SYNAPSE 58:249–257 (2005)

Binding Characteristics of the 5-HT_{2A} Receptor Antagonists Altanserin and MDL 100907

HEIDI KRISTIANSEN,[1] BETINA ELFVING,[1] PER PLENGE,[2] LARS H. PINBORG,[1] NIC GILLINGS,[3] AND GITTE M. KNUDSEN[1]*

[1]*Neurobiology Research Unit, University Hospital Rigshospitalet, Copenhagen, Denmark*
[2]*Laboratory of Neuropsychiatry, University Hospital Rigshospitalet, Copenhagen, Denmark*
[3]*PET and Cyclotron Unit, University Hospital Rigshospitalet, Copenhagen, Denmark*

KEY WORDS [^{18}F]altanserin; [^{3}H]MDL 100907; [^{11}C]MDL 100907; PET; receptor binding; rat brain

ABSTRACT To study the 5-HT_{2A} receptors in the living human brain, using positron emission tomography (PET), two selective radiotracers are currently in use: [^{18}F]altanserin and [^{11}C]MDL 100907. It is, however, currently unknown to what extent data obtained with either tracer are directly comparable. The aim of this study was to compare binding characteristics of these two radiotracers in rat brain with respect to affinity (K_d), receptor binding density (B_{max}), binding potential (BP), and nonspecific binding. Further, binding kinetics, sensitivity towards competition with the endogenous transmitter serotonin, and the competitive/noncompetitive interaction between the two radioligands were evaluated. In addition, the selectivity of [^{18}F]altanserin for the 5-HT_{2A} receptor was assessed.

The K_d value of [^{18}F]altanserin and [^{3}H]MDL 100907 was in the order of 0.3 nM. B_{max} in frontal cortex was 523 and 527 fmol/mg protein, respectively. The binding of [^{18}F]altanserin was not influenced by blocking either the 5-$HT_{2B/2C}$ or the α_1-adrenergic receptors. At 37°C the association $t_{1/2}$ was 2.8 and 2.7 min and the dissociation $t_{1/2}$ was 11 and 13.5 min for [^{18}F]altanserin and [^{3}H]MDL 100907, respectively.

Both radioligands were displaced by 5-HT, only at high concentrations; the K_i value of 5-HT ranging between 650 and 3,300 nM. This indicates that binding of both radioligands in PET studies is not directly influenced by changes in endogenous 5-HT.

Overall, the binding of [^{18}F]altanserin and [^{3}H]MDL 100907 to the 5-HT_{2A} receptor was very comparable, showing selective high affinity binding in the subnanomolar range. **Synapse 58:249–257, 2005.**

INTRODUCTION

The serotonin$_{2A}$ (5-HT_{2A}) receptors are thought to be pathophysiologically implicated in several psychiatric disorders, including schizophrenia, depression (Naughton et al., 2000), and obsessive-compulsive disorder (Adams et. al, 2005). To study the 5-HT_{2A} receptors in both healthy and diseased living human brain, several radiotracers for use in PET have been developed. Currently, two selective 5-HT_{2A} receptor radiotracers are used for PET-studies: [^{18}F]altanserin and [^{11}C]MDL 100907. In addition, [^{18}F]setoperone has been used, but its moderate affinity for the dopamine (DA) D_2-receptor only allows for interpretation of 5-HT_{2A} receptor binding in brain regions with low D_2-receptor density (Maziere et al., 1988; Meyer et al., 1999, 2001).

[^{18}F]altanserin has been widely used in clinical studies as a PET tracer for the 5-HT_{2A} receptor (Adams et al., 2004; Biver et al., 1994; Mintun et al., 2004; Pinborg et al., 2003; Sadzot et al., 1995; van Dyck et al., 2000). The limitation with this tracer, namely the production of lipophilic radiolabeled metabolites that cross the blood–brain barrier, can be counteracted either through the use of a steady-state approach that allows for a simple subtraction of nonspecific binding (Pinborg et al., 2003) or by using a multi-input functional approach (Price et al., 2001).

Contract grant sponsors: The 1991 Pharmacy Foundation, Health Insurance Foundation, Lundbeck Foundation, and NOVO Foundation.

*Correspondence to: Gitte M. Knudsen, Neurobiology Research Unit, Rigshospitalet, N9201, Blegdamsvej 9, 2100 Copenhagen, Denmark.
E-mail: heidi@nplab.dk

Received 17 December 2004; Accepted 5 July 2005

DOI 10.1002/syn.20205

Published online in Wiley InterScience (www.interscience.wiley.com).

For the more recently developed PET tracer [^{11}C]MDL 100907, only a few human studies have been published so far (Ito et al., 1998; Talvik-Lotfi et al., 2000). Both altanserin and MDL 100907 bind to the 5-HT_{2A} receptor with high affinity; altanserin K_i values in the rat brain are 0.13–0.3 nM (Janssen, 1985; Leysen, 1989; Tan et al., 1999), and K_d values of [^{3}H]MDL 100907 are between 0.19 and 0.56 nM in the rat brain (Johnson et al., 1996; Lam et al., 2001; Lopez-Gimenez et al., 1997), 0.16–0.19 nM in monkey brain, and 0.14–0.19 nM in the human brain, respectively (Lopez-Gimenez et al., 1998). Altanserin is known to have a moderate to low affinity for 5-HT receptors other than the 5-HT_{2A} receptor (K_i values: rat 5-HT_{1A}, 1,570 nM; rat 5-HT_{1B}, >10,000 nM; rat 5-HT_{2C}, 6.0 nM; human 5-HT_6, 1,756 nM; and human 5-HT_7, 15 nM), as well as for receptors outside the serotonergic system (K_i values in rat: histamine-H_1, 20 nM; DA-D_2, 62 nM; adrenergic-α_1, 4.55 nM) (Leysen, 1989; Tan et al., 1999). Displacement binding studies with [^{3}H]MDL 100907 have shown that MDL 100907 is highly selective for the 5-HT_{2A} receptor (Johnson et al., 1996) and its very low nonspecific binding in both rat and primate brains makes it particularly well-suited for in vitro studies (Hall et al., 2000; Johnson et al., 1996; Lopez-Gimenez et al., 1997, 1998). It is, however, unknown whether the differences in selectivity between the two radioligands have an impact on their usefulness in PET studies.

In 5-HT_{2A} receptor PET studies, cerebellum is usually employed as a reference region to define the nonspecific binding of radioligand, as this brain region is considered to be almost devoid of 5-HT_{2A} receptors. In a previous PET study with [^{18}F]altanserin, it has been shown that cerebellum is a suitable reference region for determination of nonspecific binding (Pinborg et al., 2003). By contrast, PET studies in humans and in nonhuman primates with [^{11}C]MDL 100907 have not consistently confirmed the suitability of cerebellum as a reference region. Several authors have reported a specific binding of [^{11}C]MDL 100907 in cerebellum (Ito et al., 1998; Talvik-Lotfi et al., 2000; Watabe et al., 2000), except for Lundkvist et al. (1996) who found no such specific cerebellar binding of [^{11}C]MDL 100907 in Cynomolgus monkeys.

In the present study, the binding characteristics of [^{18}F]altanserin and [^{3}H]MDL 100907 to rat 5-HT_{2A} receptors in cerebrum, frontal cortex, and cerebellum were evaluated and compared. In addition, the sensitivity of the two radioligands towards competition with the endogenous ligand, 5-HT, was investigated.

MATERIALS AND METHODS

Compounds

Altanserin tartrate (R 53 200) [(+)-3-[2-[4(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,3-dihydro-2-thioxo-4(1H0-quinazolinone); [R-(R*,R*)]2,3-dihyroxybutanedioate (1:1)] was kindly donated by Janssen Pharmaceuticals, Belgium. MDL 100907 [R(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorphenyl)-ethyl]-4-piperidin-methanol] was kindly donated by Professor C. Halldin, Department of Clinical Neuroscience, Karolinska Hospital, Sweden. MDL 100151 [(±)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorphenyl)ethyl]-4-piperidin-methanol] was kindly donated by H. Lundbeck A/S (Copenhagen, Denmark). The following drugs were also used: Ketanserin tartrate [3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl]-2,4[1H,3H]quinazolinedione tartrate] (Tocris Cookson Ldt, Bristol, UK), pargyline hydrochloride [*N*-methyl-*N*-2.propynylbenzylamine hydrochloride] (Research biochemicals international, MA, USA), SB 206553 [5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indol], prazosin hydrochloride [1-[4-amino-6,7-dimethoxy-2-quinazolinyl]-4-[[2-furanylcarbonyl]piperazine] hydrohcloride], and serotonin hydrochloride [5-hydroxytryptamine hydrochloride] (Sigma-Aldrich, Copenhagen, Denmark).

Radioligands

[^{18}F]altanserin was synthesized and radiolabeled at the PET and Cyclotron Unit, University Hospital Copenhagen, Rigshospitalet, Denmark, according to the method of Lemaire et al. (1991). The mean specific radioactivity was 160 ± 12 GBq/μmol, with a radiochemical purity greater than 99%. [^{3}H]MDL 100907 was kindly donated by Professor C. Halldin, Department of Clinical Neuroscience, Karolinska Hospital, Sweden. The specific activity of [^{3}H]MDL 100907 was determined to be 75 Ci/mmol (2.8 GBq/μmol).

Brain membrane preparation

Male Sprague–Dawley rats (Taconic M & B, Denmark or Charles River, Germany) weighing 200–300 g were used. All animals were allowed to acclimatize for at least 5 days after arrival before use. The study was approved by the Danish State Research Inspectorate (j. No. 2002/561-527). The preparation of membranes was done according to Elfving et al. (2001). Brain tissue from several animals, depending on the investigated brain region (cerebrum, frontal cortex (3–4 mm, including cingulate cortex), or cerebellum) was pooled. During preparation, the membrane pellet was lysed and incubated at room temperature for 20 min to ensure removal of endogenous 5-HT. Membrane protein content was determined using Peterson's modification of the Lowry method (Peterson, 1977).

Saturation binding assay

Saturation binding was performed as previously described by Elfving et al. (2001), using six different concentrations of [^{18}F]altanserin (0.05–2 nM) and [^{3}H]MDL 100907 (0.03–0.9 nM), respectively. Nonspecific binding was determined as the nondisplaceable

radioligand binding in the presence of 1 μM ketanserin or 1 μM MDL 100151 (racemic form of MDL 100907). All incubations were carried out at 37°C for 2 h. To reduce nonspecific binding to plastic and glass materials, 0.3% bovine serum albumine (BSA) was added to the incubation and wash buffer in the [^{18}F]altanserin assays. Affinity (K_d) and receptor density (B_{max}) were determined by Grafit 4 (Erithaus Software). The density of binding sites was expressed in terms of fmol/mg protein (Bylund and Yamamura, 1990). The BP was calculated as B_{max}/K_d, with K_d and B_{max} expressed in nM and picomol/g original wet weight of tissue (=nM, assuming brain tissue density of 1 g/ml) (Laruelle et al., 1994).

To evaluate the binding of [^{18}F]altanserin to the 5-HT$_{2B/2C}$ receptors and the α_1-adrenoceptor, additional saturation experiments were performed in the presence or absence of 0.1 μM SB 206553 (Adlersberg et al., 2000), a selective 5-HT$_{2B/2C}$ antagonist (Kennett et al., 1996), or 0.3 μM prazosin (Hoyer et al., 1987), a selective α_1-receptor antagonist. To mimic a PET situation where the radioligand is distributed throughout the brain, a membrane preparation of cerebrum was used.

The interaction between [^{18}F]altanserin and [^{3}H]MDL 100907 was assessed through saturation binding analysis of frontal cortex tissue in the presence or absence of its counterpart nonlabeled ligand.

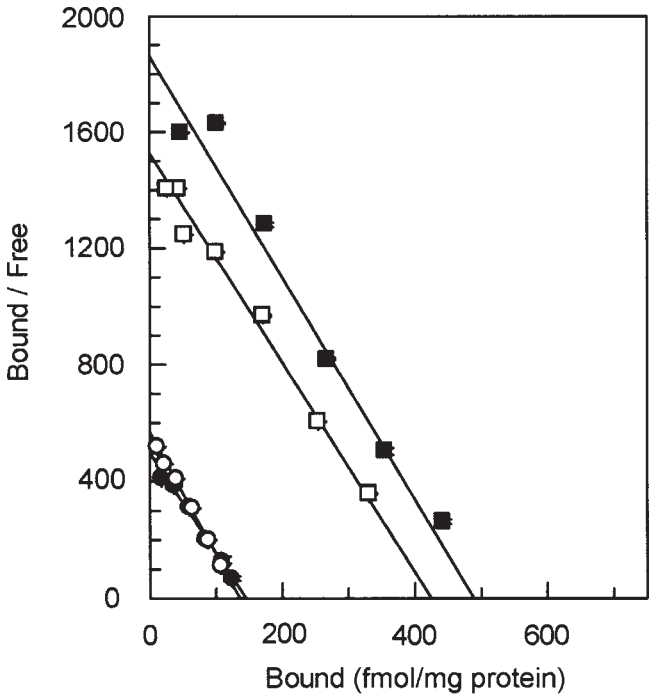

Fig. 1. Scatchard transformation of saturation binding data for [^{18}F]altanserin (closed) and [^{3}H]MDL 100907 (open) in cerebrum (circle) and frontal cortex (square) at 37°C. Each point represents the mean of at least three independent experiments (see Table I).

Association and dissociation rate determination

Association and dissociation rate constants were determined according to Elfving et al. (2001). Incubations were carried out at 37°C in cerebrum membranes, using radioligand concentrations of about 0.20 nM. Association was measured at increasing periods of time between 1 and 100 min. Nonspecific binding was determined at three time points (5, 50, and 100 min) by addition of 1 μM ketanserin. Dissociation was initiated after equilibrium was reached (40 min of preincubation) by addition of 1 μM ketanserin at different time points between 0 and 100 min. To define nonspecific binding, 1 μM ketanserin was added at the beginning of the preincubation. The association and dissociation constants were determined using Grafit 4 (Erithaus Software). The association and dissociation half-time ($t_{1/2}$) was calculated as ln 2/k_{on} and ln 2/k_{off}.

Displacement binding studies

Competition studies were performed with nine different concentrations of competing drug. To evaluate the effect of 5-HT competition, final radioligand concentrations of 0.3 nM [^{18}F]altanserin and 0.2 nM [^{3}H]MDL 100907 were used. The radioligands were incubated with 5-HT at a final concentration of 10^{-8} to 10^{-4} M. Incubations were carried out for 40 min at 37°C in a buffer containing 50 mM Tris-HCl, 4 mM $CaCl_2$, 10 μM pargyline, 0.1% ascorbic acid, and pH 7.7 (Leonhardt et al., 1992; Lyon et al., 1988), using a membrane suspension of frontal cortex.

For displacement binding studies of [^{3}H]MDL 100907 with altanserin and [^{18}F]altanserin with MDL 100151, radioligand concentrations of 0.2 and 0.13 nM were used, respectively. The concentration of nonlabeled ligands was 10^{-12} to 10^{-6} M. Incubations were carried out, as described earlier in a buffer composition of 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, and with a pH 7.4. The nonspecific binding was defined in the presence of 1 μM ketanserin.

The IC_{50} values were estimated by Grafit 4 (Erithaus Software). K_i values were calculated as $K_i = IC_{50}/(1 + [L]/K_d)$, where K_d is the affinity of either [^{18}F]altanserin or [^{3}H]MDL 100907 to rat frontal cortex membranes at 37°C and [L] is the concentration of free radioligand in the assay (Cheng and Prusoff, 1973).

Statistics

For statistical analysis, student's t-test was used. Whenever the normality test was not passed, the nonparametric Mann-Whitney test was used. $P < 0.05$ was considered statistically significant. All values are given as mean ± SEM.

RESULTS

Average saturation binding curves for [^{18}F]altanserin and [^{3}H]MDL 100907 in rat cerebrum and frontal

TABLE I. Saturation binding characteristics of [^{18}F]altanserin and [^{3}H]MDL 100907 to homogenates of rat cerebrum, frontal cortex, and cerebellum at 37°C

	[^{18}F]Altanserin			[^{3}H]MDL 100907		
	Cerebrum ($n = 8$)	Frontal cortex ($n = 5$)	Cerebellum ($n = 3$)	Cerebrum ($n = 6$)	Frontal cortex ($n = 5$)	Cerebellum ($n = 4$)
K_d (nM)	0.30 ± 0.03	0.24 ± 0.023	0.33 ± 0.08*	0.24 ± 0.02	0.32 ± 0.024	0.48 ± 0.05
B_{max} (fmol/mg protein)	145 ± 7	523 ± 23	7.9 ± 1.14	137 ± 9	527 ± 59	8.2 ± 0.57
BP	4	14[a]	0.19	4	11	0.09
NSB (%)	14.1 ± 1.1** ($n = 41$)	9.6 ± 0.9** ($n = 17$)	70.6 ± 3.9* ($n = 13$)	8.4 ± 0.7 ($n = 36$)	4.0 ± 0.4 ($n = 17$)	61.1 ± 1.9 ($n = 22$)

Values given are mean ± SEM. n, = number of experiments; BP, binding potential; NSB, nonspecific binding.
*$P < 0.05$ for difference between [^{18}F]altanserin and [^{3}H]MDL 100907; students t-test.
**$P < 0.05$; Mann-Whitney nonparametric test.
[a]$n = 3$.

cortex tissue are presented in Figure 1. The saturation binding data are presented in Table I. Both [^{18}F]altanserin and [^{3}H]MDL 100907 showed high affinity binding properties, with linear Scatchard transformation, confirming the presence of a single binding site. The average K_d values of [^{18}F]altanserin and [^{3}H]MDL 100907 in cerebrum and frontal cortex were 0.28 ± 0.02 nM and 0.27 ±0.02 nM, respectively. For both ligands, the receptor binding density was similar in all examined brain regions. B_{max} of [^{18}F]altanserin and [^{3}H]MDL 100907 in cerebellum constituted ~6% of B_{max} in cerebrum and below 2% of B_{max} in frontal cortex.

In all brain regions, the nonspecific binding of [^{3}H]MDL 100907 was statistically significantly lower than that for [^{18}F]altanserin ($P < 0.05$). The nonspecific [^{18}F]altanserin binding was the same, whether measured using ketanserin ((9.6 ± 0.9)%, $n = 18$) or MDL 100907 ((11.3 ± 1.5)%, $n = 17$) as blocking agents.

Association and dissociation rates

The association and dissociation rates, k_{on} and k_{off}, for [^{18}F]altanserin and [^{3}H]MDL 100907 are given in Table II. The association and dissociation curves are shown in Figure 2. The association rates were similar for the two radioligands, with curves reaching a plateau at 35 min for [^{18}F]altanserin and at 40 min for [^{3}H]MDL 100907 (Fig. 2A). The dissociation of [^{18}F]altanserin and [^{3}H]MDL 100907 was reached after approximately 90 and 100 min, respectively. The kinetically derived K_d value in cerebrum, calculated as k_{off}/k_{on}, was in accordance with the K_d value obtained through the saturation experiments (Tables I and II).

Selectivity of [^{18}F]altanserin

In the presence of the selective 5-HT$_{2B/2C}$ receptor antagonist SB 206553, the K_d and B_{max} values of [^{18}F]altanserin were similar to that obtained in the absence of this ligand, with K_d values of 0.35 ± 0.04 nM vs. 0.28 ± 0.03 nM and B_{max} values of 156 ± 10 fmol/mg protein vs. 153 ± 5 fmol/mg protein, respectively. Likewise, saturation binding of [^{18}F]altanserin in the presence or absence of the selective α_1-receptor antagonist prazosin showed identical K_d values of 0.34 ± 0.06 nM and 0.36 ± 0.04 nM and B_{max} values of 143 ± 10 fmol/mg protein and 140 ± 17 fmol/mg protein, respectively.

TABLE II. The association and dissociation parameters for [^{18}F]altanserin and [^{3}H]MDL 100907 to rat cerebrum membranes at 37°C

	[^{18}F]Altanserin	[^{3}H]MDL 100907
k_{on} (nM^{-1} min^{-1})	0.248 ± 0.018 ($n = 3$)	0.256 ± 0.045 ($n = 2$)
k_{off} (min^{-1})	0.062 ± 0.001 ($n = 2$)	0.052 ± 0.002 ($n = 2$)
K_d (k_{off}/k_{on}) (nM)	0.25	0.20
$T_{1/2}$ association (min)	2.8	2.7
$T_{1/2}$ dissociation (min)	11.2	13.5

Values given are mean ± SEM ($n = 2$). k_{on}, association rate constant; k_{off}, dissociation rate constant; K_d, kinetically derived dissociation equilibrium constant; $T_{1/2}$, half-time for association or dissociation.

Competition studies

Displacement binding of [^{18}F]altanserin and [^{3}H]MDL 100907 with 5-HT was repeated four times with each radioligand. On two occasions, the 5-HT displacement of [^{18}F]altanserin and [^{3}H]MDL 100907 was done simultaneously, and on the other two occasions, the experiments with the two radioligands were done separately. Comparison of the K_i values of 5-HT obtained from the two simultaneously performed experiments showed that the K_i values of 5-HT were not statistically significantly different using either radioligands; 903 ± 297 nM vs. 656 ± 95 nM ($n = 3$) and 3044 ± 112 nM vs. 2624 ± 169 nM ($n = 3$) (Fig. 3) for [^{18}F]altanserin and [^{3}H]MDL 100907, respectively. The experiments performed separately showed noncomparable K_i values for 5-HT. Thus, the 5-HT K_i values while using [^{18}F]altanserin were found to be 1382 ± 99 nM ($n = 2$) and 3345 ± 459 ($n = 3$). While using [^{3}H]MDL 100907, the 5-HT K_i values were found to be 283 ± 18 nM ($n = 3$), and 2555 ± 133 nM ($n = 3$). Overall, these results shows that the K_i values of 5-HT for both [^{18}F]altanserin and [^{3}H]MDL 100907 were subject to great variance between repeat experiments.

Displacement curves of [^{18}F]altanserin with MDL 100151 (the racemic form of MDL 100907) and [^{3}H]MDL 100907 with altanserin are shown in Figure 4. The corresponding K_i values of altanserin and MDL 100151 were 1.32 ± 0.06 nM and 1.77 ± 0.2 nM ($n = 3$).

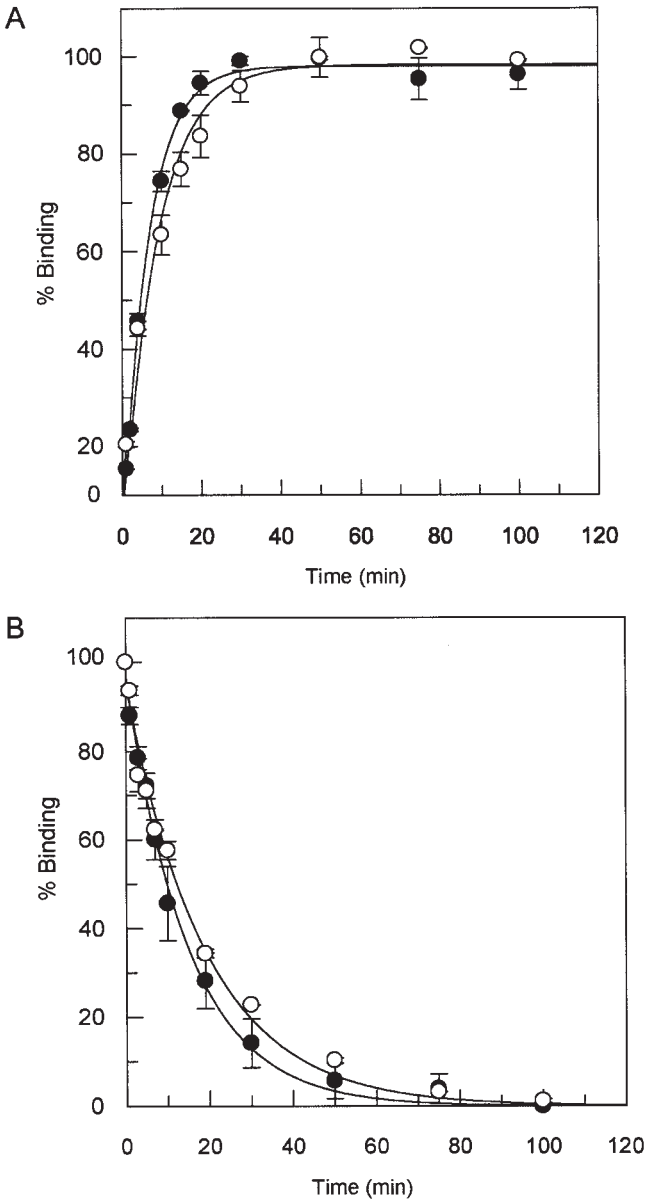

Fig. 2. (**A**) The association and (**B**) dissociation of [^{18}F]altanserin (closed) and [^{3}H]MDL 100907 (open) in cerebrum homogenate at 37°C. Each point represents the mean of two or three independent experiments. The vertical bars indicate the SEM value of each point.

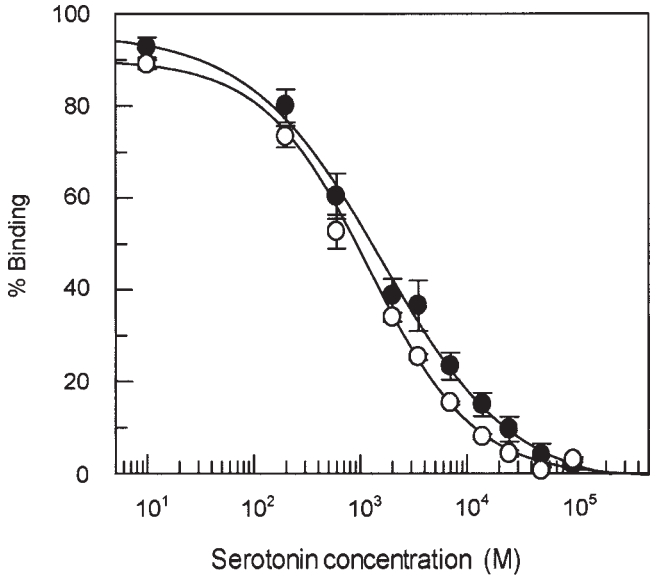

Fig. 3. Example of 5-HT displacement curves of [^{18}F]altanserin (closed) and [^{3}H]MDL 100907 (open) with 5-HT in frontal cortex homogenates at 37°C. Each point represents the mean of three independent experiments. The vertical bars indicate the SEM value of each point.

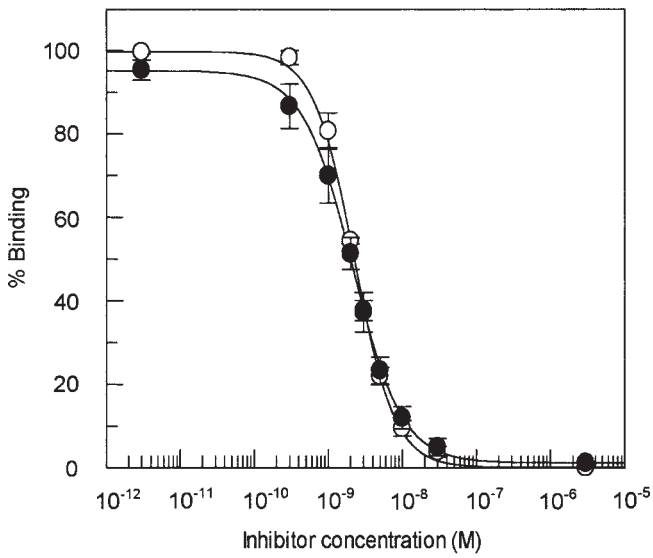

Fig. 4. Displacement binding of [^{18}F]altanserin with MDL 100151 (closed) and [^{3}H]MDL 100907 with altanserin (open) in frontal cortex homogenates at 37°C. Each point represents the mean of three independent experiments. The vertical bars indicate the SEM value of each point.

Competitive vs. noncompetitive interaction

Saturation binding data of [^{18}F]altanserin and [^{3}H]MDL 100907 in the presence of counterpart nonlabeled ligand are shown in Table III. The presence of competing nonlabeled ligand increased the K_d value of both radioligands. The increase in K_d value was statistically significant for [^{3}H]MDL 100907 ($P < 0.001$). The decrease in affinity of [^{18}F]altanserin in the presence of MDL 100907 was 33% and not statistically significant. The B_{max} value of [^{3}H]MDL 100907 increased by 11% in the presence of altanserin, while B_{max} of [^{18}F]altanserin increased by 17% in the presence of MDL 100907 (Table III). These changes in binding pattern indicate a competitive interaction between the two ligands.

DISCUSSION

Saturation binding

Both [^{18}F]altanserin and [^{3}H]MDL 100907 showed high binding affinity to the rat 5-HT$_{2A}$ receptor, in accordance with previously reported K_i and K_d values

TABLE III. Competitive/noncompetitive interaction. Saturation binding characteristics of [^{18}F]altanserin and [^{3}H]MDL 100907 in the presence of counterpart nonlabeled ligand to homogenate of rat frontal cortex at 37°C

	[^{18}F]Altanserin + 0.2 nM MDL100907 ($n = 3$)	[^{18}F]Altanserin (from table I) ($n = 5$)	[^{3}H]MDL100907 + 0.3 nM altanserin ($n = 3$)	[^{3}H]MDL100907 (from table I) ($n = 5$)
K_d (nM)	0.36 ± 0.11	0.24 ± 0.023	1.06 ± 0.05*	0.32 ± 0.024
B_{max} (fmol/mg protein)	629 ± 111	523 ± 23	591 ± 15	527 ± 59

Values given are mean ± SEM. n = number of experiments.
*$P < 0.001$ for difference between K_d of [^{3}H]MDL 100907 in the presence and absence of altanserin.

(Janssen, 1985; Johnson et al., 1996; Lam et al., 2001; Leysen, 1989; Lopez-Gimenez et al., 1997; Tan et al., 1999). The observed relative differences in receptor density between brain regions were in accordance with the known 5-HT$_{2A}$ receptor distribution (Cornea-Hebert et al., 1999; Pazos et al., 1985), with high receptor density in frontal cortex and a very low receptor density in cerebellum. The 5-HT$_{2A}$ receptor densities measured in both cerebrum and frontal cortex with both radioligands were in agreement with previous findings of 120 fmol/mg protein in rat cerebum (Lam et al., 2001), and 515 fmol/mg protein in rat frontal cortex (Johnson et al., 1996) for [^{3}H]MDL 100907. Also, the linearity of the Scatchard plot (Fig. 1), the ability of the two ligands to fully displace each other (Fig. 4), and the competitive interaction observed between these two ligands (Table III) support the notion that altanserin and MDL 100907 bind to the same single affinity site.

Binding potential

The BP (B_{max}/K_d) reflects the capacity of a given tissue for receptor–ligand binding interaction. As it is assumed that the affinity for a given ligand is similar throughout the brain, the BP becomes a measure of the receptor density of a given radioligand. The BP is an outcome measure frequently used in quantitative PET studies where K_d and B_{max} cannot be separated on the basis of a single scan (Mintun et al., 1984). Calculation of BP in in vitro studies, thus, allows for a reasonable comparison with the parameters measured in in vivo PET experiments (Laruelle et al., 1994). As expected from the known 5-HT$_{2A}$ receptor distribution, the BP was highest in frontal cortex, measured with both radioligands (11–14), and very low in cerebellum (~0.1–0.2). Previous PET studies in humans have reported considerably lower BPs for both radioligands in cortical areas than those observed here in vitro: 2.4–3.4 for [^{18}F]altanserin (Biver et al., 1994; Sadzot et al., 1995) and 6–16 for [^{11}C]MDL 100907 (Ito et al., 1998). This divergence probably reflects the species difference in the density of 5-HT$_{2A}$ receptors and also the somewhat higher affinity found for [^{3}H]MDL 100907 in human brain tissue (Lopez-Gimenez et al., 1998).

Nonspecific binding

For both radioligands, the nonspecific binding was acceptably low in cerebrum and frontal cortex, but somewhat higher in cerebellum. In all examined brain areas, the nonspecific binding of [^{18}F]altanserin was significantly higher than that for [^{3}H]MDL 100907. This finding is not well-explained by the estimated lipophilicity for the two radioligands, as their log P values are similar (altanserin 3.5, MDL 100907 3.8; own data), but it is also known that the degree of nonspecific binding cannot be predicted from this parameter alone (Laruelle et al., 2003). Others have, however, found the log P value of MDL 100907 to be somewhat lower than what we observed, i.e., 2.7 (Ito et al., 1998) and 1.9 (Laruelle et al., 2003).

For brain imaging of 5-HT$_{2A}$ receptors, the nonspecific binding is often assessed using cerebellum as a reference region. Immunohistochemical staining of the 5-HT$_{2A}$ receptor as well as membrane binding and PET studies has, however, revealed detectable levels of 5-HT$_{2A}$ receptors in this brain region in rat (Cornea-Hebert et al., 1999; Maeshima et al., 1998; Pazos et al., 1985), monkey (Watabe et al., 2000), and human tissues (Eastwood et al., 2001; Ito et al., 1998); thereby, questioning the use of cerebellum as a suitable reference region. Pazos et al., (1985) found a specific [^{3}H]ketanserin binding of around 12% in rat cerebellum compared to frontal cortex. With both [^{18}F]altanserin and [^{3}H]MDL 100907, we found a small specific radioligand binding within cerebellum, 6 and 2% of the binding density observed in cerebrum and in frontal cortex, respectively. This result, together with the PET study of Pinborg et al., (2003) where blocking of 5-HT$_{2A}$ receptors with ketanserin did not change the cerebellar binding, supports the notion that cerebellar binding is mainly of nonspecific origin, and therefore confirms the suitability of cerebellum as a reference region.

Association and dissociation rates

For both radioligands, the association to the 5-HT$_{2A}$ receptors was relatively fast, reaching binding equilibrium within 35–40 min, and the dissociation of both radioligands was relatively slow ($t_{½(off)}$ 11–14 min) (Fig. 2). The time for [^{3}H]MDL 100907 to reach equilibrium reported in Table II is twice the value previously reported by Johnson et al. (1996), who found that [^{3}H]MDL 100907 reached equilibrium after 15 min at 37°C in cortical tissue. A quite likely explanation of this discrepancy is that a higher [^{3}H]MDL 100907 concentration was use by Johnson et al. (0.25–

2 nM) compared to our study (0.2 nM). The higher the radioligand concentration, the faster the equilibrium is reached (Bylund and Yamamura, 1990). The obtained dissociation for [^{18}F]altanserin is slightly faster than that previously found using unlabeled altanserin, where the dissociation half-time was reported to be 15 ± 6 min (Leysen and Gommeren, 1986).

The kinetic behavior of the two radioligands should be seen in the context of radiolabeling with different isotopes when used in PET, which seems to be one of the major differences between altanserin and MDL 100907, i.e., altanserin being labeled with ^{18}F ($t_{1/2}$ = 110 min) and MDL 100907 being labeled with ^{11}C ($t_{1/2}$ = 20 min). In combination with the relatively slow dissociation from the 5-HT$_{2A}$ receptor of MDL 100907 (~13.5 min), one could anticipate that it might be difficult to obtain reversibility within the experimental time of a PET experiment. This problem is well-known from other radioligands, e.g., the DA-D$_2$ antagonist [^{11}C]N-Methylspiperone that has a dissociation $t_{1/2}$ of 15 min (Hall et al., 1990). In this respect, [^{18}F]altanserin has the advantage of having a much longer half-life, allowing for a scan period of up to 2–3 h.

Selectivity of [^{18}F]altanserin

Previous studies have questioned the selectivity of altanserin in PET studies, since a moderate affinity of altanserin for both the α_1-adrenergic receptor (K_i = 4.55 nM) (Leysen, 1989) and the 5-HT$_{2C}$ receptor (K_i = 6.0 nM) (Tan et al., 1999) have been found. We had not found that K_d or B_{max} of [^{18}F]altanserin was altered when the α_1-adrenergic or the 5-HT$_{2C}$ receptors were blocked. Further, the nonspecific binding of [^{18}F]altanserin remained unaltered, regardless of whether specific binding was blocked with either ketanserin (NSB, ~10%), which is not completely selective for the 5-HT$_{2A}$ receptor, or MDL 100151 (NSB, ~11%), which is considered selective. These findings verify that at least at low concentrations (<2 nM), the binding in cerebrum of [^{18}F]altanserin is not influenced by the binding to either 5-HT$_{2C/2B}$ or α_1-adrenergic receptor.

Sensitivity for 5-HT competition

When the tracers' sensitivity for competition with 5-HT was assessed, we found that only at very high concentrations of 5-HT could the binding of [^{18}F]altanserin and [^{3}H]MDL 100907 be displaced. The K_i values of 5-HT varied considerably (900–3400 nM using [^{18}F]altanserin, 290–2600 nM using [^{3}H]MDL100907), even though a monoamine oxidase inhibitor (pargyline) and ascorbic acid were applied to lessen degradation of 5-HT. These variations are in accordance with previous studies where K_i values of 5-HT between 70 and 3000 nM using [^{3}H]ketanserin have been reported (PDSP K_i – database; http://pdsp.cwru.edu/pdsp.asp).

One reason for these variable results could be resealing of cells in the membrane preparation, leading to a considerable reuptake of 5-HT into the neurons, and thereby, lowering the free concentration of 5-HT available for displacement of the radioligand. To test this hypothesis, we conducted an additional displacement experiment where the 5-HT reuptake site was blocked by S-citalopram (2 μM). Addition of S-citalopram did not, however, alter the K_i value for 5-HT, significantly, for either [^{3}H]MDL 100907 (n = 3) or [^{18}F]altanserin (n = 1). We believe that fast degradation of 5-HT is causing the variable results. However, it is noteworthy that for experiments conducted simultaneously, the 5-HT K_i values were without statistically significant using either radioligand. Associating the results to in vivo displacement studies, the measured K_i values must be seen in the context of the extracellular concentration of 5-HT. In microdialysis studies, the baseline extracellular 5-HT concentration in rat cerebral cortex is about 4 nM (Hume et al., 2001). Acute treatment with SSRIs such as citalopram has been reported to elevate the dialysate concentration of 5-HT in rat brain up to fourfold, if administered concurrently with 5-HT$_{1A}$ receptor antagonist or partial agonist (Arborelius et al., 1996). With the high concentrations of 5-HT needed to displace [^{18}F]altanserin or [^{3}H]MDL 100907, we find it unlikely that physiologically relevant changes in 5-HT could affect radioligand binding to a measurable extent in in vivo studies. This has also been confirmed by our recently performed human PET studies using [^{18}F]altanserin (Pinborg et al., 2004) and recently published data by Hirani et al. (2003), using [^{3}H]MDL 100907 in a small animal PET scanning setting. The results of these studies imply that since none of the radiotracers seem to be sensitive to changes in endogenous levels of 5-HT, both [^{18}F]altanserin and [^{3}H]MDL 100907 can be used clinically to detect a possible alteration in the 5-HT$_{2A}$ receptor level in a state of disease or during therapy, without the need to consider a simultaneous change in 5-HT (Hirani et al., 2003).

CONCLUSION

In conclusion, the two radioligands [^{18}F]altanserin and [^{3}H]MDL 100907 possess highly comparable characteristics for binding to the rat 5-HT$_{2A}$ receptor. The low nonspecific binding of [^{3}H]MDL 100907 favors the use of this ligand in in vitro studies of the 5-HT$_{2A}$ receptor. For PET image purposes, [^{18}F]altanserin can be considered sufficiently selective for the 5-HT$_{2A}$ receptor to obtain a selective image. Also, [^{18}F]altanserin may have the advantage of being labeled with [^{18}F], which permits for a longer scan duration. Additionally, for both radioligands, the binding seen in PET studies is not directly influenced by changes in endogenous 5-HT levels.

ACKNOWLEDGMENTS

The authors thank Inge Møller and Karin Stahr for excellent technical assistance. Also thanks to the staff at the PET and Cyclotron Unit, especially to Hedi Pedersen, Lone Agerskov, and Anne Sørensen; to Professor Christer Halldin, Department of Clinical Neuroscience, Karolinska Hospital, Stockholm, Sweden, for the kind donation of MDL 100907 and [^{3}H]MDL 100907; to Cand. Pharm., Berith Bjørnholm, Ph.D., Department of Computational Chemistry, Lundbeck A/S, Copenhagen, for calculating LogP values of altanserin and MDL 100907; and to Janssen pharmaceuticals, Belgium for donating altanserin.

REFERENCES

Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, Madsen K, Frokjaer V, Martiny L, Paulson OB, Knudsen GM. 2004. A database of [^{18}F]-altanserin binding to 5-HT2A receptors in normal volunteers: normative data and relationship to physiological and demographic variables. Neuroimage 21:1105–1113.

Adams KH, Hansen ES, Pinborg LH, Hasselbalch S, Svare C, Holm S, Bolwig TG, Knudsen GM. 2005. Patients with obsessive-compulsive disorder have increased 5-HT$_{2A}$ receptor binding in right caudate nucleus. Int J Neuropsychopharmacol 8:391–401.

Adlersberg M, Arango V, Hsiung S, Mann JJ, Underwood MD, Liu K, Kassir SA, Ruggiero DA, Tamir H. 2000. In vitro autoradiography of serotonin 5-HT(2A/2C) receptor-activated G protein: guanosine-5′-(gamma-[(35)S]thio)triphosphate binding in rat brain. J Neurosci Res 61:674–685.

Arborelius L, Nomikos GG, Hertel P, Salmi P, Grillner P, Hook BB, Hacksell U, Svensson TH. 1996. The 5-HT1A receptor antagonist (S)-UH-301 augments the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. Naunyn Schmiedebergs Arch Pharmacol 353:630–640.

Biver F, Goldman S, Luxen A, Monclus M, Forestini M, Mendlewicz J, Lotstra F. 1994. Multicompartmental study of fluorine-18 altanserin binding to brain 5HT2 receptors in humans using positron emission tomography. Eur J Nucl Med 21:937–946.

Bylund DB, Yamamura HI. 1990. Methods for receptor binding. In: Yamamura HI, Enna SJ, Kuhar MJ, editors. Methods in Neurotransmitter Receptor Analysis. New York: Raven Press p 1–35.

Cheng Y, Prusoff WH. 1973. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108.

Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L. 1999. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. J Comp Neurol 409:187–209.

Eastwood SL, Burnet PW, Gittins R, Baker K, Harrison PJ. 2001. Expression of serotonin 5-HT(2A) receptors in the human cerebellum and alterations in schizophrenia. Synapse 42:104–114.

Elfving B, Bjřrnholm B, Ebert B, Knudsen GM. 2001. Binding characteristics of selective serotonin reuptake inhibitors with relation to emission tomography studies. Synapse 41:203–211.

Hall H, Wedel I, Halldin C, Kopp J, Farde L. 1990. Comparison of the in vitro receptor binding properties of N-[3H]methylspiperone and [3H]raclopride to rat and human brain membranes. J Neurochem 55:2048–2057.

Hall H, Farde L, Halldin C, Lundkvist C, Sedvall G. 2000. Autoradiographic localization of 5-HT(2A) receptors in the human brain using [(3)H]M100907 and [(11)C]M100907. Synapse 38:421–431.

Hirani E, Sharp T, Sprakes M, Grasby P, Hume S. 2003. Fenfluramine evokes 5-HT2A receptor-mediated responses but does not displace [11C]MDL 100907: small animal PET and gene expression studies. Synapse 50:251–260.

Hoyer D, Vos P, Closse A, Pazos A, Palacios JM, Davies H. 1987. [3H]ketanserin labels 5-HT2 receptors and alpha 1-adrenoceptors in human and pig brain membranes. Naunyn Schmiedebergs Arch Pharmacol 335:226–230.

Hume S, Hirani E, Opacka-Juffry J, Myers R, Townsend C, Pike V, Grasby P. 2001. Effect of 5-HT on binding of [(11)C] WAY 100635 to 5-HT(IA) receptors in rat brain, assessed using in vivo microdialysis and PET after fenfluramine. Synapse 41:150–159.

Ito H, Nyberg S, Halldin C, Lundkvist C, Farde L. 1998. PET imaging of central 5-HT2A receptors with carbon-11-MDL 100907. J Nucl Med 39:208–214.

Janssen PA. 1985. Pharmacology of potent and selective S2-serotonergic antagonists. J Cardiovasc Pharmacol 7(Suppl. 7):S2–S11.

Johnson MP, Siegel BW, Carr AA. 1996. [3H]MDL 100907: a novel selective 5-HT2A receptor ligand. Naunyn Schmiedebergs Arch Pharmacol 354:205–209.

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP. 1996. In vitro and in vivo profile of SB 206553, a potent 5-HT2C/5-HT2B receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434.

Lam HR, Plenge P, Jørgensen OS. 2001. Effects of white spirits on rat brain 5-HT receptor functions and synaptic remodeling. Neurotoxicol Tetratol 23:603–608.

Laruelle M, Giddings SS, Zea-Ponce Y, Charney DS, Neumeyer JL, Baldwin RM, Innis RB. 1994. Methyl 3β-(4-[125I]iodophenyl)tropane-2β-carboxylate in vitro binding to dopamine and serotonin transporters under "physiological" conditions. J Neurochem 62: 978–986.

Laruelle M, Slifstein M, Huang Y. 2003. Relationships between radiotracer properties and image quality in molecular imaging of the brain with positron emission tomography. Mol imaging biol 5:363–375.

Lemaire C, Cantineau R, Guillaume M, Plenevaux A, Christiaens L. 1991. Fluorine-18-altanserin: a radioligand for the study of serotonin receptors with PET: radiolabeling and in vivo biologic behavior in rats. J Nucl Med 32:2266–2272.

Leonhardt S, Gorospe E, Hoffman BJ, Teitler M. 1992. Molecular pharmacological differences in the interaction of serotonin with 5-hydroxytryptamine1C and 5-hydroxytryptamine2 receptors. Mol Pharmacol 42:328–335.

Leysen JE. 1989. Use of 5-HT receptor agonists and antagonists for the characterization of their respective receptor site. In: Boulton AA, Baker GB, Juorio AV, editors. Drugs as Tools in Neurotransmitter Research. Clifton, New Jersey: Humana Press. Neuromethods, vol 12. p 299–350.

Leysen JE, Gommeren W. 1986. Drug-receptor dissociation time, new tool for drug research: Receptor binding affinity and drug-receptor dissociation profiles of serotonin-S2, dopamine-D2, histamine-H1 antagonists, and opiates. Drug Dev Res 8:119–131.

Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT. 1997. Selective visualization of rat brain 5-HT2A receptors by autoradiography with [3H]MDL 100907. Naunyn Schmiedebergs Arch Pharmacol 356:446–454.

Lopez-Gimenez JF, Vilaro MT, Palacios JM, Mengod G. 1998. [3H]MDL 100907 labels 5-HT2A serotonin receptors selectively in primate brain. Neuropharmacology. 37:1147–1158.

Lundkvist C, Halldin C, Ginovart N, Nyberg S, Swahn CG, Carr AA, Brunner F, Farde L. 1996. [11C]MDL 100907, a radioligland for selective imaging of 5-HT(2A) receptors with positron emission tomography. Life Sci 58(10):PL187–PL192.

Lyon RA, Titeler M, Seggel MR, Glennon RA. 1988. Indolealkylamine analogs share 5-HT2 binding characteristics with phenylalkylamine hallucinogens. Eur J Pharmacol 145:291–297.

Maeshima T, Fumihiro S, Hamada S, Senzaki K, Hamaguchi-Hamada K, Ito R, Okado N. 1998. Serotonin2A receptor-like immunoreactivity in rat cerebellar purkinje cells. Neurosci Lett 252:72–74.

Maziere B, Crouzel C, Venet M, Stulzaft O, Sanz G, Ottaviani M, Sejourne C, Pascal O, Bisserbe JC. 1988. Synthesis, affinity and specificity of 18F-setoperone, a potential ligand for in-vivo imaging of cortical serotonin receptors. Int J Rad Appl Instrum B 15: 463–468.

Meyer JH, Kapur S, Houle S, DaSilva J, Owczarek B, Brown GM, Wilson AA, Kennedy SH. 1999. Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. Am J Psychiatry 156:1029–1034.

Meyer JH, Kapur S, Eisfeld B, Brown GM, Houle S, DaSilva J, Wilson AA, Rafi-Tari S, Mayberg HS, Kennedy SH. 2001. The effect of paroxetine on 5-HT(2A) receptors in depression: an [18F]setoperone PET imaging study. Am J Psychiatry 158:78–85.

Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. 1984. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. Ann Neurol 15:217–227.

Mintun MA, Sheline YI, Moerlein SM, Vlassenko AG, Huang Y, Snyder AZ. 2004. Decreased hippocampal 5-HT$_{2A}$ receptor binding in major depressive disorder: in vivo measurement with [^{18}F]altanserin positron emission tomography. Biol Psychiatry 55:217–224.

Naughton M, Mulrooney JB, Leonard BE. 2000. A review of the role of serotonin receptors in psychiatric disorders. Hum Psychopharmacol 15:397–415.

Pazos A, Cortes R, Palacios JM. 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 346:231–249.

Peterson GL. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 83: 346–356.

Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, Madsen J, Knudsen GM. 2003. Quantification of 5-HT2A receptors in the human brain using [18F]altanserin and the bolus/infusion approach. J Cereb Blood Flow Metab 23:985–996.

Pinborg LH, Adams KH, Yndgaard S, Hasselbalch SG, Holm S, Kristiansen H, Paulson PB, Knudsen GM. 2004. [18F]altanserin binding to human 5HT2A receptors is unaltered following citalopram and pindolol challenge. J Cereb Blood Flow Metab 24:1037–1045.

Price JC, Lopresti BJ, Meltzer CC, Smith GS, Mason NS, Huang Y, Holt DP, Gunn RN, Mathis CA. 2001. Analysis of [18F]altanserin bolus injection PET data: consideration of radiolabelled metabolites in humans. Synapse 41:11–21.

Sadzot B, Lemaire C, Maquet P, Salmon E, Plenevaux A, Degueldre C, Hermanne JP, Guillaume M, Cantineau R, Comar D, Franck G. 1995. Serotonin 5HT2 receptor imaging in the human brain using positron emission tomography and a new radioligand, [18F]altanserin: results in young normal controls. J Cereb Blood Flow Metab 15:787–797.

Talvik-Lotfi M, Nyberg S, Nordstrom AL, Ito H, Halldin C, Brunner F, Farde L. 2000. High 5HT2A receptor occupancy in M100907-treated schizophrenic patients. Synapse 148:400–403.

Tan PZ, Baldwin RM, Van Dyck CH, Al-Tikriti M, Roth B, Khan N, Charney DS, Innis RB. 1999. Characterization of radioactive metabolites of 5-HT2A receptor PET ligand [18F]altanserin in human and rodent. Nucl Med Biol 26:601–608.

van Dyck CH, Tan PZ, Baldwin RM, Amici LA, Garg PK, Ng CK, Soufer R, Charney DS, Innis RB. 2000. PET quantification of 5-HT2A receptors in the human brain: a constant infusion paradigm with [18F]altanserin. J Nucl Med 41:234–241.

Watabe H, Channing MA, Der MG, Adams HR, Jagoda E, Herscovitch P, Eckelman WC, Carson RE. 2000. Kinetic analysis of the 5-HT2A ligand [11C]MDL 100907. J Cereb Blood Flow Metab 20: 899–909.