ELSEVIER

European Journal of Pharmacology 281 (1995) 117–122

ejp

Neuroreceptor quantification in vivo by the steady state principle and [^{123}I]iomazenil in rats

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Received 27 October 1994; revised 29 March 1995; accepted 31 March 1995

Abstract

A steady state method for neuroreceptor quantification in vivo in small laboratory animals is described, using [^{123}I]iomazenil as tracer for the benzodiazepine receptor. The method was used for determination of the receptor equilibrium constant for a non-radioactive ligand, flumazenil, in rats and involved measurement of the nonspecific binding of [^{123}I]iomazenil. Thirty-five animals were intravenously infused for 2 h with [^{123}I]iomazenil and flumazenil in different proportions to obtain occupancies of the benzodiazepine receptor from close to 0 to about 99%. The nonspecific binding of iomazenil in brain tissue was calculated by an iterative procedure from the data for the highly blocked animals, and it was found to be 1.04 ml per ml plasma ($n = 6$). The mean cortical K_d of flumazenil was 21 ± 11 nM ($n = 19$). The method is discussed with special reference to the problems of ascertaining steady state and nonspecific binding.

Keywords: Benzodiazepine receptor; Iomazenil; Flumazenil; Steady state; K_d; Partition coefficient

1. Introduction

Previous in vivo studies of the benzodiazepine receptor system in rats have used bolus injection of tracer mixed with different amounts of a cold ligand and sampling of the brain after an arbitrary time, without any attempt to approach equilibrium conditions or to correct for the absence of equilibrium, (Benavides et al., 1988; Innis et al., 1991a,b). In the equilibrium approach, which greatly facilitates quantification of kinetic parameters (Kawai et al., 1991; Lassen, 1992; Carson et al., 1993), steady state receptor occupancy is maintained. The model described by Lassen allows one to measure the equilibrium constant (K_d) for a non-radioactive ligand by using a chemically different radiolabeled ligand that binds to the same receptor population. Our aim was to develop an in vivo steady state method applicable to rats. We determined the equilibrium constant, K_d, of a non-radioactive ligand, flumazenil, by making use of a another radiolabeled benzodiazepine receptor antagonist, [^{123}I]-iomazenil. The method involved determination of the nonspecific binding, λ, of the tracer in grey matter structures.

2. Materials and methods

In the steady state approach the experimentally measured parameter is the volume of distribution, *Vd*, defined as the equilibrium brain : plasma concentration ratio. *Vd* is measured in an unblocked situation *Vd*(0) and when the receptor is partly blocked by the unlabeled ligand *Vd*(*L*). Assuming that tracer and unlabeled ligand bind to the same receptor subtype, *Vd* of the tracer, corrected for nonspecific binding, gives a measure of non-occupied receptor sites, i.e. non-occupied by the cold ligand. This is only true for a high specific activity radiolabeled ligand, as a blockade of more than a few percent of receptors by the tracer will

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SSDI 0014-2999(95)00205-7

induce errors in the *Vd*. The occupancy of the receptor during infusion of flumazenil is denoted $O(L)$. The free fraction of receptors must be $1 - O(L)$ and this is the fraction measured by the radioactive iomazenil as the bound/free ratio relative to the maximal value of the same ratio observed when no unlabeled ligand (flumazenil) is infused so that all receptor sites are available for the tracer (Lassen, 1992).

$$1 - O(L) = \frac{[B/F]_L}{[B/F]_0} = \frac{1/f(Vd(L) - \lambda)}{1/f(Vd(0) - \lambda)} = \frac{Vd(L) - \lambda}{Vd(0) - \lambda} \quad (1)$$

or:

$$O(L) = 1 - \frac{Vd(L) - \lambda}{Vd(0) - \lambda} = \frac{Vd(0) - Vd(L)}{Vd(0) - \lambda} \quad (2)$$

where B is occupied receptors, F free, f unbound protein fraction of tracer, λ the nonspecific binding, subscript L and 0 is referring to blocked and unblocked study respectively.

The Michaelis-Menten relation for flumazenil gives:

$$O(L) = \frac{B(L)}{B_{max}} = \frac{L}{K_d + L} \vee K_d = L\frac{1 - O(L)}{O(L)} \quad (3)$$

where L is the plasma concentration of non-protein bound unlabeled ligand, and B_{max} is the receptor concentration. Inserting the tracer measured $O(L)$ and $1 - O(L)$ from Eqs. (1) and (2) gives:

$$K_d = L\frac{Vd(L) - \lambda}{Vd(0) - Vd(L)} \quad (4)$$

2.1. Material

A total of 41 male Wistar rats in three groups were included in the study. Ten animals were infused for 2 h with [^{123}I]iomazenil (Ro 16-0154; Paul Sherrer Institute, Villigen, Switzerland) for calculating *Vd* unblocked, i.e. with essentially zero occupancy, ($Vd(0)$). Six animals were examined by using an extra-long infusion schedule of 3 or 6 h in order to evaluate if steady state had been reached in the standard 2 h procedure. Nineteen rats were infused, for 2 h, with increasing concentrations of flumazenil (Ro 15-1788; Lanexat) mixed with [^{123}I]iomazenil to achieve occupancies from 10% to 75%, and six animals were infused to achieve almost 100% occupancy (high occupancy group). They received from 0.013 mg flumazenil per h to 2.035 mg flumazenil per h. The *Vd* in the group with 10% to 75% occupancy was termed $Vd(L)$ and *Vd* in the highly blocked group was termed $Vd(H)$.

2.2. Experimental procedure

The rats were anesthetized using halothane 3% for induction and halothane 0.7–1% during the surgical procedure. Catheters were placed in the femoral vein and artery for drug infusion and blood sampling. The wounds were infiltrated by lidocaine and a catheter was placed for lidocaine supplementation. The rats were allowed to wake up resting in a large-bore plastic tube. After 30 min of rest, the infusion was started. All animals were infused at a rate of about 4 MBq [^{123}I]iomazenil per h (specific activity 57–463 GBq/mg). A total of three blood samples were obtained, two for [^{123}I]iomazenil determination, at 1 h before decapitation and immediately before decapitation, and one for flumazenil determination by high pressure liquid chromatography (HPLC) immediately before decapitation. The first blood sample was followed by a transfusion of 2 ml rat blood. After decapitation the brain was rapidly taken out and placed on a ice block for further dissection. Samples from frontal cortex, temporal cortex, occipital cortex, cerebellum and striatum were collected, macroscopic blood vessels were removed, the tissues were weighed and then counted in a gamma counter (Cobra, Canberra Packard, Meriden, CT, USA).

2.3. [^{123}I]Iomazenil determination

1 ml blood samples were drawn in heparin-sodium-fluoride vials and immediately centrifuged at $3000 \times g$ for 10 min. The plasma was pipetted into a counting vial. Octanol extraction was performed by mixing 500 μl plasma with 1000 μl octanol and shaking for 2 min; 500 μl of the octanol phase was then pipetted into another counting vial. The activity in the octanol sample multiplied by 4 represents [^{123}I]iomazenil concentration per milliliter of plasma.

2.4. Flumazenil determination by HPLC

The plasma concentration of flumazenil was determined as described earlier (Videbæk et al., 1993), except that FG 8123 [1-(3-cyclopropyl-1,2,4-oxadiazol-5-yl)-11,12,13,13*a*-tetrahydro-9-oxo-9*H*-imidazo[1,5-*a*]-pyrrolo-[2,1*c*][1,4]benzodiazepine] was used as internal standard and tetrahydrofuran-100 nM potassium phosphate buffer pH 6.5-water (28:25:47 by volume) was used as mobile phase.

2.5. Plasma protein binding of flumazenil

Equilibrium dialysis was performed in a Kontron Diapack model 4000 using a cellulose membrane (Sigma Chemical Co., St. Louis, MO, USA) that retains pro-

Table 1
Results obtained as described in the calculation section, where λ initially was inserted as $Vd(H)$

Tissue	λ (a)	λ (b)	λ (c)	λ (d)	λ (e)	λ (f)	λ (g)
Striatum	1.92	1.16	1.15	1.15	1.15	1.15	1.15
Cerebellum	1.92	1.35	1.46	1.46	1.46	1.46	1.46
Temporal cortex	1.92	0.83	0.82	0.82	0.82	0.82	0.82
Frontal cortex	1.92	0.53	0.75	0.74	0.75	0.74	0.74
Average		0.96	1.05	1.04	1.04	1.04	1.04
Frontal cortex Initial λ = 5	1.92	0.75	0.74	0.75	0.74	0.74	0.74

The iterations were continued until λ values measured in ml plasma per ml brain were constant; this required 1–5 iterations (a–e). In the last row the iterations when λ initially was set to 5 are shown for frontal cortex to show that the initial λ value does not affect the result.

teins of mass weight greater than 12000. Plasma samples from four rats were spiked with [^{3}H]flumazenil (Dupont, NEN Research Products, Boston, MA, USA), and 1.5 ml was dialyzed at 37° C for 24 h against an equal volume of iso-osmol phosphate-buffer (pH 7.4). Then 50-μl samples from both compartments were supplemented with 2.0 ml of Pico-Flour (Packard Instrument, Groningen, Netherlands) and measured in a Packard 1900 Ca scintillation counter (Canberra Packard, Meriden, CT, USA). The data were all corrected for color quench. The free fraction was calculated as the dpm ratio (buffer/plasma).

2.6. Calculations

Nonspecific binding, λ

If the concentration of the non-radioactive ligand is infinitely high, no receptor sites are available for specific binding of radioactive tracer, and the distribution volume of the tracer is then due to nonspecific binding, i.e. λ is Vd when 100% of the benzodiazepine receptors are blocked. Full blockade cannot be achieved experimentally, as even the highest doses of unlabeled flumazenil leave a small fraction of receptors unoccupied. The following iterative procedure was used to correct Vd in the high occupancy group ($Vd(H)$) for the small fraction of remaining free receptors. A brain density of 1 g per ml is used in the whole study (DiResta et al., 1991).

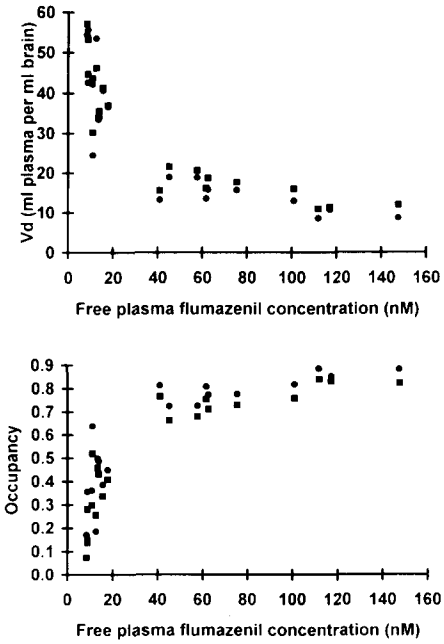

Fig. 1. The distribution volumes areas in ml plasma per ml brain, and the benzodiazepine receptor occupancy, as a function of the free plasma flumazenil concentration, for temporal cortex (●) and frontal cortex (■) in animals examined to measure K_d.

I:λ was initially set to be the mean $Vd(H)$ measured in the six animals.

II: Inserting this λ value, a preliminary value for the occupancy $O(L)$ was calculated in the 19 animals receiving cold flumazenil infusion to achieve 10% to 75% occupancy. In this calculation $Vd(0)$ was taken to be the average Vd for the ten rats studied in the unblocked condition. For animals with thus calculated preliminary occupancies between 25 and 75%, $(O(L)_{25-75\%})$, K_d was calculated for the four different regions: basal ganglia, cerebellum, temporal and frontal cortex. The initial average K_d values were 16, 13, 22, 23 nmol per liter plasma water for the four regions.

III: The still unoccupied percentage of receptors (p) in the high occupancy group was then estimated by rewriting Eq. 3, solving it for the unoccupied fraction:

$$p = 100(1 - O(L)) = 100\left[1 - \frac{L}{K_d + L}\right] \quad (5)$$

IV: p accounts for part of $Vd(H)$. This part must be subtracted from the measured $Vd(H)$ in order to esti-

Table 2
The regional and average K_d values measured in nM for three subgroups of animals

Animal group	K_d striatum	K_d cerebellum	K_d temporal cortex	K_d frontal cortex	K_d average
a (n = 19)	18 ± 13	17 ± 7	26 ± 12	23 ± 14	21
b (n = 10)	18 ± 7	19 ± 7	25 ± 5	20 ± 4	21
c (n = 9)	16 ± 8	15 ± 7	28 ± 16	27 ± 14	21

Group a: all animals; group b: animals with free flumazenil plasma concentrations higher than 20 nM; and group c: animals with receptor blockade between 25% and 75%. Values are means ± S.D.

Table 3
Brain: blood partition coefficients for lipophilic substances

Substance	Partition coefficient
Chloroform	1.1
Ethylene	1.2
Ethyl ether	1.14
[^{123}I]Iomazenil	1.04
Krypton	1.1
Nitrogen	1.1
Nitrous oxide	1.0
Xenon	1.15

mate Vd in the situation of 100% blockade, λ. Vd for 1 percent of free receptors ($Vd(1\%)$) can be calculated for the partly blocked group as:

$$Vd(1\%) = \frac{1}{100} \cdot \frac{Vd(L) - \lambda}{1 - O(L)_{25-75}} \quad (6)$$

V: The estimated percentage of still unbound receptors in the high occupancy animals is then termed $p \cdot Vd(1\%)$. Subtracting this value from the measured $Vd(H)$ gives a new and more correct λ. The algorithm is iterated using the mean value of the new λ just calculated. After a few iterations the mean λ did not change anymore and iteration could be stopped. The calculations were performed regionally and the obtained λ values after each iteration are shown in Table 1.

The calculations could have started with any λ, but if we started presuming that λ was 5, more iterations were needed in the calculations but the final result was exactly the same.

Subgroups of animals

As the HPLC method had a standard deviation above 20% when the free plasma concentration was below 20 nM, all results were calculated for all animals ($n = 19$, subgroup a) and for animals with flumazenil concentrations above 20 nM ($n = 10$, subgroup b). Furthermore the results were calculated for animals with an occupancy between 25 and 75%, as in this interval the occupancy versus the plasma concentration curve was steepest ($n = 9$, subgroup c).

3. Results

There was no statistically significant difference in $Vd(0)$ for the infusion schedules of 2, 3 or 6 h ($n = 6$), indicating that a 2-h infusion in rats was sufficient to reach, practically, the equilibrium state for iomazenil. The plasma level of flumazenil was unchanged from 1 to 2 h after initiation of the infusion (Wilcoxon test $P > 0.05$). The nonprotein bound fraction of flumazenil averaged 0.61 ± 0.01 ($n = 4$). The 19 animals studied with increasing degree of benzodiazepine receptor blockade to determine K_d had flumazenil plasma levels between 10 nmol/l plasma water and 147 nmol/l plasma water. The group of six animals examined to measure $Vd(H)$, and hence λ, had flumazenil concentrations from 928 nmol/l plasma water to 1266 nmol/l plasma water. The regional $Vd(0)$ reflected, as expected, the receptor distribution in the brain and varied from 24 ± 6 ml plasma per ml brain in striatum to 66 ± 10 ml plasma per ml brain in cortex. Fig. 1 shows the distribution volume and occupancy for the two cortical regions as a function of the plasma flumazenil concentration in the partly blocked animals. The $Vd(H)$ varied from 1.6 in striatum to 2.2 ml plasma per ml brain in cortex. When corrected for unblocked receptors, an average λ value of 1.04 ml plasma per ml brain was found for all regions combined (Table 1). The regional calculated K_d for each subgroup of animals is shown in Table 2. There was no difference in K_d between groups, but the standard deviation was higher

Table 4
K_d flumazenil obtained in different studies using different techniques

Study	Method	K_d cerebellum (nM)	K_d cortex (nM)	Reference
Human in vitro	Cortex homogenates	–	5–6	Johnson et al., 1990
	Cortex homogenates	9	6	Kopp et al., 1990
Human in vivo	PET	13	7–16	Abadie et al., 1992
	PET	4–8	3–9	Pappata et al., 1988
	PET	–	10	Persson et al., 1989
	PET	6/15	4/12	Iyo et al., 1991
	PET	–	8-26	Savic et al., 1988
	PET	18	22	Litton et al., 1993
	PET	10	10	Price et al., 1993
	PET	12	12	Lassen et al., 1995
	SPECT	–	10	Videbæk et al., 1993
Rat in vitro (4° C)	Autoradiography	3	4	Mans et al., 1992
Rat in vivo	Tissue counting steady state	17	26	Present study

in animals with a plasma flumazenil concentration below 20 nM. The mean K_d value for all regions and animals, $n = 19$, was 21 ± 11 nM.

4. Discussion

The constant infusion method of Kawai et al. (1991) has in the present study been applied to study the benzodiazepine receptor in rats. We modified the method in two ways. First, we used chemically different ligands, flumazenil and iomazenil, that bind to the same receptor. With this approach all non-radioactive ligands, both benzodiazepines and β-carbolines, that bind to the benzodiazepine receptor can be studied using iomazenil, with the understanding that the measured occupancies and K_d values only pertain to sites binding iomazenil. The second modification consisted of avoiding a programmed infusion, using instead a constant infusion rate throughout the study. More detailed studies are needed to show if a two-step infusion program would change the results significantly, or allow a significantly shorter infusion duration than the 2 h we used and that we validated in the prolonged infusion studies lasting 3 and 6 h.

4.1. [^{123}I]Iomazenil as tracer, for measuring occupancy

The steady state method is easy to perform using continuous infusion of a mixture of tracer and unlabeled ligand. It requires two study sessions, unblocked and blocked. When the studies are performed by tissue counting, we must rely on $Vd(0)$ being almost the same for all animals. In our study the frontal cortex $Vd(0)$ had a mean of 66 ml plasma per ml brain with a standard deviation of 10 ml plasma per ml brain. Inter-individual variations in $Vd(0)$ may be one of the reasons for the variation in K_d measurements. The specific activity of the tracer was reported by the manufacturer to be 57–463 GBq/mg. The maximal chemical amounts of tracer in the brain could be calculated as the maximal counts, about 4 cps per mg tissue divided by 57 GBq/mg and with a counting efficiency of 0.5 (exceptionally low). With a molecular weight of 411, this gives a maximal iomazenil concentration of ca. 0.3 nM. This means that the chemical amount of bound iomazenil is very small compared to the B_{max}, which is above 100 nM (Mans et al., 1992), corresponding to a maximal tracer occupancy of 0.3%.

4.2. Flumazenil as cold ligand

The HPLC method to determine plasma flumazenil had a standard deviation above 20% when the free plasma concentration was below 20 nM. This might imply that K_d determined in animals with a low receptor occupancy (low plasma flumazenil) is less reliable. Excluding the animals with a plasma flumazenil concentration below 20 nM in subgroup b reduced the standard error on K_d by almost 50% to 6 nM, but the average K_d remained 21 nM.

4.3. λ for [^{123}I]iomazenil

A major methodological problem for in vivo receptor studies concerns the value of the nonspecific uptake, λ, relative to the total uptake, Vd. A tracer with a low λ to $Vd(0)$ ratio is preferable. The $Vd(0)$ for [^{123}I]iomazenil was high, above 60 ml plasma per ml brain in cortex and the λ was about 1 ml plasma per ml brain, thus when [^{123}I]iomazenil is used as tracer, the λ problem is negligible, as λ is only about 1–3% of $Vd(0)$ in rats. In human studies the λ error is even smaller, as in the cortex the $Vd(0)$ is higher than 100 ml plasma per ml brain (Videbæk et al., 1993), while λ must be assumed to be practically the same as in rat brain. A λ value close to unity was expected a priori as the gross chemical composition of blood and brain does not differ much, and because λ for several other lipophilic substances are of same order of magnitude, see Table 3. For [^{11}C]flumazenil the $Vd(0)$ of the cortex is about 7 ml plasma per ml brain while λ is 0.8 ml plasma per ml brain (Lassen et al., 1995). This 10-fold higher $\lambda/Vd(0)$ ratio means that flumazenil, in principle, gives less accurate results than iomazenil in in vivo studies.

4.4. K_d for flumazenil

Data on the receptor binding of flumazenil are available from in vitro studies and from in vivo studies using emission tomography as summarized in Table 4. In vitro studies with human cortex (Johnson et al., 1990; Kopp et al., 1990) at 37° C have shown K_d values between 5 and 10 nM. Positron emission tomography (PET) studies in humans, using [^{11}C]flumazenil at pseudoequilibrium, or tracer kinetic modeling (Abadie et al., 1992; Persson et al., 1989; Pappata et al., 1988; Litton et al., 1993; Iyo et al., 1991; Price et al., 1993) have measured K_d values ranging from 4 to 28 nM, with the majority of results around 10 nM. The only PET and single photon emission computer tomography (SPECT) studies performed using the steady state method with bolus tracer injection of [^{11}C]flumazenil and [^{123}I]iomazenil respectively, measured the cortical K_d for flumazenil to be 12 nM (Lassen et al., 1995) and 10 nM (Videbæk et al., 1993). These two studies used a different free fraction of flumazenil, 0.64 vs. 0.52 respectively. Recalculation of the results in accordance with this makes them agree exactly. In rat, in vitro results obtained at 4° C by Mans et al. (1992) indicated the K_d to be 3 nM in striatum, between 3 and 3.6 in frontal and parietal layer VI and 4.7 in frontal and

parietal cortical layer IV. We found the same tendency as Mans et al. (1992), that the K_d in striatum was lower than in cortex, but our mean K_d value was somewhat higher than theirs. The temperature and milieu difference may be at least a part of the generally found tendency that K_d is larger in vivo than in vitro. Johnson et al. (1990) found with human tissue in vitro a 7-fold increase in K_d by elevating the temperature from 0° C to 37° C but Hansen et al. (1991) found only a 3-fold increase elevating the temperature from 4° C to 37° C in rats. We conclude that the steady state method makes it possible to measure K_d in vivo using one tracer and another cold ligand.

Acknowledgements

This work is supported by grants from the Danish Medical Research Council and the Lundbeck Foundation. We thank Dorthe Givard for her secretarial assistance and Kent Pedersen and Kim P. Gadegaard for their technical assistance.

References

Abadie, P., J.C. Baron, J.C. Bisserbe, J.P. Boulenger, P. Rioux, J.M. Travère, L. Barré, M.C. Petit-Taboué and E. Zarifian, 1992, Central benzodiazepine receptors in human brain: estimation of regional B_{max} and K_d values with positron emission tomography, Eur. J. Pharmacol. 213, 107.

Benavides, J., B. Peny, A. Dubois, G. Perrault, E. Morel, B. Zivkovic and B. Scatton, 1988, In vivo interaction of zolpidem with central benzodiazepine (BZD) binding sites (as labeled by [^{3}H]Ro 15-1788) in the mouse brain. Preferential affinity of zolpidem for the w1 (BZD1) subtype, J. Pharmacol. Exp. Ther. 245, 1033.

Carson, R.E., M.A. Channing, R.G. Blasberg, B.B. Dunn, R.M. Cohen, K.C. Rice and P. Herscovitch, 1993, Comparison of bolus and infusion methods for receptor quantification: application to [^{18}F]cyclofoxy and positron emission tomography, J. Cereb. Blood Flow Metab. 13, 24.

DiResta, G.R., J. Lee and E. Arbit, 1991, Measurement of brain tissue specific gravity using pycnometry, J. Neurosci. Methods 39, 245.

Hansen, T.D., D.S. Warner, M.M. Todd, M.T. Baker and N.F. Jensen, 1991, The influence of inhalation anesthetics on in vivo and in vitro benzodiazepine receptor binding in the rat cerebral cortex, Anesthesiology 1, 97.

Innis, R.B., S. Zoghbi, E. Johnson, S. Woods, M. Al Tikriti, R. Baldwin, J. Seibyl, R. Malison, G. Zubal, D. Charney et al., 1991a, SPECT imaging of the benzodiazepine receptor in non-human primate brain with Ro 16-0154, Eur. J. Pharmacol. 193, 249.

Innis, R.B., M.S. Al-Tikriti, S. Zoghbi, R.M. Baldwin, E.H. Sybirska, M. Laruelle, R.T. Malison, J.P. Seibyl, R.C. Zimmermann, E.W. Johnson, E.O. Smith, D.S. Charney, G.R. Heninger, S.W. Woods and P.B. Hoffer, 1991b, SPECT imaging of the benzodiazepine receptor: feasibility of in vivo potency measurements from stepwise displacement curves, J. Nucl. Med. 32, 1754.

Iyo, M., T. Itoh, T. Yamasaki, H. Fukuda, O. Inoue, H. Shinotoh, K. Suzuki, S. Fukui and Y. Tateno, 1991, Quantitative in vivo analysis of benzodiazepine binding in human brain using positron emission tomography, Neuropharmacology 3, 207.

Johnson, E.W., S.W. Woods, S. Zoghbi, B.J. McBride, R.M. Baldwin and R.B. Innis, 1990, Receptor binding characterization of the benzodiazepine radioligand [^{125}I]Ro16-0154: potential probe for SPECT brain imaging, Life Sci. 47, 1535.

Kawai, R., R.E. Carson, B. Dunn, A.H. Newman, K.C. Rice and R.G. Blasberg, 1991, Regional brain measurement of B_{max} and K_d with the opiate antagonist cyclofoxy: equilibrium studies in the conscious rat, J. Cereb. Blood Flow Metab. 11, 529.

Kopp, J., H. Hall, A. Persson and G. Sedvall, 1990, Temperature dependence of [^{3}H]RO15-1788 binding to benzodiazepine receptors in human postmortem brain homogenates, J. Neurochem. 55, 1310.

Lassen, N.A., 1992, Neuroreceptor quantification in vivo by the steady state principle using constant infusion or bolus injection of radioactive tracers, J. Cereb. Blood Flow Metab. 12, 709.

Lassen, N.A., P.A. Bartenstein, A.A. Lammertsma, M.C. Prevett, D.R. Turton, S.K. Luthra, S. Osman, P.M. Bloomfield, T. Jones, P.N. Patsalos, M.T. O'Connell, J.S. Duncan and J.V. Andersen, 1995, Benzodiazepine receptor quantification in vivo in humans using [^{11}C]flumazenil and PET: application of the steady state principle, J. Cereb. Blood Flow Metab. 15, 152.

Litton, J.E., J. Neiman, S. Pauli, L. Farde, T. Hindmarsh, C. Halldin and G. Sedvall, 1993, PET analysis of [^{11}C]flumazenil binding to benzodiazepine receptors in cronic alcohol-dependent men and healthy controls, Psychiatry Res. 1, 1.

Mans, A.M., K.M. Kukulka, K.J. McAvoy and N.C. Rokosz, 1992, Regional distribution and kinetics of three sites on the GABA-A receptor: lack of effect of portacaval shunting, J. Cereb. Blood Flow Metab. 12, 334.

Pappata, S., Y. Samson, C. Chavoix, C. Prenant, M. Mazière and J.C. Baron, 1988, Regional specific binding of [^{11}C]RO 15 1788 to central type benzodiazepine receptors in human brain: quantitative evaluation by PET, J. Cereb. Blood Flow Metab. 8, 304.

Persson, A., S. Pauli, C. Halldin, S. Stone-Ellander, L. Farde, I. Sjøgren and G. Sedvall, 1989, Saturation analysis of specific [^{11}C]RO 15-1788 binding to the human neocortex using positon emission tomography, Hum. Psychopharmacol. 4, 21.

Price, J.C., H.S. Mayberg, R.F. Dannals, A.A. Wilson, H.T. Ravert, B. Sadzot, A. Kimball, M.A. Feldman and J.J. Frost, 1993, Measurement of benzodiazepine receptor number and affinity in humans using tracer kinetic modeling, positron emission tomography and [^{11}C]flumazenil, J. Cereb. Blood Flow Metab. 13, 656.

Savic, I., A. Persson, P. Roland, S. Pauli, G. Sedvall and L. Widén, 1988, In-vivo demonstration of reduced benzodiazepine receptor binding in human epileptic foci, Lancet 863.

Videbæk, C., L. Friberg, S. Holm, S. Wammen, C. Foged, J.V. Andersen, L. Dalgaard and N.A. Lassen, 1993, Benzodiazepine receptor equilibrium constants in humans for flumazenil and midazolam determined using the steady-state principle and the SPECT-tracer [^{123}I]iomazenil, Eur. J. Pharmacol. 249, 43.