Biochemical Pharmacology, Vol. 51, pp. 71–76, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02122-1

Radiolabelling of the Human 5-HT_{2A} Receptor with an Agonist, a Partial Agonist and an Antagonist: Effects on Apparent Agonist Affinities

Andrew J. Sleight,§ Nico J. Stam,†*
Vincent Mutel and Patrick M. L. Vanderheyden‡*

*PHARMA DIVISION, PRECLINICAL RESEARCH, F. HOFFMANN-LA ROCHE LTD, 4002-BASEL, SWITZERLAND; †DEPARTMENTS OF BIOTECHNOLOGY; AND ‡NEUROPHARMACOLOGY, ORGANON INTERNATIONAL B.V., POST BOX 20, 5340 BH OSS, THE NETHERLANDS

ABSTRACT. Previous work has shown that 5-hydroxytryptamine (5-$HT)_{2A}$ receptors can be radiolabelled with various radioligands, including partial agonists, such as [^{125}I]-DOI and [^{3}H]-DOB, and antagonists, such as [^{3}H]-ketanserin and [^{3}H]-spiperone. Because 5-HT has high affinity for the 5-HT_{2A} receptor when displacing [^{3}H]-DOB, the purpose of the present study was to determine whether or not the receptor could be labelled with [^{3}H]-5-HT and what would be the effect of labelling the receptor with various radioligands having differing efficacies at the receptor. Consequently, the human 5-HT_{2A} receptor stably expressed in NIH 3T3 cells was radiolabelled with the endogenous agonist [^{3}H]-5-HT, the partial agonist [^{3}H]-DOB, and the antagonist [^{3}H]-ketanserin. The receptor could be radiolabelled with [^{3}H]-5-HT with a K_d value of 1.3 ± 0.1 nM and a B_{max} value of 3461 ± 186 fmoles/mg protein and the radiolabelling was sensitive to the stable guanosine 5′-triphosphate (GTP) analogue guanylyl-imidodiphosphate (GMP-PNP). Ketanserin labeled significantly more receptors (K_d = 1.1 ± 0.1 nM: B_{max} = 27684 ± 1500 fmoles/mg protein) than [^{3}H]-DOB (K_d = 0.8 ± 0.08 nM: B_{max} = 8332 ± 16 fmoles/mg protein) which, in turn, labelled significantly more receptors than [^{3}H]-5-HT. The apparent affinity of antagonists did not change when the receptor was radiolabelled with either [^{3}H]-agonists or [^{3}H]-antagonists; however, agonists had a higher apparent affinity for [^{3}H]-agonist-labeled receptors than for [^{3}H]-antagonist-labeled receptors. Therefore, the apparent affinity of agonists for the 5-HT_{2A} receptor estimated from displacement experiments depends on the intrinsic efficacy of the radioligand used. BIOCHEM PHARMACOL 51;1: 71–76, 1996.

KEY WORDS. 5-HT_{2A} receptor; [^{3}H]-DOB; [^{3}H]-5-HT; [^{3}H]-ketanserin; agonist binding

The neurotransmitter 5-HT[‖] exerts its effect through a number of receptor subtypes [1]. These were originally classified in the periphery by functional studies [2] and in the central nervous system by radioligand receptor binding [3]. These receptor-binding studies in animal or human brain membranes are difficult to interpret due to the presence of multiple 5-HT receptor subtypes expressed in the same tissue. 5-HT receptor binding sites were initially classified as either 5-HT_1 or 5-HT_2 on the basis of their affinity for 5-HT and spiperone (5-HT_2 receptors have high affinity for spiperone and low affinity for 5-HT and 5-HT_1 receptors have high affinity for 5-HT and low affinity for spiperone [3]). Other 5-HT receptors have subsequently been characterized and the 5-HT_2 receptor identified by Peroutka and Snyder has now been renamed the 5-HT_{2A} receptor [4, 5]. The 5-HT_{2A} receptor can be labeled with radioligands such as [^{125}I]-DOI, [^{3}H]-DOB, and [^{3}H]-ketanserin [6–8]. Because 5-HT_2 agonists such as 5-HT, DOI, and DOB displaced [^{125}I]DOI and [^{3}H]DOB binding with higher affinity than that seen with [^{3}H]ketanserin binding [6–8], it was suggested that [^{3}H]-DOI and [^{3}H]-DOB labeled either subtypes of the 5-HT_2 receptor or different affinity states of the receptor. In studies using the cloned rat 5-HT_{2A} receptor, similar differences for agonist affinity values were observed, suggesting that the 5-HT_{2A} receptor does, indeed, display different agonist affinity states [7].

In the present study, we investigated whether or not human 5-HT_{2A} receptors expressed in NIH 3T3 cells can be labeled with [^{3}H]-5-HT as well as with [^{3}H]-DOB and [^{3}H]-ketanserin. Furthermore, we investigated the effect of using these radioligands with different intrinsic activities on the apparent affinities of agonists and antagonists estimated from displacement experiments. The binding studies were performed on a single preparation of membranes prepared from an NIH 3T3 fibroblast cell line expressing the human 5-HT_{2A} receptor [9] using fermentation techniques.

§ Corresponding author. Tel. (41) 61 6884274; FAX (41) 61 6884484.

‖ Abbreviations: 5-HT, 5-hydroxytryptamine; DOI, 4-iodo-2,5-dimethoxyphenylisopropylamine; DOB, 4-bromo-2,5-dimethoxyphenylisopropylamine; GMP-PNP, guanylyl-imidodiphosphate; PSB, phosphate buffered saline; mCPP, meta chlorophenyl piperazine.

Received 6 April 1995; accepted 4 September 1995.

MATERIALS AND METHODS

Cell Culture and Membrane Preparation

We have previously reported the transfection and functional expression of the gene encoding the human 5-HT_{2A} receptor in NIH 3T3 mouse fibroblasts [9]. Pools of transfected cells were tested for expression by [^{3}H]ketanserin binding. Limited dilution was used to generate a panel of stably growing cell clones. Dissociation constants (K_d) and the maximum number of binding sites (B_{max}) were determined in saturation experiments. One of the clones (clone 9), expressing 27 pmole/mg protein, was selected for radioligand studies. To obtain large quantities of membranes, the recombinant cell clone was cultured in 3.6 l DMEM/Ham's F12 medium (1:1) containing 5% (v/v) newborn bovine serum (hyclone) on cultisphere-G (5 g/L) microcarriers in a celligen bioreactor at 37°C pH 7–7.4, 100–110 rpm. Fresh medium was continuously perfused at a rate of 50% of the initial volume per day until a final cell density of approximately 5×10^6 cells/mL was reached. The carriers containing the cells were washed twice with phosphate-buffered saline (PBS) NaCl (8 g/L), KCl (0.2 g/L), Na_2HPO_4 (1.15 g/L) at room temperature and subsequently incubated in 100 mL Hank's balanced salt solution containing 2.5 mg/mL trypsin (Flow) for 5 min at 37°C. The reaction was stopped by adding 100 mL PBS containing 1.7 mg/mL soybean trypsin inhibitor (Sigma) and the cells were washed with PBS by 2 centrifugations for 10 min at 3000 × g. The resulting pellet was resuspended in 250 mL of ice-cold 50 mM Tris-HCl containing 10 mM $MgCl_2$, 0.5 mM EDTA, and 0.1 mM phenylmethylsulphoyl fluoride (PMSF) using a Polytron homogenizer (15 sec at maximal speed), this homogenate was incubated for 10 min at room temperature. Subsequently, it was centrifuged at 20000 × g for 20 min and the pellets resuspended in the homogenization buffer to which 20% (w/v) glycerol was added to obtain a concentration corresponding to 4×10^7 cells/mL. Aliquots of the homogenate were frozen and stored at −80°C.

Radioligand Binding Assays

On the day of the experiment, membranes were thawed and resuspended in 10 × the original volume of assay buffer. This assay buffer consisted of Tris-HCl 50 mM, pargyline 10^{-5} M, $MgCl_2$ 5 mM and ascorbic acid 0.1%, pH 7.4. All compounds were dissolved in either water or 10% DMSO and diluted in assay buffer. Assays were identical for each radioligand used and consisted of 100 µL of tissue preparation, 50 µl of the radioligand, and 50 µl of a displacing drug or assay buffer. For saturation and displacement analyses, nonspecific binding was defined in the presence of 10^{-5} M methysergide and the specific binding defined as the total binding minus nonspecific binding. All incubations were performed at room temperature for 1 h and the reactions stopped by rapid filtration through Whatmann GF/B filters. The filters were washed with 3 × 2 mL Tris-HCl (50 mM, pH 7.4). The radioactivity retained on the filters was measured by scintillation spectroscopy in 2 mL of scintillation fluid. All experiments were performed in triplicate and repeated at least 3 times.

Saturation analyses were performed for [^{3}H]-5-HT, [^{3}H]-DOB, and [^{3}H]-ketanserin using at least 8 concentrations of each radioligand (concentrations ranging from 0.05–15 nM). K_d and B_{max} were calculated using the EBDA/LIGAND [10, 11] program. The 5-HT_{2A} receptor was labeled with either [^{3}H]-5-HT (3 nM), [^{3}H]-DOB (1 nM), or [^{3}H]-ketanserin (1 nM) and displacement curves were constructed with each radioligand for a number of agonists and antagonists using 7 concentrations of the displacing agents (1 data point per log unit of concentration: 10^{-11}M to 10^{-5} M). Displacement curves were analysed using EBDA/LIGAND to calculate Hill coefficients and pK_i values, respectively.

Experiments with GMP-PNP

In separate experiments, the effect of the stable GTP analogue, GMP-PNP, on the dissociation constant and maximum number of binding sites for [^{3}H]-5-HT was studied. Saturation experiments were performed as described above in the presence and absence of GMP-PNP (200 µM). In another experiment, [^{3}H]-ketanserin was displaced from the 5-HT_{2A} receptor by 19 concentrations of 5-HT (3 data points per log unit of concentration) in the presence and absence of GMP-PNP (200 µM). Each of these experiments was repeated 4 times.

Statistical Analyses

Differences between K_d and B_{max} values for radioligands were analyzed statistically using the unpaired Student's t-test. K_d and B_{max} values are expressed as the arithmetic mean ± SEM. pK_i values are also expressed as the mean ± SEM of at least 3 separate determinations. Data from experiments where 3 data points were used per log unit of concentration were analyzed with both one-site and two-site models using the LIGAND program and the calculated F ratios were used to determine whether a two-site model fitted the data significantly better than a one-site model.

Drugs

Radioligands were purchased from either New England Nuclear or Ammersham. The specific activity for [^{3}H]-5-HT, [^{3}H]-DOB, and [^{3}H]-ketanserin were 27.9, 16.0, and 61.0 Ci/mmole, respectively. All compounds used were synthesised at either Hoffmann-La Roche or Organon or were purchased from either Sigma or Research Biochemicals Inc.

RESULTS

We have previously reported the cloning of the gene encoding the human 5-HT_{2A} receptor and its functional expression in NIH 3T3 cells [9]. From pools of stably transfected cells a single cell line expressing a high density of receptors was selected. To produce large quantities of membranes from this cell line, the fermentation technique was used to scale up the cell culture. When grown on cultispher-G microbeads, a 3.6l fermentor yielded approximately 2×10^{10} cells. As described in

the Methods section, a membrane batch from freshly cultured cells was prepared and used for the binding experiments.

Saturation Experiments

To verify the presence of [^{3}H]-agonist and [^{3}H]-antagonist binding sites, saturation binding experiments with agonist [^{3}H]-5-HT, the partial agonist [^{3}H]-DOB, and the antagonist [H]-ketanserin were performed. The corresponding K_d and B_{max} values are given in Table 1. Each of the radioligands labeled the binding site with nanomolar affinity and yielded linear Scatchard plots. Figure 1 shows Scatchard plots from one set of experiments that were representative of the other experiments. Furthermore, [^{3}H]-ketanserin labeled recombinant receptors with a similar affinity with which it labels rat cortical 5-HT_{2A} receptors [8]. The number of binding sites labeled with [^{3}H]-5-HT and [^{3}H]-DOB was significantly smaller than the number of sites labeled by [^{3}H]-ketanserin. Furthermore, [^{3}H]-DOB labeled significantly more sites than [^{3}H]-5-HT (Table 1).

Displacement Experiments

To determine the affinity of a number of 5-HT receptor agonists and antagonists for the human 5-HT_{2A} receptor, competition binding studies using [^{3}H]-5-HT, [^{3}H]-DOB, and [^{3}H]-ketanserin as radioligands were performed. The corresponding affinities and Hill coefficients are shown in Table 2. In all experiments, Hill slopes were not significantly different from unity, consistent with the labeling of a single population of binding sites. The labeling of the 5-HT_{2A} receptor with different radioligands did not affect the apparent affinities of the antagonists tested for the binding site. This, however, was not the case for the agonists tested. The affinities of the agonists varied in some cases by over a log unit between the [^{3}H]-agonist and [^{3}H]-antagonist labeled receptor. The pK_i values for 5-HT, DOI, mCPP, and quipazine determined by competition with [^{3}H]-5-HT were higher than those observed with [^{3}H]-DOB which, in turn, were greater than those found with [^{3}H]-ketanserin (Table 2).

Experiments with GMP-PNP

In the absence of GMP-PNP, the K_d and B_{max} values for [^{3}H]-5-HT were 1.3 nM and 3461 fmoles/mg protein, respectively.

TABLE 1. Binding parameters calculated from saturation experiments performed with [^{3}H]5-HT, [^{3}H]DOB, and [^{3}H]ketanserin

	[^{3}H]-5-HT	[^{3}H]-DOB	[^{3}H]-ketanserin
K_d (nM)	1.3 ± 0.03	0.8 ± 0.05	1.15 ± 0.1
B_{max} (fmol/mg protein)	3461 ± 186 *†	8332 ± 16 *	27684 ± 1500

Values are given as means ± SEM of 3 determinations.
* $P < 0.005$ compared to [^{3}H]ketanserin data.
†$P < 0.005$ compared to [^{3}H]DOB data.

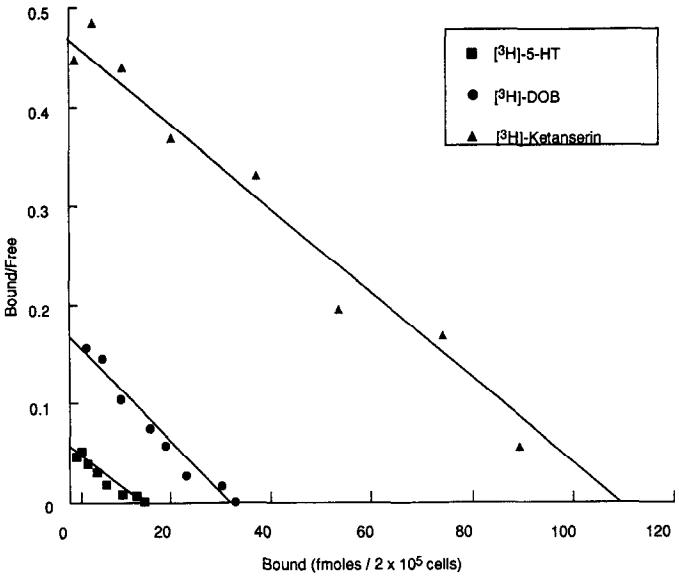

FIG. 1. Examples of Scatchard plots of the saturation experiments using [^{3}H]-5-HT, [^{3}H]-DOB, and [^{3}H]-ketanserin. Eight concentrations of each radioligand were used. Nonspecific binding was measured in the presence of 10 μM methysergide in all cases and specific binding was defined as total binding minus nonspecific binding. The data are taken from a single experiment that was repeated 3 times.

In the presence of GMP-PNP (200 μM), insufficient binding remained to permit analysis (data not shown). These experiments were repeated 3 times and similar results were obtained in each experiment. The displacement of [^{3}H]-ketanserin by 19 concentrations of 5-HT (3 data points per log unit of concentration) from the 5-HT_{2A} receptor was studied in the presence and absence of GMP-PNP. The pKi values of 5-HT for the [^{3}H]-ketanserin binding site in these experiments were 6.87 and 6.94 in the presence and absence of GMP-PNP, respectively. These data agree with those shown in Table 2. Furthermore, in the absence of GMP-PNP, the Hill coefficient was 0.98 and a 2-site model did not fit the data significantly better than a 1-site model ($F = 2.14$ (d.f. 16,14) $P = 0.16$). GMP-PNP (200 μM) had no significant effect on either the affinity of 5-HT or on the slope of the displacement curve. Again, this experiment was repeated 4 times and displacement curves from one of these experiments is shown in Fig. 2.

DISCUSSION

Originally, the 5-HT_{2A} receptor subtype was classified by its low affinity for 5-HT [3]. High-affinity binding sites for the partial agonists [^{3}H]-DOB and [^{125}I]-DOI [12] have subsequently been described in cell lines transfected with the rat 5-HT_{2A} receptor and in animal tissues [6–8]. In the present study, we report for the first time that human recombinant 5-HT_{2A} receptors display high affinity for [^{3}H]-5-HT, in addition to the more classical radioligands used for this receptor such as [^{3}H]-DOB and [^{3}H]-ketanserin. Furthermore, the affinities of some reference compounds were estimated when the receptor was labeled with radioligands displaying different in-

TABLE 2. Affinities of 5-HT_2 agonists and antagonists at 5-HT_{2A} receptor binding sites labeled with either [^{3}H]5-HT, [^{3}H]DOB, or [^{3}H]ketanserin

Compound	pK_i		
	[^{3}H]-5-HT	[^{3}H]-DOB	[^{3}H]-ketanserin
5-HT	8.42 ± 0.03 (0.91)	7.72 ± 0.03 (0.86)	6.84 ± 0.08 (0.98)
DOI	9.22 ± 0.18 (0.97)	8.78 ± 0.02 (0.83)	7.97 ± 0.03 (0.86)
mCPP	7.29 ± 0.18 (0.90)	7.14 ± 0.02 (0.93)	6.82 ± 0.01 (0.93)
Quipazine	7.51 ± 0.08 (0.94)	6.75 ± 0.04 (0.89)	5.81 ± 0.10 (0.94)
Spiperone	8.60 ± 0.17 (0.81)	8.57 ± 0.05 (0.91)	8.71 ± 0.07 (0.81)
Mesulergine	7.49 ± 0.02 (0.83)	7.02 ± 0.03 (0.94)	7.26 ± 0.02 (1.1)
Ketanserin	8.49 ± 0.14 (0.82)	8.26 ± 0.11 (0.96)	8.63 ± 0.03 (1.2)

Data represent the mean ± SEM of 3–5 experiments. Hill coefficients are given in parentheses.

trinsic efficacies for the 5-HT_{2A} receptor (i.e. the antagonist [^{3}H]-ketanserin [12–16], the partial agonist [^{3}H]-DOB [12], and the endogenous agonist [^{3}H]-5-HT [12–16].

For this study, we have used a recombinant cell line transfected with the human 5-HT_{2A} receptor gene that was previously cloned [9]. To perform [^{3}H]-agonist and [^{3}H]-antagonist binding on a single membrane preparation devoid of other 5-HT receptor subtypes, the production of a stably transfected NIH 3T3 cell line expressing a high density of 5-HT_{2A} receptors was scaled up using the fermentation techniques described in the present report.

In these membranes, we demonstrated that human 5-HT_{2A} receptors could be labeled with [^{3}H]-5-HT with a K_d value of 1.3 nM. This is in agreement with calculated K_i values obtained by labeling the 5-HT_{2A} receptor with [^{3}H]-DOB or [^{125}I]-DOI [6–8]. These findings are in contrast with the original classification of the 5-HT_{2A} receptor as having low affinity for 5-HT [3]. Of the 3 radioligands used, [^{3}H]-5-HT labeled significantly fewer binding sites than did [^{3}H]-DOB which, in turn, labeled significantly fewer binding sites than [^{3}H]-ketanserin (Fig. 1). This relationship appears to be in the order of the intrinsic efficacies of the radioligands in the 5-HT_{2A} receptor-mediated stimulation of phosphatidylinositol metabolism [12]. It has previously been shown that there was no significant binding detected in cell lines transfected with vector DNA only [9].

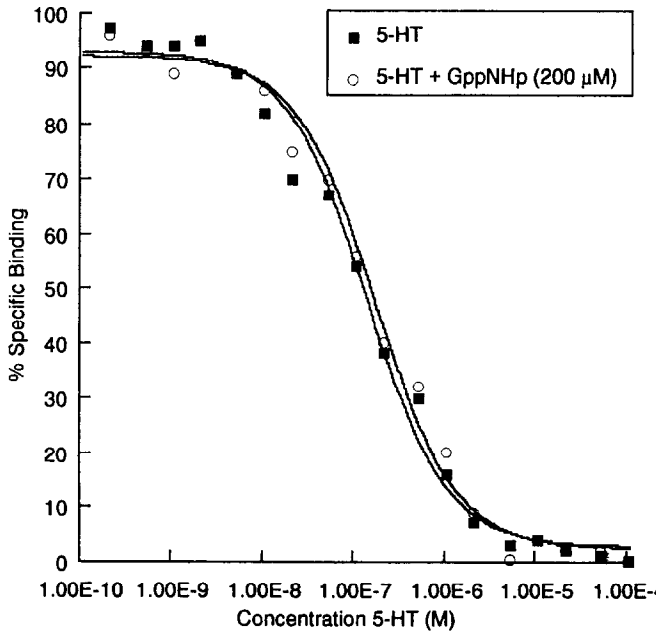

FIG. 2. Displacement of [^{3}H]-ketanserin from 5-HT_{2A} receptors by 5-HT in the presence and absence of GMP-PNP (200 μM). Nonspecific binding was measured in the presence of methysergide (10 μM) and specific binding was defined as total binding minus nonspecific binding. All values are expressed as percentage of specific binding. The data are taken from a single experiment that was repeated 4 times.

To further characterize the [^{3}H]-agonist and [^{3}H]-antagonist labeled 5-HT_{2A} receptor binding sites, displacement studies with a number of agonists and antagonists were performed. Labeling the human 5-HT_{2A} receptor with the various radioligands did not appear to have any significant effect on the estimated affinity of the antagonists tested, although spiperone and mesulergine did have lower affinity for the human receptor than has been reported for the rat receptor [17, 18]. In the case of mesulergine, a single amino acid difference in transmembrane segment V between the human and rat receptor has been found to be responsible for this species difference [19]. The affinities of the agonists tested, however, did vary with the radioligand used. Agonists had highest affinity for receptors labeled with the agonist [^{3}H]-5-HT and lowest affinity for receptors labeled with the antagonist [^{3}H]-ketanserin.

These findings can be explained on the basis of the G-protein coupling with the receptor. Agonists potentiate the coupling of the receptor to G-proteins which, in turn, allows the receptor to have high affinity for agonists. According to this hypothesis, a full agonist such as 5-HT would predominantly label the high-affinity state, whereas an antagonist such as ketanserin would have equal affinity to both the high- and low-affinity states of the receptor. Our finding that the B_{max} for [^{3}H]-5-HT is much smaller than that found for [^{3}H]-ketanserin is in line with this theory. It is, therefore, not surprising that the B_{max} for the partial agonist [^{3}H]-DOB is between that of a full agonist and that of a full antagonist because it will also label some low-affinity sites. Therefore, if displacement studies are performed in the presence of a partial agonist or an antagonist, it may be less likely that the receptor is in its

G-protein coupled high-affinity state and, hence, will have lower affinity for a displacing agonist.

Theoretically, the displacement of [^{3}H]-ketanserin by 5-HT should be biphasic and modified by GTP analogues; however, in the present study, the Hill coefficient of the displacement was 0.98 and the curve was monophasic (Fig. 2). Furthermore, although specific [^{3}H]-5-HT binding was reduced by GMP-PNP, the GTP analogue did not modify the displacement of [^{3}H]-ketanserin by 5-HT. These data are in agreement with previously published reports [20]. The absence of a GMP-PNP effect on the 5-HT displacement of [^{3}H]-ketanserin in the cell line used in the present study may be due to the small proportion of high-affinity sites (the number of sites labeled by [^{3}H]-5-HT is approximately 13% of the number labeled by [^{3}H]-ketanserin). Therefore, the proportion of high-affinity sites seen in the displacement of [^{3}H]-ketanserin by 5-HT is probably too small to be detected by the present binding techniques. However, the data do suggest that in our cell line, and in other cell lines expressing high numbers of receptors of which only a small proportion are in the high-affinity state, the radioligand is more important than the displacing drug in determining whether the receptor is in its high- or low-affinity state. Similar findings have not been reported for the 5-HT_{2C} receptor, where the affinities of agonists are similar when the binding site is labeled with either an agonist [^{3}H]-5-HT or an antagonist [^{3}H]-mesulergine [21–23]. Unfortunately, in the present study, it was not possible to determine whether or not this discrepancy between the 5-HT_{2A} and the 5-HT_{2C} receptors is due to differences in the pharmacology of the 2 receptors or to some intrinsic properties of the radioligands used because [^{3}H]-mesulergine was found to be a poor radioligand for human 5-HT_{2A} receptors (Sleight, 1994, unpublished observations). Nevertheless, it is interesting to speculate that one of the main differences between the 5-HT_{2A} and the 5-HT_{2C} receptors is not their affinity for 5-HT, but the differences between their high- and low-affinity states.

In summary, our study shows that human 5-HT_{2A} receptors expressed in NIH 3T3 cells can be labeled with [^{3}H]5-HT and that radioligands with varying intrinsic efficacies label different numbers of receptor binding sites expressed in the same cell line. Furthermore, the affinities of agonists for the 5-HT_{2A} receptor also appear to depend on the intrinsic efficacy of the radioligand and, therefore, care should be taken with the choice of radioligand used to estimate the affinity of agonists for the 5-HT_{2A} receptor. As always, it should not be forgotten that this study was performed with recombinant receptors expressed at high levels and that care should be taken when extrapolating these effects to those that may occur in native receptors.

The authors are grateful to Dr. P. Levering (N.V. Organon) for performing fermentation cultures of the clonal cell line NIH 3T3-5HT_{2A}-5 and Alan Rudler, Valerie Meyer, Elizabeth Bixel, and Catherine Carolo for their excellent technical assistance with the radioligand binding assays.

References

1. Boess FG and Martin IL, Molecular biology of 5-HT receptors. *Neuropharmacol* **133:** 275–317, 1994.
2. Gaddum JH and Picarelli ZP, Two kinds of tryptamine receptor. *Br J Pharmacol Chemother* **12:** 323–328, 1957.
3. Peroutka SJ and Snyder SH, Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid and [^{3}H]spiperone. *Mol Pharmacol* **16:** 687–699, 1979.
4. Humphrey PPA, Hartig P and Hoyer D, A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol Sci* **14:** 233–236, 1993.
5. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR and Humphrey PPA, International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* **46:** 157–204, 1994.
6. Titeler M, Herrick K, Lyon RA, McKenney JD and Glennon RA, [^{3}H]DOB: A specific agonist radioligand for 5-HT_2 serotonin receptors. *Eur J Pharmacol* **117:** 145–146, 1985.
7. Teitler M, Leonhardt S, Weisberg EL and Hoffman BJ, 4-[^{125}I]iodo-(2,5-dimethoxy)phenylisopropylamine and [^{3}H]ketanserin labeling of 5-hydroxytryptamine$_2$ (5-HT_2 receptors in mammalian cells transfected with a rat 5-HT_2 cDNA: Evidence for multiple states and not multiple 5-HT_2 receptor subtypes. *Mol Pharmacol* **38:** 594–598, 1990.
8. McKenna DJ and Peroutka SJ, Differentiation of 5-hydroxytryptamine$_2$ receptor subtypes using ^{125}I-R-(-)2,5-dimthoxy-4-iodophenylisopropylamine and [^{3}H]-ketanserin. *J Neurosci* **9:** 3482–3490, 1989.
9. Stam NJ, Van Huizen F, Van Alebeck C, Brands J, Dijkema R, Tonnaer JADM and Olijve W, Genomic organization, coding sequence and functional expression of human 5-HT_2 and 5-HT_{1A} receptor genes. *Eur J Pharmacol Mol Pharmacol Section* **227:** 153–162, 1992.
10. McPherson GA, *KINETIC, EBDA, LIGAND, LOWRY: A Collection of Radioligand Binding Analysis Programmes*, p. 23, Biosoft, Cambridge, 1985.
11. Munson PJ and Rodbard D, LIGAND: a versatile computerized approach for characterisation of ligand-binding systems. *Anal Biochem* **107:** 220–239, 1980.
12. Pierce PA and Peroutka SJ, Antagonism of 5-hydroxytryptamine$_2$ receptor-mediated phosphatidylinositol turnover by d-lysergic acid diethylamide. *J Pharmacol Exp Ther* **247:** 918–925, 1988.
13. Conn PJ and Sanders-Bush E, Serotonin-stimulated phosphoinositide turnover: Mediation by the S_2 binding site in rat cerebral cortex but not in subcortical regions. *J Pharmacol Exp Ther* **234:** 195–203, 1985.
14. Conn PJ and Sanders-Bush E, Selective 5HT-2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. *Neuropharmacol* **23:** 993–996, 1984.
15. Conn PJ and Sanders-Bush E, Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT-2 binding site. *J Neurosci* **6:** 3669–3675, 1986.
16. Chaffoy de Courcelles D, Leysen JE, De Clerck F, Van Belle H and Janssen PAJ, Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S_2 receptor sites. *J Biol Chemistry* **260:** 7603–7608, 1985.
17. Hoyer D, Engel G and Kalkman HO, Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (-)[^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. *Eur J Pharmacol* **118:** 13–23, 1985.
18. Choudhary MS, Craigo S and Roth BL, A single point mutation (Phe^{340}-Leu^{340}) of a conserved phenylalanine abolishes 4-[^{125}I]iodo-(2,5-dimethoxy)phenylisopropylamine and [^{3}H]mesulergine but not [^{3}H]ketanserin binding to 5-hydroxytryptamine$_2$ receptors. *Mol Pharmacol* **43:** 755–761, 1993.
19. Johnson MP, Loncharich RJ, Baez M and Nelson DL, Species variation in transmembrane region V of the 5-hydroxytrypta-

mine type 2A receptor alter the structure-activity relationship of certain ergolines and tryptamines. *Mol Pharmacol* **45:** 277–286, 1994.
20. Szele FG and Pritchett DB, High affinity agonist binding to cloned 5-hydroxytryptamine$_2$ receptor is not sensitive to GTP analogs. *Mol Pharmacol* **43:** 915–920, 1993.
21. Havlik S and Peroutka SJ, Differential radioligand binding properties of [^{3}H]5-hydroxytryptamine and [^{3}H]mesulergine in a clonal 5-hydroxytryptamine$_{1C}$ cell line. *Brain Res* **584:** 191–196, 1992.
22. Leonhardt S, Gorospe E, Hoffman BJ and Titeler M, Molecular pharmacological differences in the interaction of serotonin with 5-hydroxytryptamine$_{1C}$ and 5-hydroxytryptamine$_2$ receptors. *Mol Pharmacol* **42:** 328–335, 1992.
23. Stam NJ, Vanderheyden PMLl, van Alebeek C, Klomp J, de Boer T, van Delft AML and Olyve W, Genomic organisation and functional expression of the gene encoding the human serotonin 5-HT$_{2C}$ receptor. *Eur J Pharmacol Mol Pharmacol Section* **269:** 339–348, 1994.