboons (Chavoix et al., 1991; Horton et al., 1978; Valin et al., 1991). This effect was associated with a reduced GABA concentration in the cerebrospinal fluid by inhibition of the glutamic acid decarboxylase activity (Horton et al., 1978; Valin et al., 1991). It is therefore highly probable that the proconvulsant effect observed with DL-allylglycine in our study results from a reduced endogenous GABA concentration.

In control experiments, the time course of cerebral radioactivity was similar in the treated and untreated animals, indicating that [^{11}C]flumazenil brain kinetics are not modified by DL-allylglycine pre-treatment. Since the brain kinetics of [^{11}C]flumazenil depend on its bioavailability for the cerebral tissue and its binding parameters with the benzodiazepine receptors (Delforge et al., 1993), this result has two important implications for our study. First, this indicates that the bioavailability of the radiotracer for the brain tissue is not altered by the treatment with DL-allylglycine and thus, the pharmacological parameters between the two experimental conditions can be compared. The second implication which arises from the similar radiotracer kinetics is that in our study, DL-allylglycine does not alter the interaction of [^{11}C]flumazenil with benzodiazepine receptors. This is consistent with in vitro binding studies showing that binding of benzodiazepine receptor antagonists such as flumazenil is not modified in the presence of GABA (Möhler and Richards, 1981).

In our study, we observed that the dose-displacement curves obtained for both agonists (Fig. 1) tend to be shifted to the right when animals were pre-treated with DL-allylglycine. This was confirmed by the significantly increased ID_{50} for both drugs in these animals when compared to untreated animals. This decreased potency of benzodiazepine receptor agonists is likely due to a decreased benzodiazepine receptor affinity since DL-allylglycine appears to have no interaction with the pharmacokinetics of concurrently administered drugs (see above). This 'GABA shift' suggested in vivo is consistent with in vitro findings showing that GABA increases the affinity of benzodiazepine receptor agonists (Sieghart, 1995). Biochemical studies showed that binding of [^{3}H]flunitrazepam, a benzodiazepine receptor agonist, to brain membranes was stimulated by GABA or GABA mimetics and this stimulation was inhibited by the GABA receptor antagonist bicuculline (Olsen, 1982; Tallman et al., 1978). GABA was also shown to enhance the potency of benzodiazepine receptor agonists for displacing the [^{3}H]flumazenil (Möhler and Richards, 1981). All these studies show that the extent of the shift is directly related to the intrinsic efficacy of the benzodiazepine receptor ligand. The range of GABA concentration variations used in in vitro studies to evidence the GABA shift was 10^3 to 10^4. For ethical reasons, such a huge decrease in the endogenous GABA concentration was not possible in vivo. The conditions used in our study may only reduce GABA by 2- to 10-fold (see Valin et al., 1991) and this may explain why, although the IC_{50} values are significantly increased, the GABA shift is small and does not appear proportional to the intrinsic efficacy of the benzodiazepine receptor ligands.

As expected, in vivo Hill coefficients, calculated for both diazepam and bretazenil in untreated animals, were close to unity indicating that these drugs recognise in a similar manner all subtypes of benzodiazepine receptors. This is consistent with in vitro and previous in vivo data (Brouillet et al., 1991; Haefely et al., 1985). In the treated animals, when endogenous GABA concentration is lowered, the in vivo Hill coefficients obtained for both drugs were still close to unity. This indicates that both studied agonists still bind to all subtypes of benzodiazepine receptors with similar affinity. This suggests that, in vivo, modifications induced by low endogenous GABA concentration occur in the same proportion for all benzodiazepine receptor subtypes.

The slope of the relationship between the fractional benzodiazepine receptor occupancy by a dose of a benzodiazepine receptor agonist and the resulting pharmacological effect, is a reliable index of the intrinsic efficacy of this substance (Bottlaender et al., 1994; Brouillet et al., 1991). As shown in Fig. 2 (solid lines), in untreated animals, diazepam, which is often considered to be a full agonist, possesses a high positive intrinsic efficacy (slope of 4.05) whereas bretazenil, a partial agonist, possesses a low positive intrinsic efficacy (slope of 1.10). After pre-treatment with DL-allylglycine, the slopes of the relationships for both the full agonist and the partial agonist were not modified (Fig. 2; dotted lines). This indicates that the intrinsic efficacy of both agonists is not modified under conditions which may reduce endogenous GABA concen-

tration and thus, that the ability of the benzodiazepine receptor agonists to change the benzodiazepine receptor to an active form is not altered in this condition.

From a pharmacological point of view, our study shows that the anticonvulsant effect of benzodiazepine receptor agonists is reduced in DL-allylglycine-treated animals due to reduced basal rate of the GABAergic transmission and to decreased affinity of benzodiazepine receptor agonists. These results have therapeutic implications for a number of human pathological processes.

In conclusion, our study brings new insight into the mechanisms in vivo of the allosteric coupling between benzodiazepine receptor and GABA binding site. We show, in vivo, a probable reduction of the affinity of benzodiazepine receptors for their agonists in animals under a condition which is known to reduce endogenous GABA concentration and this occurs without altering the intrinsic capability of agonists to modulate GABAergic transmission.

Acknowledgements

We are grateful to Hoffmann-La Roche (Basel, Switzerland) for generously providing flumazenil and *nor*-flumazenil. We wish to thank V. Brulon for his technical assistance, J. Cayla, C. Jouy and all the staff for their outstanding care of the primate colony. The authors are very grateful to Dr. K. Moya for his comments on the manuscript.

References

Bottlaender, M., E. Brouillet, M. Varastet, C. Le Breton, L. Schmid, C. Fuseau, R. Sitbon, C. Crouzel and M. Mazière, 1994, In vivo high intrinsic efficacy of triazolam: a positron emission tomography study in nonhuman primates, J. Neurochem. 62, 1102.

Brouillet, E., C. Chavoix, M. Bottlaender, M. Khalili-Varasteh, P. Hantraye, D. Fournier, R.H. Dodd and M. Mazière, 1991, In vivo bidirectional modulatory effect of benzodiazepine receptor ligands on GABAergic transmission evaluated by positron emission tomography in non-human primate, Brain Res. 557, 167.

Chavoix, C., E. Brouillet, M. Kunimoto, V. De la Sayette, M. Khalili-Varasteh, P. Hantraye, R.H. Dodd, B. Guibert, C. Prenant, R. Naquet and M. Mazière, 1991, Relationships between benzodiazepine receptors, impairment of GABAergic transmission and convulsant activity of β-CCM: a PET study in the baboon *Papio papio*, Epilepsy Res. 8, 1.

Delforge, J., A. Syrota, M. Bottlaender, M. Varastet, C. Loc'h, B. Bendriem, C. Crouzel, E. Brouillet and M. Mazière, 1993, Modeling analysis of [^{11}C]flumazenil kinetics studied by PET: application to a critical study of the equilibrium approaches, J. Cereb. Blood Flow Metab. 13, 454.

Haefely, W. and P. Polc, 1986, Physiology of GABA enhancement by benzodiazepines and barbiturates, in: Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties, eds. R.W. Olsen and J.C. Venter (Alan Liss, New York, NY) p. 97.

Haefely, W., E. Kyburz, M. Gerecke and H. Möhler, 1985, Recent advances in molecular pharmacology of benzodiazepine receptors and in the structure-activity relationships of their agonists and antagonists, Adv. Drug Res. 14, 165.

Hantraye, P., E. Brouillet, B. Guibert, C. Chavoix, H. Fukuda, C. Prenant, M. Crouzel, R. Naquet and M. Mazière, 1987, Pentylenetetrazol-induced seizure is not mediated by benzodiazepine receptors in vivo, Neuropharmacology 26, 1509.

Horton, R.W., A.G. Chapman and B.S. Meldrum, 1978, Regional changes in cerebral GABA concentration and convulsions produced by D and by L-allylglycine, J. Neurochem. 30, 1501.

Mazière, M., P. Hantraye, C. Prenant, J. Sastre and D. Comar, 1984, Synthesis of ethyl 8-fluoro-5,6-dihydro-5-[^{11}C]methyl-6-oxo-4*H*-imidazo[1,5-*a*] [1,4]benzodiazepine-3-carboxylate (Ro 15 1788-[^{11}C]): a specific radioligand for the in vivo study of central benzodiazepine receptors by positron emission tomography, Int. J. Appl. Radiat. Isot. 35, 973.

Miller, L.G., D.J. Greenblatt, J.G. Barnhill, W.R. Summer and R.I. Shader, 1988, 'GABA shift' in vivo: enhancement of benzodiazepine binding in vivo by modulation of endogenous GABA, Eur. J. Pharmacol. 148, 123.

Möhler, H. and J.G. Richards, 1981, Agonist and antagonist benzodiazepine receptor interaction in vitro, Nature 294, 24.

Olsen, R.W. 1982, Drugs interactions at the GABA receptor-ionophore complex, Annu. Rev. Pharmacol. Toxicol. 22, 245.

Samson, Y., J. Bernuau, S. Pappata, C. Chavoix, J. Baron and M. Mazière, 1987, Cerebral uptake of benzodiazepine measured by positron emission tomography in hepatic encephalopathy, New Engl. J. Med. 12, 414.

Sieghart, W. 1992, $GABA_A$ receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites, Trends Pharmacol. Sci. 13, 446.

Sieghart, W., 1995, Structure and pharmacology of γ-aminobutyric $acid_A$ receptors subtypes, Pharmacol. Rev. 47, 181.

Sigel, E., R. Baur, G. Trube, H. Möhler and P. Malherbe, 1990, The effect of subunit composition of rat brain $GABA_A$ receptors on channel function, Neuron 5, 703.

Tallman, J.F., J.W. Thomas and D.W. Gallager, 1978, GABAergic modulation of benzodiazepine binding site sensitivity, Nature 274, 383.

Valin, A., C. Voltz, R. Naquet and K.G. Lloyd, 1991, Effects of pharmacological manipulation on neurotransmitter and other amino acid levels in the CSF of the Senegalese baboon *Papio papio*, Brain Res. 538, 15.