British Journal of Pharmacology (2000) 130, 1108–1114 
www.nature.com/bjp

# 5-$HT_{1A}$ receptor agonist-antagonist binding affinity difference as a measure of intrinsic activity in recombinant and native tissue systems

*,[1]J. Watson, [1]L. Collin, [1]M. Ho, [1]G. Riley, [1]C. Scott, [1]J.V. Selkirk & [1]G.W. Price

[1]Neuroscience Research, SmithKline Beecham Pharmaceuticals, NFSP, Harlow, Essex, CM19 5AW

**1** It has been reported that radiolabelled agonist : antagonist binding affinity ratios can predict functional efficacy at several different receptors. This study investigates whether this prediction is true for recombinant and native tissue 5-$HT_{1A}$ receptors.

**2** Saturation studies using [$^3$H]-8-OH-DPAT and [$^3$H]-MPPF revealed a single, high affinity site ($K_D \sim 1$ nM) in HEK293 cells expressing human 5-$HT_{1A}$ receptors and rat cortex. In recombinant cells, [$^3$H]-MPPF labelled 3–4 fold more sites than [$^3$H]-8-OH-DPAT suggesting the presence of more than one affinity state of the receptor. [$^3$H]-Spiperone labelled a single, lower affinity site in HEK293 cells expressing h5-$HT_{1A}$ receptors but did not bind to native tissue 5-$HT_{1A}$ receptors. These data suggest that, in transfected HEK293 cells, human 5-$HT_{1A}$ receptors exist in different affinity states but in native rat cortical tissue the majority of receptors appear to exist in the high agonist affinity state.

**3** Receptor agonists inhibited [$^3$H]-MPPF binding from recombinant 5-$HT_{1A}$ receptors in a biphasic manner, whereas antagonists and partial agonists gave monophasic inhibition curves. All compounds displaced [$^3$H]-8-OH-DPAT and [$^3$H]-spiperone binding in a monophasic manner. In rat cortex, all compounds displaced [$^3$H]-MPPF and [$^3$H]-8-OH-DPAT in a monophasic manner.

**4** Functional evaluation of compounds, using [$^{35}$S]-GTP$\gamma$S binding, produced a range of intrinsic activities from full agonism, displayed by 5-HT and 5-CT to inverse agonism displayed by spiperone.

**5** [$^3$H]-8-OH-DPAT : [$^3$H]-MPPF $pK_i$ difference correlated well with functional intrinsic activity ($r = 0.86$) as did [$^3$H]-8-OH-DPAT : [$^3$H]-spiperone $pK_i$ difference with functional intrinsic activity ($r = 0.96$).

**6** Thus agonist : antagonist binding affinity differences may be used to predict functional efficacy at human 5-$HT_{1A}$ receptors expressed in HEK293 cells where both high and low agonist affinity states are present but not at native rat cortical 5-$HT_{1A}$ receptors in which only the high agonist affinity state was detectable.

*British Journal of Pharmacology* (2000) **130,** 1108–1114

**Keywords:** 5-$HT_{1A}$ receptor; [$^3$H]-8-OH-DPAT; [$^3$H]-MPPF, [$^3$H]-spiperone; radioligand binding; intrinsic activity

**Abbreviations:** h, human; HEK, human embryonic kidney; 8-OH-DPAT, 8-Hydroxy-dipropylaminotetralin hydrobromide

## Introduction

The classical ternary complex model assumes that there is a direct relationship between the ability of a drug to stabilize the active state of a receptor and its ability to elicit a response (De Lean *et al*., 1980). This concept is also used for more recent models such as the two-state model of agonist action, which suggests that ligands which bind with high affinity to the active form of a receptor are agonists whereas antagonists do not discriminate between the active or inactive state of the receptor. Ligands which bind with higher affinity to the inactive state of the receptor have been demonstrated to be inverse agonists (Sammama *et al*., 1993; Leff, 1995). These different agonist states have been shown to exist for several receptors such as serotonin, dopamine, muscarinic and $\beta$-adrenoreceptors (Clawges *et al*., 1997; Freedman *et al*., 1988; Garnier *et al*., 1998; Houston & Howlett, 1998; Lahti *et al*., 1992; Tayebati *et al*., 1999). It has previously been reported that the ratio of the affinity of a compound for high and low agonist affinity states of muscarinic receptors is a reasonable prediction of intrinsic activity (Freedman *et al*., 1988; Loudon *et al*., 1997; Tayebati *et al*., 1999). Similar studies have also shown that efficacy at cannabinoid $CB_1$, $D_2$ receptors and human 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors also correlate well with high affinity agonist binding (Fitzgerald *et al*., 1999; Kearn *et al*., 1999; Lahti *et al*., 1992).

Human 5-$HT_{1A}$ receptors have also been shown to exist in high and low agonist affinity states in transfected cell lines (Clawges *et al*., 1997; Price *et al*., 1998). We have investigated the different states of the human recombinant 5-$HT_{1A}$ receptor expressed in HEK293 cells and native 5-$HT_{1A}$ receptors in rat cortex using [$^3$H]-8-OH-DPAT as an agonist radioligand (Sijbesma *et al*., 1991) and [$^3$H]-MPPF and [$^3$H]-spiperone as antagonist radioligands (Kung *et al*., 1996; Sundaram *et al*., 1992). We have then compared compound-induced inhibition of binding of these radioligands with compound efficacy, measured by human recombinant 5-$HT_{1A}$ receptor stimulation of [$^{35}$S]-GTP$\gamma$S binding, to determine if a relationship exists between binding affinity differences and functional intrinsic activity.

*Author for correspondence.

## Methods

### *Receptor binding*

HEK (human embryonic kidney) 293 cells stably expressing human (h)5-HT$_{1A}$ receptors were homogenized and washed twice with ice-cold TRIS buffer (TRIS 50 mM $MgCl_2$ 10 mM, pH 7.4) (Watson *et al*., 1998). Rat cerebral cortex was excised, homogenized in TRIS buffer and incubated at 37°C for 15 min prior to washing twice. Both sets of membranes were stored at −80°C until required.

Membranes (from $5 \times 10^5$ cells or 10 mg original wet weight tissue per well) were incubated in TRIS buffer containing [$^3$H]-8-OH-DPAT (2 nM), [$^3$H]-MPPF (0.3 nM) or [$^3$H]-spiperone (1 nM) with/without compounds at 37°C for 45 min. Non-specific binding was defined by buspirone (10 $\mu$M). In saturation studies increasing concentrations of radioligand were used: [$^3$H]-8-OH-DPAT (0.1–40 nM), [$^3$H]-MPPF (0.1–15 nM) and [$^3$H]-spiperone (1.5–100 nM). Incubations were terminated by filtration over GF/B filters, followed by $6 \times 1$ ml washes with ice cold TRIS buffer. Bound radioactivity was measured by liquid scintillation spectrometry.

### *[$^{35}$S]-GTP$\gamma$S binding*

HEK293 cells stably expressing recombinant h5-HT$_{1A}$ receptors were prepared and assayed according to the method used by Watson *et al*., 1998. Briefly, membranes were pre-incubated in 20 mM HEPES containing 3 mM $MgCl_2$, 100 mM NaCl, 0.2 mM ascorbate and 10 $\mu$M GDP. The reaction was started by the addition of 100 pM [$^{35}$S]-GTP$\gamma$S and incubated for a further 30 min. The reaction was terminated by rapid filtration over GF/B filters and bound radioactivity was measured by scintillation spectrometry. Intrinsic activity was calculated as per cent stimulation of basal specific [$^{35}$S]-GTP$\gamma$S binding and expressed as a fraction of the maximal 5-HT response.

### *Materials*

Drugs and reagents were purchased from Sigma-Aldrich (Poole, U.K.), Calbiochem (Nottingham, U.K.), Bio-Rad (Hemel Hempstead, U.K.), Fisons Scientific Equipment (Loughborough, U.K.), Research Biochemicals International (Poole, U.K.), Tocris Cookson Ltd. (Bristol, U.K.) and GibcoBRL (Paisley, U.K.). [$^{35}$S]-GTP$\gamma$S, [$^3$H]-8-OH-DPAT, [$^3$H]-MPPF and [$^3$H]-spiperone were supplied by Amersham International (Little Chalfont, U.K.).

### *Data analysis*

Data from both radioligand binding studies and [$^{35}$S]-GTP$\gamma$S binding studies were fitted by a 4-parameter logistic equation using GRAFIT (Erithacus Software Ltd.) to yield $IC_{50}$ and $EC_{50}$ values respectively. In binding studies, pK$_i$ values were calculated from $IC_{50}$ values using the correction for radioligand concentration described by Cheng & Prusoff (1973).

## Results

### *Receptor binding studies*

*Saturation studies* In HEK293 cells expressing h5-HT$_{1A}$ receptors, [$^3$H]-8-OH-DPAT and [$^3$H]-MPPF appeared to bind to a single population of sites, however [$^3$H]-MPPF labelled over three times as many sites as [$^3$H]-8-OH-DPAT (Table 1). [$^3$H]-spiperone also appeared to bind a single component and labelled a similar number of sites as [$^3$H]-8-OH-DPAT. In rat cortex, [$^3$H]-8-OH-DPAT and [$^3$H]-MPPF bound in a monophasic manner and labelled a similar number of binding sites. However, [$^3$H]-spiperone failed to give a robust, specific binding signal in rat cortex (data not shown), presumably because of the low affinity that spiperone displays for this native receptor (see below).

### *Inhibition of binding by agonists and antagonists*

*Cloned h5-HT$_{1A}$ receptors* The agonists, 5-CT, 5-HT and 8-OH-DPAT displayed over 10 fold greater affinity at displacing [$^3$H]-8-OH-DPAT binding than [$^3$H]-MPPF or [$^3$H]-spiperone (Figure 1a,c,e and Table 2). Monophasic inhibition curves were generated with agonists using both [$^3$H]-8-OH-DPAT and [$^3$H]-spiperone, however biphasic inhibition curves were observed with [$^3$H]-MPPF. Data from 2-site curve fitting revealed that the high affinity agonist component represented 25% of specific binding which correlated well with the [$^3$H]-8-OH-DPAT saturation studies, where the agonist radiolabel appeared to label only 0.27 of the total number of binding sites. The 5-HT$_{1A}$ receptor antagonists, WAY100635, MPPI and spiperone displayed monophasic inhibition curves with comparable potency, with all three radioligands (Table 2).

*Rat cortex* Agonists and antagonists inhibited [$^3$H]-8-OH-DPAT and [$^3$H]-MPPF binding with similar affinity and gave monophasic inhibition curves (Figure 1b,d and Table 2).

### *Functional studies – [$^{35}$S]-GTP$\gamma$S binding studies*

The full agonist 5-HT, stimulated [$^{35}$S]-GTP$\gamma$S binding by approximately 60% above basal with a $pEC_{50} = 8.4$. Other compounds displayed a range of intrinsic activities when their response (per cent stimulation) was expressed as a fraction of the maximal 5-HT response (Figure 2a,b and Table 3). Inverse agonist activities were expressed relative to spiperone which was designated as a full inverse agonist.

### *Correlation between functional intrinsic activity (I.A.) and agonist : antagonist pK$_i$ difference at h5-HT$_{1A}$ receptors*

[$^3$H]-8-OH-DPAT: [$^3$H]-MPPF pK$_i$ difference for standard compounds correlated well with functional I.A. resulting in a correlation coefficient *(r)* of 0.86 (Figure 3a). [$^3$H]-8-OH-DPAT: [$^3$H]-spiperone pK$_i$ difference for standards showed a

**Table 1** Saturation studies in HEK293 cells expressing h5-HT$_{1A}$ receptors and native tissue

| | *[$^3$H]-8-OH-DPAT* | | *[$^3$H]-MPPF* | | *[$^3$H]-spiperone* |
|---|---|---|---|---|---|
| | *5-HT$_{1A}$/HEK* | *Rat cortex* | *5-HT$_{1A}$/HEK* | *Rat cortex* | *5-HT$_{1A}$/HEK* |
| $K_d$ (nM) | 1.30±0.07 | 1.4±0.2 | 0.8±0.04 | 1.4±0.1 | 10.9±1.1 |
| $B_{max}$ (pmol mg$^{-1}$ protein) | 2.4±0.32 | 0.3±0.01 | 8.7±0.9 | 0.4±0.01 | 2.9±0.4 |

Data are expressed as mean±s.e.mean from three individual experiments, each performed in duplicate.

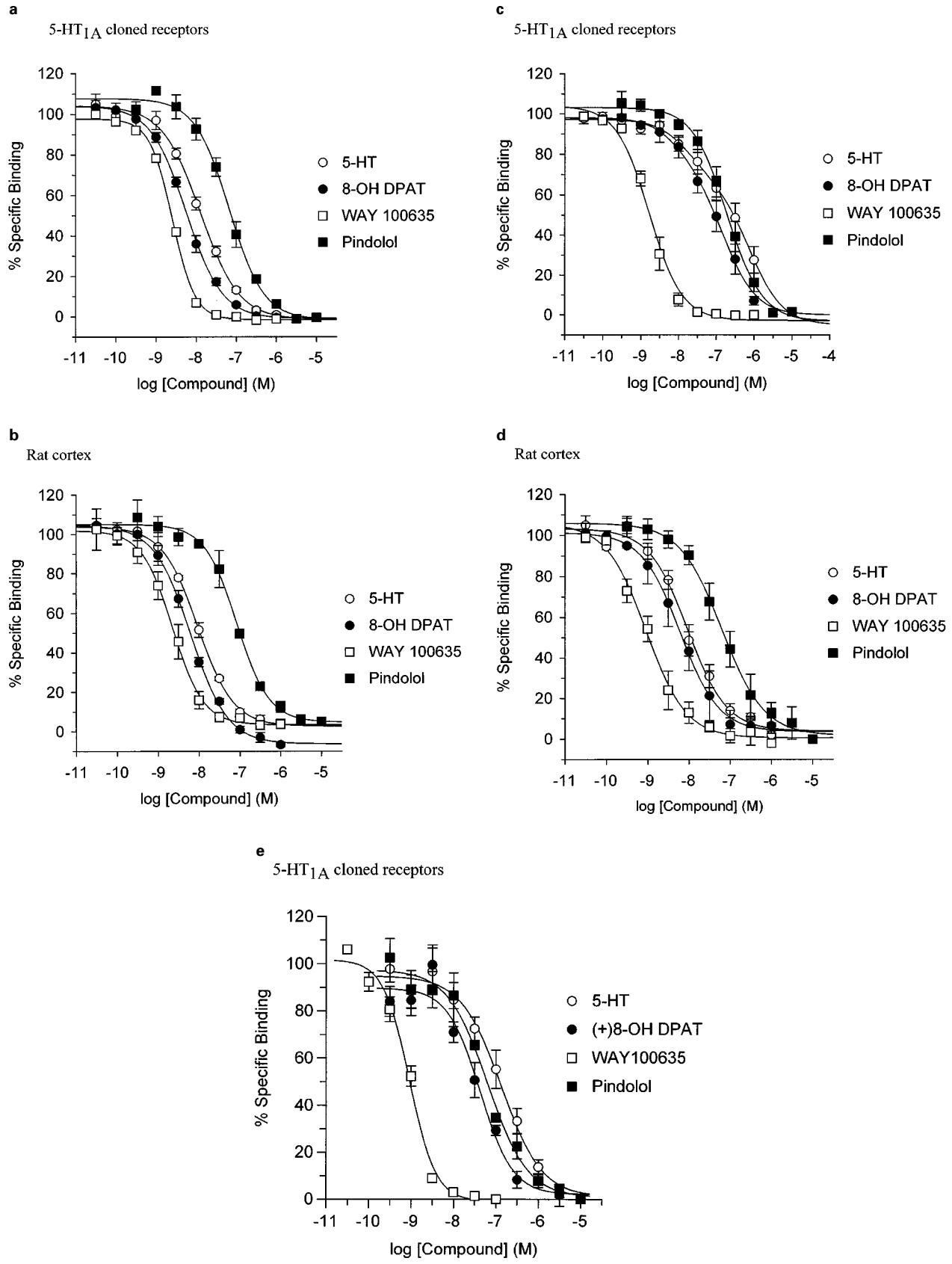


**Figure 1** Displacement of agonist and antagonist radioligand binding by compounds with affinity for 5-$HT_{1A}$ receptors. (a and b) Shows inhibition of binding of [$^3$H]-8-OH-DPAT from human 5-$HT_{1A}$ receptors expressed in HEK293 cells and rat cortex, respectively. (c and d) Shows inhibition of [$^3$H]-MPPF binding from human 5-$HT_{1A}$ receptors expressed in HEK293 cells and rat cortex, respectively. (e) Shows inhibition of [$^3$H]-spiperone binding from cloned 5-$HT_{1A}$ receptors. Binding to rat cortex could not be detected (data not shown). Data are expressed as the mean$\pm$s.e.mean from at least three individual experiments, each point performed in duplicate. All curves were fitted by a 4-parameter logistic equation.

**Table 2** Agonist and antagonist binding affinities at cloned h5-HT$_{1A}$ receptors and native tissue 5-HT$_{1A}$ receptors

| | *[$^3$H]-8-OH-DPAT* | | *[$^3$H]-MPPF* | | *[$^3$H]-spiperone* |
|---|---|---|---|---|---|
| | *5-HT$_{1A}$/HEK* | *Rat cortex* | *5-HT$_{1A}$/HEK* | *Rat cortex* | *5-HT$_{1A}$/HEK* |
| 5-HT | 8.2 | 8.3 | 8.3 6.6# | 8.7 | 6.9 |
| 5-CT | 9.0 | 9.2 | 9.5 7.4# | 8.8 | 7.6 |
| (+)8-OH-DPAT | 8.5 | 8.6 | 7.4 | 8.2 | 7.4 |
| (−)8-OH-DPAT | 8.4 | 8.5 | 7.5 | 8.2 | 7.4 |
| (+)8-OH-DPAT | 8.5 | 8.5 | 8.4 6.8# | 8.8 | nd |
| Pindolol | 7.4 | 7.3 | 7.3 | 7.8 | 7.2 |
| MPPI | 8.6 | 8.2 | 8.8 | 8.5 | 8.8 |
| WAY100135 | 8.3 | 8.1 | 8.3 | 7.5 | nd |
| WAY100635 | 8.9 | 8.9 | 9.3 | 9.6 | 9.0 |
| Spiperone | 7.4 | 6.9 | 7.9 | 8.4 | 8.2 |

Data are expressed as mean pK$_i$ from three individual experiments, s.e.mean $\leqslant$ 0.1. #represents pK$_i$ of low affinity site labelled by [$^3$H]-MPPF (from biphasic curve fitting) and data is used in Table 3.

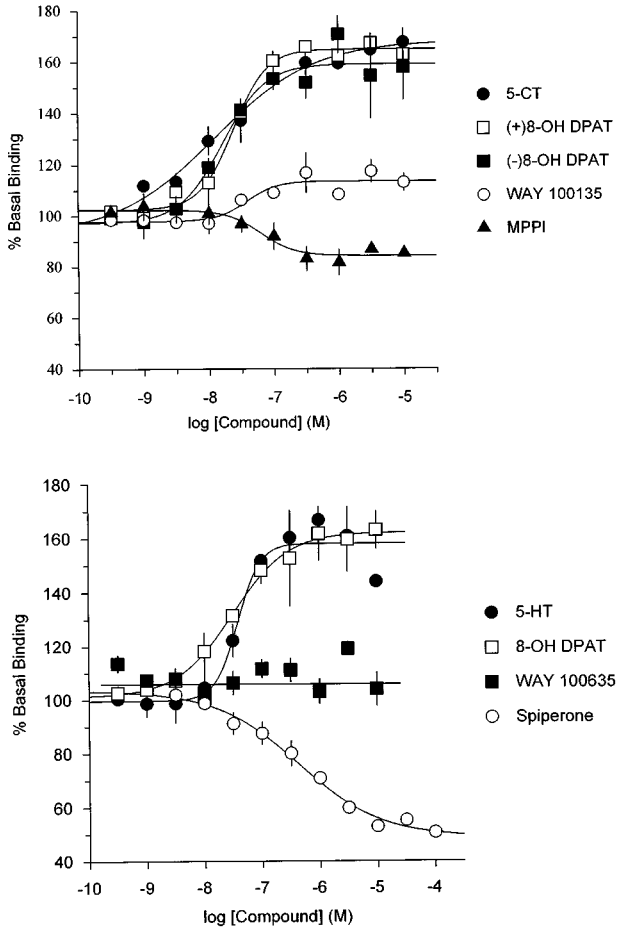


**Figure 2** [$^{35}$S]-GTP$\gamma$S binding to HEK293 cells expressing human 5-HT$_{1A}$ receptors. Data are expressed as per cent of basal [$^{35}$S]-GTP$\gamma$S binding and are the mean $\pm$ s.e.mean from three individual experiments, each performed in duplicate. All curves were fitted by a 4-parameter logistic equation.

linear relationship with functional I.A. resulting in $r = 0.96$ (Figure 3b). The pK$_i$ difference between [$^3$H]-8-OH-DPAT and either antagonist radioligands, for standard compounds, correlated well with $r = 0.96$ (data not shown).

## Discussion

Evaluation of agonist activity at receptors has been based, primarily, on functional tests performed in cell lines expressing cloned receptors and/or native tissue preparations. In most cases, especially for native tissue, functional assessment can be time consuming and complicated to perform. The report of a correlation between [$^3$H]-N-methyl scopolamine (muscarinic antagonist) and [$^3$H]-Oxotremorine-M (muscarinic agonist) binding affinity ratio and functional assessment of muscarinic drugs in rat cortex, which was presumed to be primarily of the M$_1$ receptor sub-type was, therefore, of interest (Freedman *et al*., 1988). These studies resulted in the development of a simple radioligand binding assay, the results of which could estimate the relative intrinsic activity of muscarinic compounds at M$_1$ receptors. Later studies also confirmed this correlation (Loudon *et al*., 1997). These studies were possible since, in rat cortex, muscarinic receptors existed in high and low agonist affinity states (Birdsall *et al*., 1978; 1980), a phenomenon common to several G protein coupled receptors (Clawges *et al*., 1997; De Lean *et al*., 1980; Garnier *et al*., 1998; Houston & Howlett, 1998; Lahti *et al*., 1992). The studies of muscarinic receptors alluded to above were limited to the analysis of the profile of compounds at M$_1$ receptors but recent reports have shown that antagonist : agonist radioligand binding affinity ratios can predict the intrinsic activity of compounds at native tissue cannabinoid and M$_2$ receptors and recombinant 5-HT$_2$ receptors (Fitzgerald *et al*., 1999; Kearn *et al*., 1999; Tayebati *et al*., 1999).

It has been reported that human 5-HT$_{1A}$ receptors, expressed in cultured cells, can also exist in both high and low agonist affinity states (Clawges *et al*., 1997; Price *et al*.,

**Table 3** Comparison of functional activity and agonist : antagonist binding difference in h5-HT$_{1A}$ receptor clones

| | *Intrinsic activity* | *Ag: Antag difference* (8-OH-DPAT: MPPF) | *Ag: Antag difference* (8-OH-DPAT: spiperone) |
|---|---|---|---|
| 5-HT | 1.0 | 1.56 | 1.3 |
| 5-CT | 0.95 | 1.57 | 1.4 |
| (+/−)DPAT | 0.87 | 1.75 | nd |
| (+)DPAT | 0.93 | 1.13 | 1.1 |
| (−)DPAT | 0.88 | 0.87 | 1.0 |
| Pindolol | 0.41 | 0.1 | 0.2 |
| MPPI | −0.4 (inverse) | −0.11 | −0.2 |
| WAY100135 | 0.23 | −0.02 | nd |
| WAY100635 | 0.00 | −0.45 | −0.1 |
| Spiperone | −1.0 (inverse) | −0.46 | −0.8 |

Ag : Antag = pK$_i$ ([$^3$H]-8-OH-DPAT) : pK$_i$([$^3$H]-MPPF)# or pK$_i$ ([$^3$H]-8-OH-DPAT) : pK$_i$ ([$^3$H]spiperone) (data from Table 2). Intrinsic activity is from the [$^{35}$S]-GTP$\gamma$S binding studies, relative to the full agonist 5-HT. Inverse = inhibition of basal [$^{35}$S]-GTP$\gamma$S binding, intrinsic activities relative to spiperone.

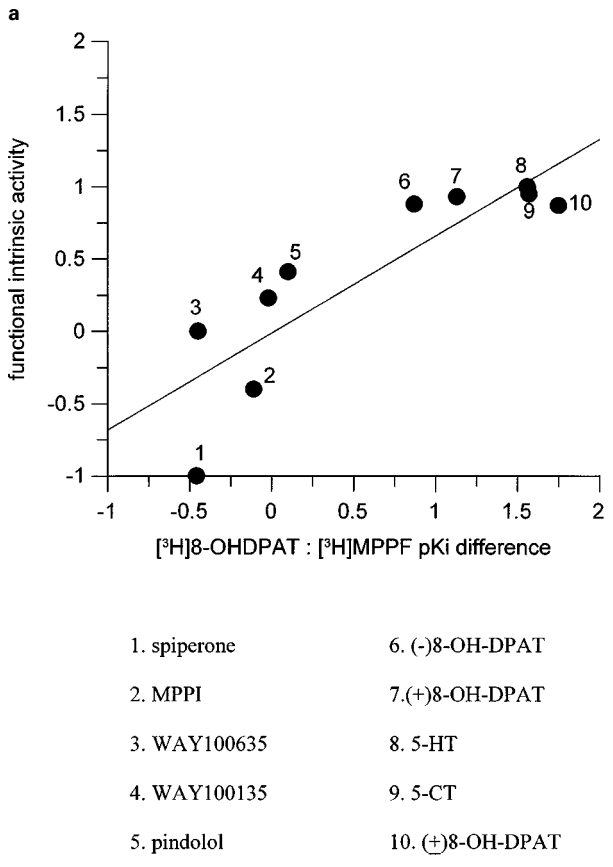


**Figure 3** Relationship between agonist: antagonist binding affinity difference and functional intrinsic activity (I.A.), in HEK293 cells expressing human 5-HT$_{1A}$ receptors. Functional efficacy was determined by [$^{35}$S]-GTP$\gamma$S binding assays. (a) Shows the relationship between [$^3$H]-8-OH-DPAT : [$^3$H]-MPPF pK$_i$ difference and I.A. The correlation between [$^3$H]-8-OH-DPAT : [$^3$H]-spiperone pK$_i$ difference and I.A. is shown in (b).

1998). The aim of our study was, therefore, to determine if the agonist : antagonist binding affinity difference for a selection of compounds predicted functional intrinsic activity at recombinant human 5-HT$_{1A}$ receptors. Similar studies in rat cortex were carried out in parallel. From saturation analysis, the 5-HT$_{1A}$ receptor agonist radioligand, [$^3$H]-8-OH-DPAT (Sijbesma *et al*., 1991), appeared to label a single, high agonist affinity site in both HEK293 cells expressing h5-HT$_{1A}$ receptors and rat cortex. The density of sites in the recombinant system was 8 fold greater than that of the native preparation, a common feature of many cloned receptors. The 5-HT$_{1A}$ antagonist radioligand, [$^3$H]-MPPF (Kung *et al*., 1996), also labelled recombinant and native receptors with high affinity but in the recombinant system, it labelled 3–4 fold more sites than [$^3$H]-8-OH-DPAT. Data from agonist inhibition curves suggested that h5-HT$_{1A}$ receptors, expressed in HEK293 cells, exist in both high and low agonist affinity states, the majority of which are in the low agonist affinity state. The antagonist radioligand, [$^3$H]-spiperone, appeared to selectively label the low agonist affinity site in the HEK cells expressing h5-HT$_{1A}$ receptors but did not bind to native 5-HT$_{1A}$ receptors (data not shown).

In recombinant cells and native tissue, a range of compounds with affinity for 5-HT$_{1A}$ receptors inhibited [$^3$H]-8-OH-DPAT binding in a monophasic manner. However, agonists inhibited [$^3$H]-MPPF binding to human receptors in a biphasic manner, whereas antagonist inhibition curves were monophasic. This supports the above finding that h5-HT$_{1A}$ receptors exist in two agonist affinity states in HEK293 cells. [$^3$H]-MPPF labelled both of these states with similar affinity but agonists, and to a lesser extent partial agonists, discriminated between the two states.

Both saturation and competition studies in rat cortex suggested that the 5-HT$_{1A}$ receptor high affinity state predominates in this preparation. Both agonist and antagonist radioligands labelled a similar number of receptors and agonists produced monophasic inhibition curves with high affinity, with either ligand. This phenomenon has also been reported for native 5-HT$_{1B}$ receptors (Selkirk *et al*., 1997).

In HEK cells expressing h5-HT$_{1A}$ receptors, both agonists and antagonists displaced [$^3$H]-spiperone binding in a monophasic manner but the binding affinity for agonists was lower than those obtained when displacing [$^3$H]-8-OH-DPAT binding or [$^3$H]-MPPF from the high affinity site. There was a good correlation between agonist affinity measured by [$^3$H]-spiperone and the 'low affinity' component of [$^3$H]-MPPF binding. From the studies using [$^3$H]-8-OH-DPAT it was evident that the high agonist affinity state exists in native tissue and so the fact that [$^3$H]-spiperone did not bind to rat cortex and the aforementioned data, together suggest that [$^3$H]-spiperone only binds to the low agonist affinity state. An observation which is consistent with its inverse agonist activity (Sammama *et al*., 1993; Leff, 1995).

[$^{35}$S]-GTP$\gamma$S binding studies were carried out in HEK293 cells expressing h5-HT$_{1A}$ receptors to measure the functional intrinsic activity of the compounds evaluated in radioligand binding studies. A range of intrinsic activities was observed, from full agonism (5-HT, 5-CT) to inverse agonism (spiperone) and the correlation between these efficacy values and binding affinity difference was assessed. The data showed a linear relationship between functional intrinsic activity and [$^3$H]-8-OH-DPAT : [$^3$H]-MPPF and [$^3$H]-8-OH-DPAT : [$^3$H]-spiperone binding affinity differences with correlation coefficients ranging from 0.86–0.96. The results also revealed a good correlation between [$^3$H]-8-OH-DPAT : [$^3$H]-MPPF and [$^3$H]-8-OH-DPAT : [$^3$H]-spiperone binding affinity differences suggesting

that either [$^3$H]-MPPF or [$^3$H]-spiperone could be used as an antagonist radioligand for these particular studies.

It has been suggested that receptor reserve may influence the relationship we are describing in this study (Fitzgerald *et al*., 1999) in that, for systems with high reserve, the I.A. for a partial agonist may increase, with little effect on the high and low-affinity binding values. This certainly appears to be the case in our study. Weak partial agonists such as pindolol and WAY100135, which in many native tissue studies fail to display any intrinsic activity, demonstrate significant efficacy in the [$^{35}$S]-GTP$\gamma$S assay. Interestingly, these compounds show very little difference in their displacement of agonist and antagonist radioligands, consistent with very low efficacy/antagonism. This suggests that the binding affinity ratio is giving a better approximation of tau in a system with high 5-HT$_{1A}$ receptor reserve. The very high receptor expression in this recombinant system is presumably responsible for this receptor reserve. Paradoxically, it is probably this same receptor reserve which allows expression of the low agonist affinity state. A finite number of G proteins exist in recombinant cells and so, assuming receptor : G protein interaction is 1 : 1, if the number of receptors present exceed G protein levels then a proportion of receptors will not couple to G proteins and hence exist in a low agonist affinity state. Many other factors determine which agonist state a receptor can exist in such as $Na^+$, $Mg^{++}$ concentration, cofactors, guanine nucleotides (Houston & Howlett, 1998; Nanoff *et al*., 1995; Stiles, 1988; Vickroy *et al*., 1983) and these will dictate whether or not the studies described can be used to estimate functional activity. Basal constitutive activity may also hinder these types of studies as I.A. may be reduced in cell lines with high basal coupling present.

We have shown that human 5-HT$_{1A}$ receptors expressed in HEK293 cells exist in high and low agonist affinity states, whereas 5-HT$_{1A}$ receptors in rat cortex predominately exist in the high agonist affinity state. Functional evaluation of h5-HT$_{1A}$ receptors expressed in HEK293 cells, using [$^{35}$S]-GTP$\gamma$S binding, revealed a correlation between functional efficacy and agonist : antagonist binding affinity difference for a range of compounds. Therefore, the results of this study suggest that radioligand binding techniques may be a useful tool for predicting the functional activity of compounds at h5-HT$_{1A}$ receptors expressed in HEK293 cells, where both the high and low agonist affinity state exists. The predominance of the high agonist affinity state in rat cortex does not allow this relationship to extrapolate to native tissue 5-HT$_{1A}$ receptors in our studies but a recent report has shown that when rat hippocampal 5-HT$_{1A}$ receptors were manipulated to display both agonist affinity states, then a similar correlation was observed (Assie *et al*., 1999). In this report, antagonist radioligand binding experiments were carried out in the presence of non-hydrolazable GTP analogues to ensure 5-HT$_{1A}$ receptors existed in a low agonist affinity state. Similar studies have been performed in cerebellum membranes to manipulate agonist affinity states of cannabinoid receptors (Kearn *et al*., 1999). Our studies did not include these analogues but future studies are required to investigate their effect on 5-HT$_{1A}$ receptor affinity states in rat cortex.

## References


ASSIE, M.-B., COSI, C. & KOEK, W. (1999). Correlation between low/high affinity ratios for 5-HT1A receptors and intrinsic activity. *E. J. Pharmacol.,* **386,** 97 – 103.

BIRDSALL, N.J.M., BURGEN, A.S.V. & HUME, E.C. (1978). The binding of agonists to brain muscarinic receptors. *Mol. Pharmacol.,* **14,** 723 – 739.

BIRDSALL, N.J.M., BURGEN, A.S.V. & HUME, E.C. (1980). The character of the muscarinic receptors in different regions of the rat brain. *Proc. R. Soc.,* **207,** 1 – 12.

CHENG, Y.C. & PRUSSOF, W.H. (1973). Relationship between inhibition constant (K$_i$) and the concentration of inhibitor which causes 50% inhibition (IC$_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.,* **92,** 881 – 894.

CLAWGES, H.M., DEPREE, K.M., PARKER, E.M. & GRABER, S.G. (1997). Human 5-HT$_1$ receptor subtypes exhibit distinct G protein coupling behaviours in membranes from Sf9 cells. *Biochem.,* **36,** 12930 – 12938.

DE LEAN, A., STADEL, J.M. & LEFKOWITZ, R.J. (1980). A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled $\beta$-adrenergic receptor. *J. Biol. Chem.,* **255,** 7108 – 7117.

FITZGERALD, L.W., CONKLIN, D.S., KRAUSE, C.M., MARSHALL, A.P., PATTERSON, J.P., TRAN, D.P., IYER, G., KOSTICH, W.A., LARGENT, B.L. & HARTIG, P.R. (1999). High-affinity agonist binding correlates with efficacy (intrinsic activity) at the human serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors: evidence favouring the ternary complex and two-state models of agonist action. *J. Neurochem.,* **72,** 2127 – 2134.

FREEDMAN, S.B., HARLEY, E.A. & IVERSEN, L.L. (1988). Relative affinities of drugs acting at cholinoceptors in displacing agonist and antagonist radioligands: the NMS/Oxo-M ratio as an index of efficacy at cortical muscarinic receptors. *Br. J. Pharmacol.,* **93,** 437 – 445.

GARNIER, V., ZINI, R., MORIN, D. & TILLEMENT, J.-P. (1998). Evidence for the stabilisation of the high-affinity state of $\beta$-adrenoreceptors by an endogenous factor in rat brain. *Pharmacol. Res.,* **37,** 365 – 373.

HOUSTON, D.B. & HOWLETT, A.C. (1998). Differential receptor-G-protein coupling evoked by dissimilar cannabinoid receptor agonists. *Cell. Signal,* **10,** 667 – 674.

KEARN, C.S., GREENBERG, M.J., DICAMELLI, R., KURZAWA, K. & HILLARD, C.J. (1999). Relationship between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. *J. Neurochem.,* **72,** 2379 – 2387.

KUNG, H.F. (1996). New 5-HT$_{1A}$ receptor antagonist: [$^3$H]p-MPPF. *Synpase,* **23,** 344 – 346.

LAHTI, R.A., FIGUR, L.M., PIERCEY, M.F., RUPPEL, P.L. & EVANS, D.L. (1992). Intrinsic activity determinations at the dopamine D$_2$ guanine nucleotide-binding protein-coupled receptor: utilisation of receptor state binding affinities. *Mol. Pharmacol.,* **42,** 432 – 438.

LEFF, P. (1995). The two-state model of receptor activation. *Trends. Pharmacol. Sci.,* **16,** 89 – 97.

LOUDON, J.M., BROMIDGE, S.M., BROWN, F., CLARK, M.S.G., HATCHER, J.P., HAWKINS, J., RILEY, G.J., NOY, G. & ORLEK, B.S. (1997). SB 202026 : a novel muscarinic partial agonist with functional selectivity for M$_1$ receptors. *J. Pharmacol. Exp. Ther.,* **283,** 1059 – 1068.

NANOFF, C., MITTERAUER, T., ROKA, F., HOHENEGGER, M. & FREISSMUTH, M. (1995). Species differences in A$_1$ adenosine receptor/G protein coupling: identification of a membrane protein that stabilises the association of the receptor/G protein complex. *Mol. Pharmacol.,* **48,** 806 – 817.

PRICE, G.W., HO, M., SCOTT, C., SELKIRK, J.V. & BROWN, A.M. (1998). 5-HT receptor agonist-antagonist binding affinity ratios as a measure of intrinsic activity using [$^3$H]8-OH-DPAT and [$^3$H]MPPF as radioligands. *Br. J. Pharmacol.,* **125,** 58P.

SAMMAMA, P., COTECCHIA, S., COSTA, T. & LEFKOWITZ, R.J. (1993). A mutation-induced activated state of the $\beta_2$-adrenergic receptor. Extending the ternary complex model. *J. Biol. Chem.,* **268,** 4625 – 4636.

SELKIRK, J.V., SCOTT, C., JERMAN, J.C. & PRICE, G.W. (1997). [$^3$H]GR125743 labels high and low affinity states of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. *Br. J. Pharmacol.,* **120,** 295P.

SIJBESMA, H. (1991). Species differences in the distribution of central 5-HT$_1$ binding sites: a comparative autoradiographic study between rat and guinea-pig. *Brain Res.,* **555,** 295 – 304.

STILES, G.L. (1988). A$_1$ adenosine receptor-G protein coupling in bovine brain membranes:effects of guanine nucleotides, salt and solubilisation. *J. Neurochem.,* **51,** 1592 – 1598.

SUNDARAM, H., NEWMAN-TANCREDI, A. & STRANGE, P.G. (1992). Pharmacological characterisation of the 5-$HT_{1A}$ serotonin receptor using the agonist [$^3$H]8-OH-DPAT, and the antagonist [$^3$H]spiperone. *Biochem. Soc. Trans.,* **20,** 145S.

TAYEBATI, S.K., PIERGENTILI, A., NATALE, D. & AMENTA, F. (1999). Evaluation of an agonist index: affinity ratio for compounds active on muscarinic cholinergic $M_2$ receptors. *J. Auton. Pharmacol.,* **19,** 77–84.

VICKROY, T.W., YAMAMURA, H.I. & ROESKE, W.R. (1983). Differential regulation of high-affinity agonist binding to muscarinic sites in the rat heart, cerebellum and cerebral cortex. *Biochem. Biophys. Res. Comm.,* **116,** 284–290.

WATSON, J., BROUGH, S., COLDWELL, M.C., GAGER, T., HO, M., HUNTER, A.J., JERMAN, J., MIDDLEMISS, D.N., RILEY, G.J. & BROWN, A.M. (1998). Functional effects of the muscarinic receptor agonist, xanomeline, at 5-$HT_1$ and 5-$HT_2$ receptors. *Br. J. Pharmacol.,* **125,** 1413–1420.

*Received January 26, 2000*
*Revised March 27, 2000*
*Accepted March 27, 2000*