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# Cloned human 5-HT$_{1A}$ receptor pharmacology determined using agonist binding and measurement of cAMP accumulation

Najam A. Sharif, Colene D. Drace, Gary W. Williams and Julie Y. Crider

## Abstract

Twenty agonists and nine antagonists were evaluated for their ability to compete for [$^3$H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([$^3$H]-8-OH-DPAT) binding to the cloned human serotonin-1A (ch-5-HT$_{1A}$) receptor expressed in Chinese hamster ovary cells and for their ability to alter adenylyl cyclase activity in the same cells. The most potent full agonists of high affinity included *N,N*-dipropyl-5-carboxamidotryptamine (pEC50 = 9.6 ± 0.1), MDL 73005EF (pEC50 = 9.3 ± 0.2), 5-methyl-urapidil (pEC50 = 9.2 ± 0.1), 5-carboxamidotryptamine (pEC50 = 9.1 ± 0.2), *R*(+)-8-OH-DPAT (pEC50 = 8.6 ± 0.1) and BMY-7378 (pEC50 = 8.6 ± 0.1). WB-4101 (pEC50 = 8.3 ± 0.2; IA = 79%), clozapine (pEC50 = 8.1 ± 0.3; IA = 29%), (buspirone (pEC50 = 7.6 ± 0.2; IA = 79%), quipazine (pEC50 < 5; IA = 45%) and *R*-DOI (pEC50 < 5; IA = 31%) were weaker agonists with partial agonist properties. The most potent antagonists were WAY-100,635 (pK$_i$ = 10.2 ± 0.1), methiothepin (pK$_i$ = 8.8 ± 0.2), spiperone (pK$_i$ = 8.7 ± 0.2) and NAN-190 (pK$_i$ = 8.5 ± 0.2). The receptor affinities and functional potencies were well correlated (r = 0.88; $P < 0.0001$). Our binding data correlated well with the pharmacology of endogenous 5-HT$_{1A}$ receptors in the rabbit iris-ciliary body (r = 0.91; $P < 0.001$) and rat hippocampus (r = 0.93, $P < 0.0001$). Our functional cAMP data correlated well with other cAMP accumulation data (r = 0.8, $P < 0.01$ vs calf hippocampus) but less so with [$^{35}$S]-GTP$\gamma$S binding to the ch-5-HT$_{1A}$ receptor as a functional activity read-out (r = 0.58, $P < 0.05$). The present study provides a detailed pharmacological characterization of the ch-5-HT$_{1A}$ receptor using binding and functional assays.

## Introduction

Serotonin is a major neurotransmitter in the central and peripheral nervous system and is involved in numerous physiological and pathological functions in the mammalian body (Hoyer et al 1994; Barnes & Sharp 1999). Serotonin (5-HT) receptors are currently divided into seven subtypes (5-HT$_{1–7}$), which have all been cloned and pharmacologically characterized to some extent (Hoyer et al 1994; Barnes & Sharp 1999). The 5-HT$_1$ and 5-HT$_5$ receptor subtypes (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_{5A}$ and 5-HT$_{5B}$) are negatively coupled to adenylyl cyclase. 5-HT$_2$ (5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$) receptor signal transduction is accomplished through phospholipase C and the stimulation of phosphoinositide turnover and intracellular calcium mobilization (Gerhardt & van Heerikuizen 1997). The 5-HT$_4$, 5-HT$_6$ and 5-HT$_7$ receptors are positively coupled to adenylyl cyclase via the G-protein G$_S$, and the 5-HT$_3$ receptor is a ligand-gated ion channel (Adham et al 1998; Crider et al 2003).

The human 5-HT$_{1A}$ receptor consists of 422 amino acids and displays approximately 89% sequence homology with the rat 5-HT$_{1A}$ receptor (Albert et al 1990). There are three proposed extracellular glycosylation sites and three possible phosphorylation sites residing in the intracellular loops of the human 5-HT$_{1A}$ receptor. The terminal C-chain is hypothesized to play a role in the coupling of the 5-HT$_{1A}$ receptor to the G$_i$ protein (Olivier et al 1999). Experimental evidence suggests that G$_i$ proteins, perhaps preferentially G$_3$, mediate the effects of serotonin to inhibit adenylyl cyclase and also may stimulate phospholipase C (Fargin et al 1991). The 5-HT$_{1A}$ receptor is also proposed to regulate cAMP levels by inhibiting its degradation by phosphodiesterases or some other mechanism (Wang et al 1999). In addition to the inhibition of adenylyl cyclase, 5-HT$_{1A}$

Molecular Pharmacology Unit, Pharmaceutical Products Research, Alcon Research, Ltd, Fort Worth, TX 76134-2099, USA

Najam A. Sharif, Colene D. Drace, Gary W. Williams, Julie Y. Crider

**Correspondence:** Naj Sharif, Molecular Pharmacology (R2-19), Pharmaceutical Products Research, Alcon Research, Ltd, Fort Worth, TX 76134-2099, USA. E-mail: naj.sharif@alconlabs.com

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receptor activation can lead to the opening of $K^+$ channels (Andrade et al 1986; Haj-Dahmane et al 1991) and the inhibition of calcium channels (Hamon et al 1990). The 5-$HT_{1A}$ receptor may also act via the $G_z$ protein to increase the secretion of various neuroendocrine hormones (Serres et al 2000). Agonist-induced desensitization through loss of high-affinity ligand binding sites as well as functional coupling to adenylyl cyclase has been observed in the 5-$HT_{1A}$ receptor (Harrington et al 1994; Raymond et al 1999).

The 5-$HT_{1A}$ receptor mediates a diverse range of physiological and pathological responses in the mammalian body and represents a major receptor of interest for drug development. For instance, 5-$HT_{1A}$ agonists induce hypothermia in humans (Seletti et al 1995) and also stimulate hormone secretion (Lesch et al 1990). 5-$HT_{1A}$ receptor agonists possess anxiolytic and antidepressant activity (Hoyer et al 1994; Barnes & Sharp 1999) and they appear to be useful for treating attention deficit disorder (Olivier et al 1999). Neuroprotective activity has also been observed with 5-$HT_{1A}$ receptor agonists such as BAY X3702 (repinotan) (De Vry et al 1998; Semkova et al 1998), and NMDA-induced caspase-3 activity and DNA fragmentation are inhibited by 5-$HT_{1A}$ receptor agonists (Madhavan et al 2003). In addition, 5-$HT_{1A}$ receptors have been characterized in the rabbit iris-ciliary body (Chidlow et al 1995) and 5-$HT_{1A}$ receptor agonists lower intraocular pressure in this species (Chidlow et al 1999; Osborne et al 2000).

Much of the previous pharmacological work in the 5-$HT_{1A}$ receptor field undertaken to define the agonist and antagonist activity of compounds was performed using a limited number of compounds, using the receptor from various animal species, and/or using [$^{35}$S]-GTP$\gamma$S binding studies to measure functional activity. The aim of the present study was to provide comparative functional (using the inhibition of adenylyl cyclase activity) and agonist binding (using [$^3$H]-8-hydroxy-2-(di-*n*-propylamino)tetralin; [$^3$H]8-OH-DPAT) data for a large group of serotonin receptor agonists and antagonists at the cloned human 5-$HT_{1A}$ (ch-5-$HT_{1A}$) receptor expressed in Chinese hamster ovary (CHO) cells and to compare these data with the literature data. The present study has the advantage of carrying out the binding and functional studies in the same cellular system, thus providing a consistent environment for comparison of the drugs. There is a relative paucity of such comprehensive studies of this type in the literature and we hope our data will remedy this situation.

## Materials and Methods

### Chemicals

Serotonin hydrochloride, *R*(+)-8-hydroxy-dipropylaminotetralin hydrobromide, 5-carboxamidotryptamine maleate, BMY 7378 dihydrochloride, *N,N*-dipropyl-5-carboxamidotryptamine maleate, 5-methoxy-dimethyl tryptamine, 5-methoxy-$\alpha$-methyl tryptamine, RU 24969 hemisuccinate, buspirone hydrochloride, 5-methyl-urapidil, $\alpha$-methyl-5-hydroxytryptamine maleate, 5-methoxytryptamine hydrochloride, ketanserin, *R*-(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride, quipazine dimaleate, *N,N*-dimethyl-5-methoxytryptamine, *N*-methyltryptamine oxylate, WB-4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane hydrochloride), 1-(3-chlorophenyl)-piperazine hydrochloride, pergolide methanesulfonate, MDL 73005EF hydrochloride, WAY-100635, methiothepin, spiperone hydrochloride, NAN-190 hydrobromide, clozapine, pindolol, mesulergine hydrochloride and mianserin hydrochloride were purchased from Sigma/RBI (St Louis, MO, USA). Flesinoxan hydrochloride was obtained from Solvay Pharma BV (Weesp, The Netherlands). $\alpha$-Methyl-5-methoxytryptamine hydrochloride was obtained from the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program (SRI International, Menlo Park, CA, USA). Bufotenine (*N,N*-dimethyl-5-hydroxytryptamine) oxalate was obtained from Biosynth International (Naperville, IL).

### Cell culture

CHO cells expressing the human 5-$HT_{1A}$ receptor were purchased from Euroscreen (Gosselies, Belgium) and cultured in CHO-S-SFMII medium containing 0.4 mg mL$^{-1}$ geneticin, 2.5 $\mu$g mL$^{-1}$ amphotericin B, 5 units mL$^{-1}$ penicillin, 5 $\mu$g mL$^{-1}$ streptomycin and 1% dialysed fetal bovine serum. Media and other supplements were products of Bio-Whittaker (Walkersville, MD, USA).

### cAMP production in cultured cells

Direct agonist-dependent inhibition of forskolin-stimulated cAMP formation was measured as described previously (Crider et al 1998, 1999). Compounds of interest were diluted in ethanol such that the final ethanol concentration was 1%. On reaching confluence, the CHO cells expressing the ch-5-$HT_{1A}$ receptor were rinsed twice with 0.5 mL of DMEM/F-12. The cells were incubated for 20 min in DMEM/F-12 containing 0.8 mM ascorbate and 1.0 mM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; Sigma, St Louis, MO, USA) at room temperature (~22°C). Serotonin receptor agonists were added at the end of this period and the reaction was allowed to proceed for 10 min. Forskolin (10 $\mu$M) was then added (10 min incubation) to stimulate cAMP production. After aspiration of the reaction medium, ice-cold 0.1 M acetic acid (150 $\mu$L, pH ~3.5) was added for termination of cAMP synthesis and cell lysis. Finally, ice-cold 0.1 M sodium acetate (220 $\mu$L, pH 11.5–12.0) was added to neutralize the samples before analysis by an enzyme immunoassay (Crider et al 1998, 1999).

### cAMP measurements

cAMP production was measured using an enzyme immunoassay kit purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). This assay was conducted according to the package insert in an automated manner using the Biomek 2000 robot (Beckman Instruments; Fullerton, CA, USA) (Sharif et al 2001; Crider et al 2003).

### Receptor binding studies

Membranes from CHO cells expressing the ch-5-HT$_{1A}$ receptor were diluted to 2.5 units mL$^{-1}$ in 50 mM Tris buffer (pH 7.4, 4°C). The membranes were re-suspended using a Polytron equipped with a PTA10TS generator (< 20 s) (Kinematic AG, Basel, Switzerland). Drug dilutions were made in 10:10 DMSO:ethanol (v/v) using the Biomek 2000 robot. The diluted compounds (50 $\mu$L) were then added to a 96-well deep block. A volume of 400 $\mu$L of the receptor preparation was manually added to the block. The Biomek 2000 robot was then used to add 50 $\mu$L (0.25 nM final concentration) of [$^3$H]8-OH-DPAT. Non-specific binding was defined using 10 $\mu$M unlabelled 8-OH-DPAT. The 96-well blocks were sealed and incubated in a shaking water bath for 60 min at 27°C. The blocks were then transferred to a MachII/IV harvester (TomTech; Hamden, CT, USA) and the incubations were terminated by rapid vacuum filtration using Whatman GF/B glass fibre filter mats previously soaked in 0.3% polyethyleneimine (Sharif et al 1991, 1998). The samples were washed with cold 50 mM Tris buffer (pH 7.4). The samples were subsequently counted on a Wallac BigSpot $\beta$-counter for 3 min each.

### Data analyses

All functional and binding data were expressed as means ± s.e.m. of data obtained from multiple experiments. Sample optical density readings were compared with the standard curve and the cAMP content of each sample was evaluated by linear regression analysis using the Excel software package (Microsoft, Redmond, WA, USA). Functional potency (EC50 and IC50) values were computed using a sigmoidal-fit function of the Origin software package (Microcal Software, Inc., Northampton, MA, USA) (Sharif et al 1998). Linear regression analysis was also performed using Origin. Ligand binding IC50 values were calculated and converted into $K_i$ values using ActivityBase (IDBS, Surrey, UK) (Sharif et al 1991, 2001). Data were converted to −log EC50 (pEC50) and −log $K_i$ (p$K_i$) values for the construction of the correlation plots and for comparison with the literature values. The various treatments were statistically compared using non-parametric multiple hypothesis techniques (Kruskal–Wallis), assuming that a value of $P$ less than 0.05 denoted significance.

## Results

Figure 1A shows representative functional data for the inhibition of 10 $\mu$M forskolin-stimulated cAMP production in CHO cells expressing the human 5-HT$_{1A}$ receptor. Under these experimental conditions, the maximal and minimal amounts of cAMP produced with 8-OH-DPAT, the reference compound, were 20.8 ± 0.8 and 6.3 ± 0.4 pmoles/well, respectively. The rank order of potency for serotonergic agonists in the 5-HT$_{1A}$ receptor functional assay (pEC50, M) was *N,N*-dipropyl-5-CT (9.6) ≥ MDL 73005EF (9.3) ≥ 5-methyl-urapidil (9.2) ≥ 5-CT (9.1) > 8-OH-DPAT (8.6) ≥ BMY-7378 (8.5) ≥ WB-4101 (8.3) ≥ pergolide (8.2) ≥

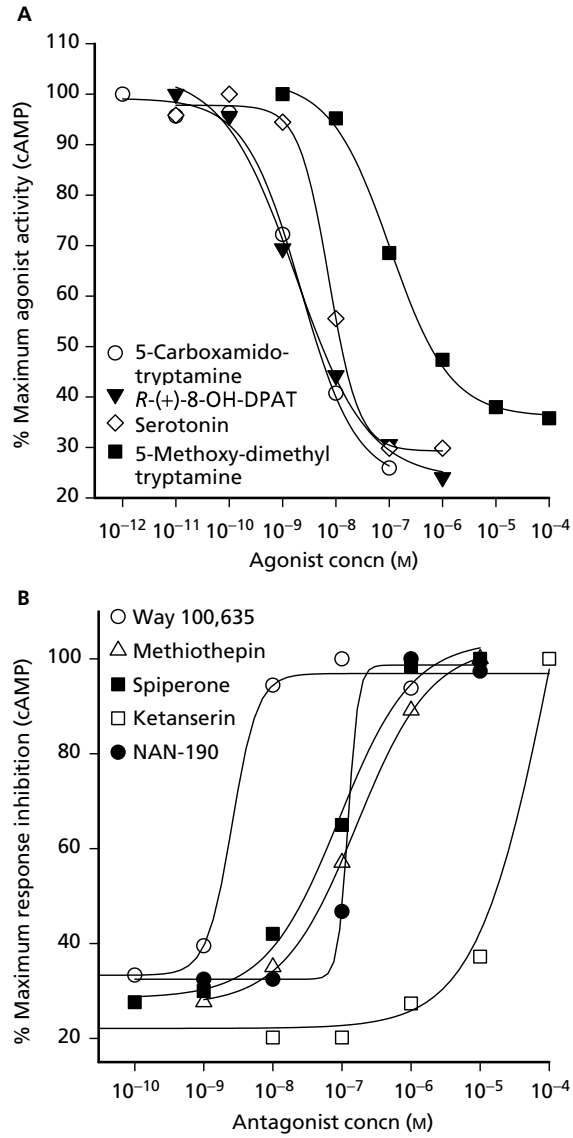


**Figure 1** A. Agonist inhibition of forskolin-stimulated cAMP production in Chinese hamster ovary (CHO) cells expressing the cloned human 5-HT$_{1A}$ (ch-5-HT$_{1A}$) receptor. The cells were incubated with the agonists (six concentrations) for 10 min followed by 10 $\mu$M forskolin for 10 min. The cells were then lysed and the cAMP generated determined with an enzyme immunoassay procedure. Representative functional concentration–response curves for several serotonergic receptor agonists in the CHO cell adenylyl cyclase assay are shown using multiple concentrations of each agent. Data points represent means of two determinations per concentration. Summary data from several such experiments are shown in Table 1. B. Antagonist inhibition of 8-OH-DPAT-mediated inhibition of adenylyl cyclase activity in CHO cells expressing the ch-5-HT$_{1A}$ receptor. The cells were pre-incubated with antagonists (six concentrations) for 20 min followed by an incubation with the 8-OH-DPAT (100 nM) for 10 min, and finally 10 $\mu$M forskolin for 10 min. The cells then were lysed and the cAMP generated determined with an enzyme immunoassay procedure. Representative functional concentration–response curves for several serotonergic receptor antagonists in the CHO cell adenylyl cyclase assay are shown. Data points represent means of two determinations per concentration. Summary data from several such experiments are shown in Table 2.

RU24969 (8.2) ≥ flesinoxan (8.1) ≥ 5-methoxy-tryptamine (8.0) ≥ 5-HT (7.8) ≥ buspirone (7.6) ≥ bufotenine (7.4) > α-methyl-5-HT (6.5) ≥ 5-methoxy-α-methyltryptamine (6.3) > quipazine (< 5.0) ≥ *R*-DOI (< 5.0) (Table 1). Several compounds exhibited partial agonist profiles, namely BMY-7378, clozapine, WB-4101, quipazine and *R*-DOI (Table 1). Figure 1B shows the effects of several 5-$HT_{1A}$ antagonists on their ability to reverse the agonist-induced changes in cAMP levels. The antagonist potency values ($pK_i$) in this ch-5-$HT_{1A}$ functional assay system were: WAY-100,635 (10.2) > methiothepin (8.8) ≥ spiperone (8.7) ≥ Nan-190 (8.5) > clozapine (7.0) > ketanserin (6.0) > mesulergine (< 5.0) ≥ mianserin (< 5.0) (Table 2).

Representative competition curves for [$^3$H]8-OH-DPAT binding to the ch-5-$HT_{1A}$ receptor are shown in Figure 2. The results from binding studies for several serotonergic compounds are shown in Tables 1 and 2. Binding affinity data at the ch-5-$HT_{1A}$ receptor were well correlated with cAMP functional data from CHO cells in the present study (Figure 3A, $r = 0.88$, $P < 0.0001$, $n = 27$). The inhibition of adenylyl cyclase in our CHO cells was highly correlated with functional data from calf hippocampus ($r = 0.8$, $P < 0.01$, $n = 12$; Hoyer et al 1994; Figure 3B) but to a lesser degree with [$^{35}$S]-GTPγS binding at the ch-5-$HT_{1A}$ receptor ($r = 0.58$, $P < 0.05$, $n = 12$; Stanton & Beer 1997 and Newman-Tancredi et al 1998 combined; Figure 3C).

Data from the present binding study were well correlated with [$^3$H]8-OH-DPAT binding in membrane preparations from rat hippocampus ($r = 0.93$, $P < 0.0001$, $n = 11$; Figure 4A) and rabbit iris-ciliary body (Chidlow et al 1995) ($r = 0.91$, $P < 0.001$, $n = 9$, data not shown). Similarly, binding data from the present study were well correlated with other literature [$^3$H]8-OH-DPAT binding experiments for ch-5-$HT_{1A}$ receptor (Khawaja et al 1997 and Dunlop et al 1998 combined) ($r = 0.8$, $P < 0.01$, $n = 14$, Figure 4B).

## Discussion

The present study has expanded on earlier work from other laboratories and provides comparative 5-$HT_{1A}$ receptor binding and functional data for 27 compounds ranging in potency ($pEC50/pK_i$) from < 5.0 to 10.3. The functional and binding data at the ch-5-$HT_{1A}$ receptor were highly correlated ($r = 0.88$ $P < 0.0001$, $n = 27$, Figure 3A). Our results corroborate the findings of Schoeffter et al (1997) in which the pEC50 values for the inhibition of forskolin-stimulated cAMP production in HAT7 cells were 7.9, 8.3 and 7.7 for 5-HT, 8-OH-DPAT and buspirone, respectively.

**Table 1** Effects of serotonergic agonists on forskolin-stimulated production of cAMP in Chinese hamster ovary cells expressing the cloned human 5-$HT_{1A}$ receptor and competition for [$^3$H]8-OH-DPAT binding to cloned human 5-$HT_{1A}$ receptors

| Compound | Reported receptor selectivity | Functional agonist potency (pEC50) and intrinsic activity (% maximum response relative to 5-CT) | 5-$HT_{1A}$ receptor binding affinity parameters ($pK_i$) |
|---|---|---|---|
| *N,N*-Dipropyl-5-carboxamidotryptamine | 5-$HT_{1A}$, 5-$HT_7$ | 9.6 ± 0.1 (97 ± 2%) | 10.0 ± 0.1 |
| MDL 73005EF | 5-$HT_{1A}$ | 9.3 ± 0.2 (92 ± 6%) | 9.8 ± 0.3 |
| 5-Methyl-urapidil | 5-$HT_{1A}$; $\alpha_1$ | 9.2 ± 0.1 (93 ± 10%) | 10.2 ± 0.2 |
| 5-Carboxamidotryptamine | 5-$HT_1$, 5-$HT_{5A}$, 5-$HT_7$ | 9.1 ± 0.2 (100 ± 2%) | 10.1 ± 0.1 |
| *R*-(+) 8-OH-DPAT | 5-$HT_{1A}$, 5-$HT_7$ | 8.6 ± 0.1 (102 ± 3%) | 9.5 ± 0.03 |
| BMY-7378 | 5-$HT_{1A}$; $\alpha_{1D}$ | 8.5 ± 0.1 (55 ± 9%) | 9.5 ± 0.2 |
| WB-4101 | 5-$HT_{1A}$; $\alpha_1$ | 8.3 ± 0.2 (79 ± 10%) | 9.1 ± 0.1 |
| RU24969 | 5-$HT_1$ | 8.1 ± 0.1 (92 ± 6%) | 8.8 ± 0.1 |
| Flesinoxan | 5-$HT_{1A}$; $\alpha_{1D}$ | 8.1 ± 0.1 (96 ± 2%) | 9.9 ± 0.2 |
| Clozapine | Mixed | 8.1 ± 0.3 (29 ± 6%) | 7.3 ± 0.2 |
| 5-Methoxy-tryptamine | Non-selective | 8.0 ± 0.02 (107 ± 8%) | 9.4 ± 0.3 |
| Pergolide | Dopamine | 8.0 ± 0.2 (79 ± 8%) | 9.1 ± 0.3 |
| 5-HT | Non-selective | 7.8 ± 0.2 (97 ± 3%) | 9.5 ± 0.2 |
| Buspirone | 5-$HT_{1A}$ | 7.6 ± 0.2 (79 ± 5%) | 8.3 ± 0.1 |
| 5-Methoxy-dimethyl tryptamine | Non-selective | 7.4 ± 0.2 102 ± 2% | 8.7 ± 0.2 |
| Bufotenine (5-hydroxy-*N,N*-di-methyltryptamine) | 5-$HT_{1A}$; 5-$HT_2$; 5-$HT_3$ | 7.4 ± 0.2 (98 ± 5%) | 8.8 ± 0.4 |
| α-Methyl 5-HT | 5-$HT_2$ | 6.5 ± 0.2 (111 ± 10%) | 8.1 ± 0.2 |
| 5-Methoxy-α-methyl tryptamine | Non-selective | 6.3 ± 0.2 (103 ± 2%) | 7.4 ± 0.1 |
| Quipazine | 5-$HT_3$ | < 5.0 (45 ± 7%) | 6.0 ± 0.2 |
| *R*-DOI | 5-$HT_2$ | < 5.0 (31%) | 5.7 ± 0.1 |

Ligand binding data are means ± s.e.m. from 3–44 experiments, each performed using duplicate tubes per drug concentration and using multiple concentrations of each test agent. Functional adenylyl cyclase activity data are means ± s.e.m. from 3–80 experiments, each also performed using duplicate determinations per drug concentration and using multiple concentrations of the test agents.

**Table 2** Antagonism of 8-OH-DPAT-mediated inhibition of adenylyl cyclase activity in Chinese hamster ovary (CHO) cells expressing the cloned human 5-$HT_{1A}$ receptor and competition for [$^3$H]8-OH-DPAT binding to cloned human 5-$HT_{1A}$ receptors

| Compound | Reported receptor selectivity | Functional antagonist potency ($pK_i$) | Receptor binding affinity parameters ($pK_i$) |
|---|---|---|---|
| WAY-100,635 | 5-$HT_{1A}$ | $10.2 \pm 0.1$ | $10.3 \pm 0.02$ |
| Methiothepin | Non-selective | $8.8 \pm 0.2$ | $9.3 \pm 0.2$ |
| Spiperone | 5-$HT_{1A}$, 5-$HT_{2A}$ | $8.7 \pm 0.2$ | $8.6 \pm 0.4$ |
| NAN-190 | 5-$HT_{1A}$ | $8.5 \pm 0.2$ | $9.6 \pm 0.2$ |
| Clozapine | Mixed | $7.0 \pm 0.2$ | $7.3 \pm 0.2$ |
| Pindolol | 5-$HT_1$, $\beta$-adrenergic | $7.0 \pm 0.2$ | $8.4 \pm 0.3$ |
| Ketanserin | 5-$HT_2$, 5-$HT_{1D}$ | $6.0 \pm 0.1$ | $6.2 \pm 0.2$ |
| Mesulergine | 5-$HT_{2C}$ | $< 5.0$ | $6.8 \pm 0.1$ |
| Mianserin | Non-selective | $< 5.0$ | $6.7 \pm 0.2$ |

Functional data shown for CHO cells are the means ± s.e.m. from 3–7 experiments each performed using duplicate wells per test agent concentration and using multiple concentrations of the agents. Binding data shown for CHO cells are the means ± s.e.m. from 3–5 experiments each performed using duplicate tubes per drug concentration and using multiple concentrations of each test agent.

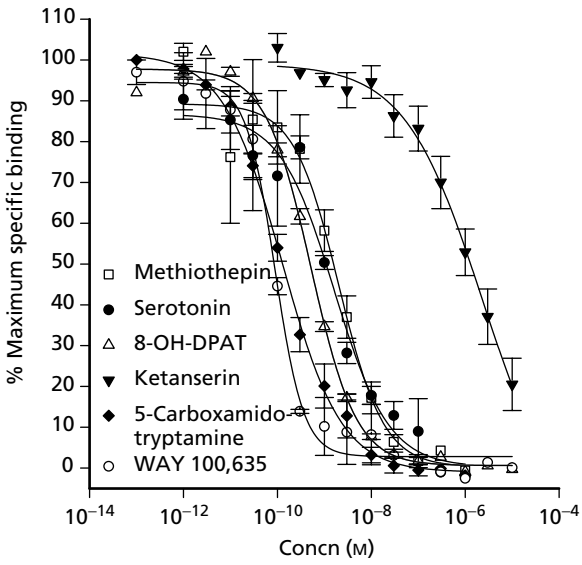


**Figure 2** [$^3$H]8-OH-DPAT binding to cloned human 5-$HT_{1A}$ receptors expressed in Chinese hamster ovary cell membranes. Competition assays were conducted with a range of serotonergic agents (10 concentrations) using [$^3$H]8-OH-DPAT (0.25 nM final concentration). Data points represent means of two determinations in duplicate. Summary affinity data from several such experiments are shown in Tables 1 and 2.

In an alternative functional assay using a microphysiometer to measure extracellular acidification rates (Dunlop et al 1998), CHO cells expressing the ch-5-$HT_{1A}$ receptor produced pEC50 values of 8.9 for 5-CT and 8.1 for 8-OH-DPAT, which were comparable with our values of 9.1 and 8.6 for the same two compounds, respectively. The present functional study for the inhibition of adenylyl cyclase in CHO cells expressing the ch-5-$HT_{1A}$ receptor was highly correlated with adenylyl cyclase studies in calf hippocampus (Hoyer et al 1994) but less so with [$^{35}$S]GTP$\gamma$S binding at the ch-5-$HT_{1A}$ receptor (Stanton & Beer 1997; Newman-Tancredi et al 1998). These findings are intriguing and suggest that the direct measurement of the cAMP as an index of receptor activation/blockade may be the preferred method of studying the functional pharmacology of the 5-$HT_{1A}$ receptor rather than the indirect procedure of measuring the interaction of [$^{35}$S]GTP$\gamma$S with the G-proteins associated with the 5-$HT_{1A}$ receptor. However, further work is needed to substantiate these conclusions.

Early experiments with [$^3$H]8-OH-DPAT binding in recombinant rat and human 5-$HT_{1A}$ receptor preparations produced $pK_i$ values of 9.4, 8.8, 6.9 and 4.6 for 8-OH-DPAT, 5-HT, spiperone and ketanserin, respectively (Albert et al 1990). The results show a similar rank order of potency to those seen in Tables 1 and 2. Our binding studies are highly correlated with [$^3$H]8-OH-DPAT binding in membrane preparations from rat hippocampus ($r = 0.93$, $P < 0.0001$, $n = 11$; Assié et al 1999). Other [$^3$H]8-OH-DPAT binding experiments using membranes from CHO cells that expressed the ch-5-$HT_{1A}$ receptor (Khawaja et al 1997; Dunlop et al 1998) also correlated well with our binding studies ($r = 0.8$, $P < 0.0001$, $n = 14$). In addition, the rank order of binding potency for our studies was similar to that observed for [$^3$H]8-OH-DPAT binding in membranes from *Escherichia coli* that expressed the recombinant human 5-$HT_{1A}$ receptor: 5-methylurapidil $\geq$ 8-OH-DPAT $\geq$ 5-HT $\geq$ buspirone (Bertin et al 1992).

Overall, the most potent compounds in both binding and functional assays were the selective 5-$HT_{1A}$ agents, followed by the non-selective serotonergic agonists. With the exception of the anxiolytic clozapine and the anti-Parkinson's agent pergolide, compounds from other serotonergic receptor classes were weak or inactive at the ch-5-$HT_{1A}$ receptor. Interestingly, we found that clozapine also acted as a partial agonist at the ch-5-$HT_{1A}$ receptor (pEC50 = 8.1, $\%E_{max} \sim 30$). This phenomenon also was observed in the 5-$HT_{1A}$ receptor [$^{35}$S]GTP$\gamma$S

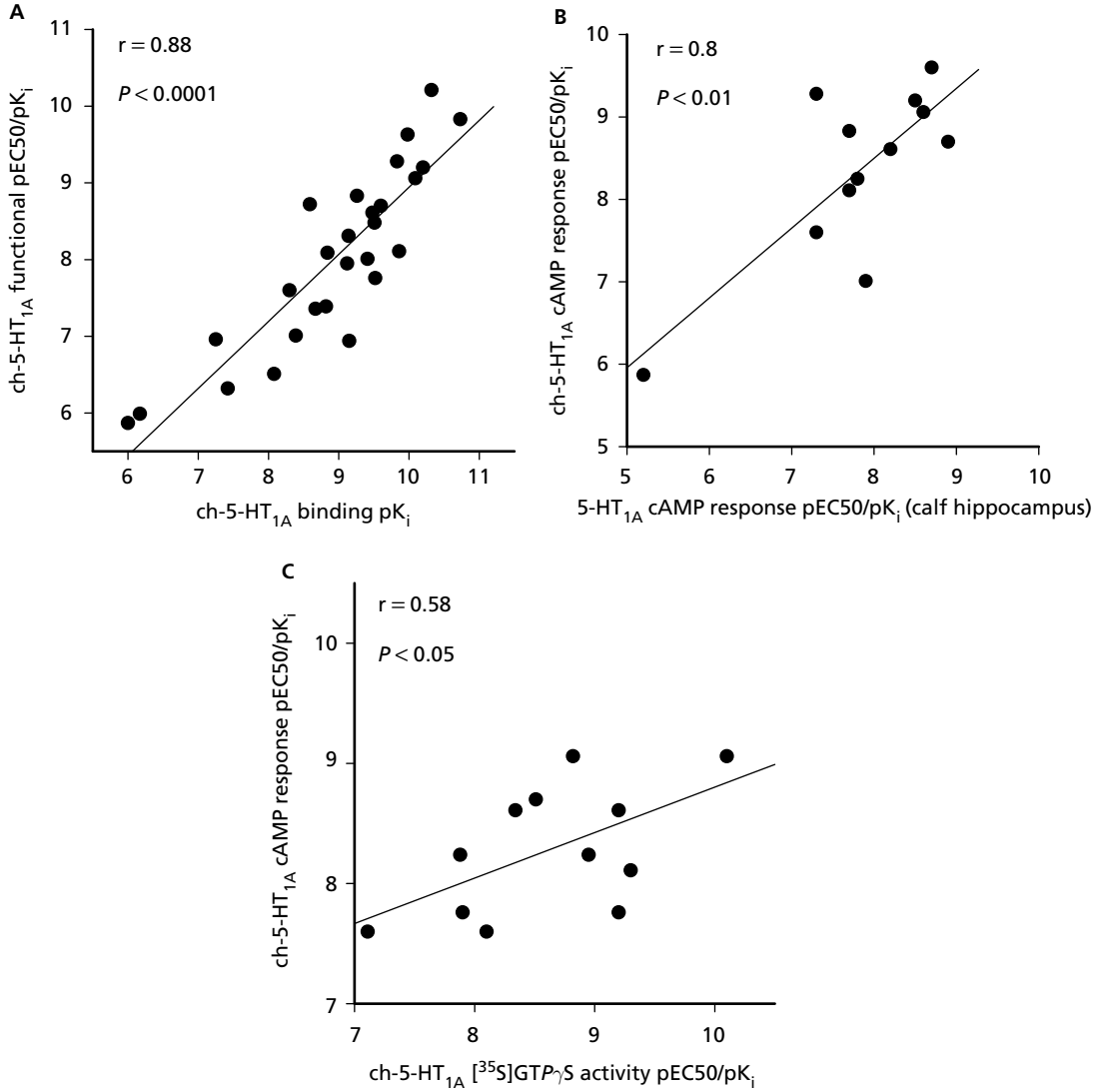


**Figure 3** A. Correlation of the functional agonist and antagonist data from Chinese hamster ovary (CHO) cells and that of binding data from the present study of [$^3$H]8-OH-DPAT using the cloned human 5-HT$_{1A}$ (ch-5-HT$_{1A}$) receptor. Assays to measure the inhibition of cAMP accumulation in CHO cells and for [$^3$H]8-OH-DPAT binding to the ch-5-HT$_{1A}$ receptor were conducted as described in the text. B. Correlation of the functional data (adenylyl cyclase) from CHO cells expressing the ch-5-HT$_{1A}$ receptor and inhibition of adenylyl cyclase in calf hippocampus (Hoyer et al 1994). Assays to measure the inhibition of cAMP accumulation in CHO cells were conducted as described in the text. C. Correlation of the functional data (inhibition of adenylyl cyclase) from CHO cells expressing the ch-5-HT$_{1A}$ receptor and inhibition of [$^{35}$S]GTP$\gamma$S binding at the ch-5-HT$_{1A}$ receptor (Stanton & Beer 1997; Newman-Tancredi et al 1998). Assays to measure the inhibition of cAMP accumulation in CHO cells were conducted as described in the text.

binding experiments with clozapine (Newman-Tancredi et al 1996). The present ch-5-HT$_{1A}$ CHO cell functional assay system was capable of distinguishing partial agonists such as BMY-7378, WB-4101, clozapine, quipazine and *R*-DOI from the other compounds tested that behaved as full agonists. This tended to suggest that the receptor density and degree of their functional coupling to the down-stream biochemical pathways in these cells was somewhat representative of the endogenous receptor expression and signal transduction mechanism(s).

The present study provides an extensive characterization of the ch-5-HT$_{1A}$ receptor using a wide range of serotonergic agonists and antagonists. Among the antagonists studied, WAY-100,635 was the most potent compound, followed by methiothepin and spiperone. It was interesting to note that whereas the majority of the antagonists yielded parallel dose–response curves for inhibiting the forskolin-induced cAMP production with Hill coefficients close to unity, NAN-190 tended to display rather steep dose–response curves (e.g. Figure 1B). The underlying reason for this difference among the antagonists is unclear but may reflect subtle differences in the way NAN-190 binds to the receptor to preclude the agonist from binding to the active site of the receptor. This needs to be studied further. However, the

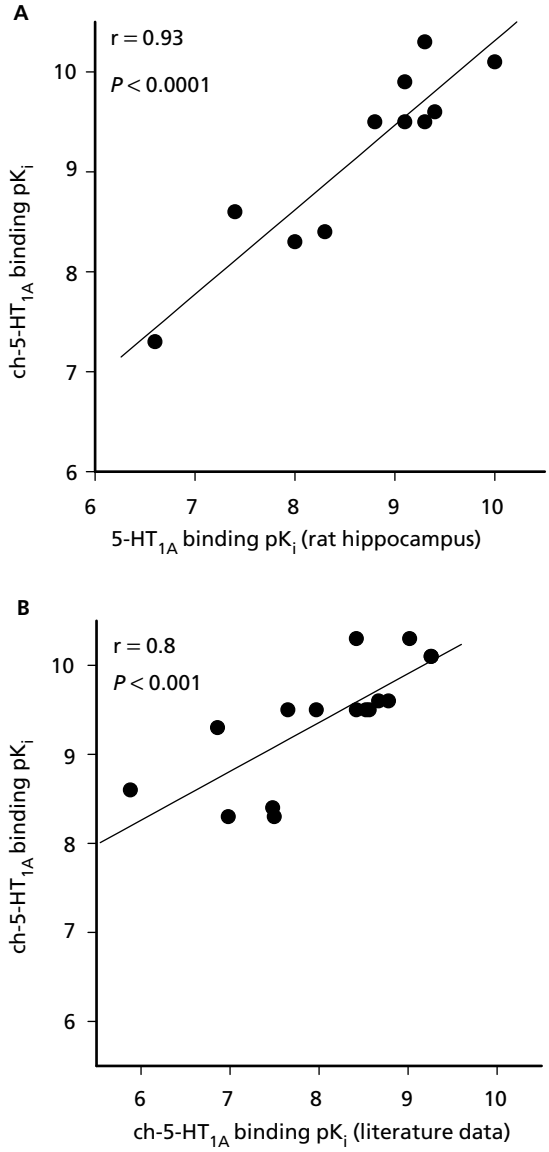


**Figure 4** A. Correlation of binding $pK_i$ values from the present study ([$^3$H]8-OH-DPAT binding to the cloned human 5-HT$_{1A}$ (ch-5-HT$_{1A}$) receptor expressed in Chinese hamster ovary cells) with binding studies using membranes preparations from rat hippocampus (Assié et al 1999). Assays to measure binding to the ch-5-HT$_{1A}$ receptor were conducted as described in the text. B. Correlation of binding $pK_i$ values from the present study ([$^3$H]8-OH-DPAT binding to ch-5-HT$_{1A}$ receptor expressed in Chinese hamster ovary cells) with similar binding studies from Khawaja et al (1997) and Dunlop et al (1998).

agonist/antagonist data were shown to be highly correlated to literature values for the inhibition of adenylyl cyclase in calf hippocampus, microphysiometer acidification studies and [$^3$H]8-OH-DPAT binding in rat hippocampus and with ch-5-HT$_{1A}$ receptor, but apparently less so with [$^{35}$S]GTP$\gamma$S binding. One advantage of the present work is the fact that we used a direct measure of adenylyl cyclase inhibition at the ch-5-HT$_{1A}$ receptor to measure functional activity associated with the ch-5-HT$_{1A}$ receptor (i.e. inhibition of cAMP production). Furthermore, it was possible to make direct comparisons between binding and functional studies since both were conducted in CHO cells that expressed the ch-5-HT$_{1A}$ receptor.

The 5-HT$_{1A}$ receptor has been shown to play an important role in a number of disease states. For example, this membrane protein is a drug target for a number of psychotherapeutic agents used to treat depression (Blier & de Montigny 1999) and attention deficit disorder (Olivier et al 1999). 5-HT$_{1A}$ receptor agonists have been shown to possess neuroprotective activity (De Vry et al 1998; Semkova et al 1998; Madhavan et al 2003). These agents also lower intraocular pressure in the rabbit and have been suggested as therapeutic agents for the treatment of glaucoma (Chidlow et al 1999; Osborne et al 2000). In conclusion, our work serves to expand the current understanding of the pharmacology of the ch-5-HT$_{1A}$ receptor and provides binding and functional data on a large number of serotonergic compounds of interest.

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