High-level stable expression of recombinant 5-HT_{1A} 5-hydroxytryptamine receptors in Chinese hamster ovary cells

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The human 5-hydroxytryptamine 5-HT_{1A} receptor gene was transfected into Chinese hamster ovary cells. A series of recombinant monoclonal cell lines expressing the receptor were isolated and the properties of one cell line that expressed receptors at a high level (2.8 pmol/mg) were studied in detail. In ligand binding assays with the selective 5-HT_{1A} receptor agonist 2-(*NN*-di[³H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene ([³H]8-OH-DPAT) only a single class of saturable high-affinity binding sites was detected, with a pharmacological profile in competition experiments essentially identical to that of the 5-HT_{1A} receptor of bovine hippocampus. [³H]8-OH-DPAT binding to the recombinant cell membranes was inhibited by GTP, showing that the receptors in the transfected cells couple to G-proteins. A series of 5-hydroxytryptamine agonists inhibited forskolin-stimulated adenylate cyclase activity in the cells and, despite the high level of receptor expression, their apparent efficacies were similar to those observed for inhibition of adenylate cyclase in brain. This recombinant cell line provides a complete model system for studying the 5-HT_{1A} receptor and its transmembrane signalling system. The recombinant cells can also be grown in suspension culture for long periods but, whereas 5-HT_{1A} receptor numbers and receptor regulation by guanine nucleotides are maintained in suspension-grown cells, the inhibition of adenylate cyclase by the 5-HT_{1A} receptor is gradually lost.

INTRODUCTION

5-Hydroxytryptamine (5-HT) is an important neurotransmitter in the brain and periphery which is involved in a variety of behavioural and physiological functions, including sleep, pain perception, control of mood, sexual behaviour and memory (Dourish, 1987). Radioligand binding assays using a range of ligands have revealed a heterogeneous population of G-protein-linked transmembrane 5-HT receptors comprising the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}, 5-HT₂ and 5-HT₄ subtypes (Dumuis *et al.*, 1988; Leonhardt *et al.*, 1989; Hibert *et al.*, 1990). Of these, the 5-HT_{1A} receptor is of interest because the new anxiolytic drug buspirone is active at this site, and buspirone seems to exhibit few of the side-effects of the benzodiazepine anxiolytics (Dourish, 1987).

The gene sequences coding for the 5-HT_{1A}, 5-HT_{1C} and 5-HT₂ receptors are now known (Kobilka *et al.*, 1987; Fargin *et al.*, 1988; Julius *et al.*, 1988; Pritchett *et al.*, 1988), permitting structure-function studies to be carried out by site-directed mutagenesis and expression in animal cell lines. Expression of these receptors in cell lines also enables comparisons of the pharmacological profile of the various subtypes to be made in an environment free of cross-reacting sites. Furthermore, information about linkage to second messengers can be gained which is particularly desirable in the case of the 5-HT_{1A} receptor, since this receptor has been reported to be linked to both stimulation and inhibition of adenylate cyclase activity (De Vivo & Maayani, 1986; Shenker *et al.*, 1987).

We report here the high-level expression of the human 5-HT_{1A} receptor in Chinese hamster ovary (CHO) cells, the characterization of the ligand-binding properties of the receptor and its linkage via G-proteins to inhibition of adenylate cyclase.

MATERIALS AND METHODS

Transfection of 5-HT_{1A} receptor gene into CHO cells

The *Xba*I-*Bam*HI fragment of the human genomic clone G21 (Fargin *et al.*, 1988), was excised from the plasmid pSP64-G21, which was kindly supplied by Dr. R. J. Lefkowitz, Duke University, Durham, NC, U.S.A., and purified by electrophoresis on low-melting-point agarose prior to ligation into the unique *Xba*I and *Bam*HI cloning sites in the eukaryotic expression vector pSVL (Pharmacia Ltd.). Highly purified pSVL-G21 was obtained by two rounds of CsCl centrifugation.

CHO cells lacking dihydrofolate reductase (DHFR) activity (DHFR⁻) (Urlaub & Chasin, 1980) were grown in Dulbecco's modified Eagle's medium supplemented with hypoxanthine, thymidine, L-proline and 10% dialysed foetal bovine serum. Cells were co-transfected with a 10:1 ratio of pSVL-G21 and an expression vector carrying the DHFR gene (pSV2-DHFR) (Urlaub & Chasin, 1980) by the calcium phosphate precipitation method (Page, 1988) using a total of 10 µg of DNA/10⁶ cells. Both pSVL-G21 and pSV2-DHFR had been linearized with the restriction enzyme *Pvu*I.

Transfected cells stably expressing DHFR were selected by omission of hypoxanthine and thymidine from the growth medium. The medium was changed every 2 or 3 days until all the cells in a control untransfected flask with selective medium had died. Transfection efficiency was approx. 1 colony per 50 000 cells. The mixed population of surviving transfectants was tested for expression of the 5-HT_{1A} receptor by binding of a 6 nM concentration of the high-affinity 5-HT_{1A}-specific agonist [³H]8-OH-DPAT to the cell homogenate. Monoclonal cell lines were isolated by dilution cloning and 35 of these were screened for receptor expression. Clonal cell lines were routinely grown in

Abbreviations used: 5-HT, 5-hydroxytryptamine; CHO, Chinese hamster ovary; DHFR, dihydrofolate reductase; cAMP, cyclic AMP; Gpp[NH]p, guanosine 5'-[βγ-imido] triphosphate; 8-OH-DPAT, 2-(*NN*-dipropylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene.

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adherent culture as described above, and except where otherwise stated all data were obtained using cells grown in adherent culture.

For growth of cells in suspension, 1.5 litres of medium (RPMI containing 10% foetal bovine serum) was inoculated with 10^7 cells grown in adherent culture. The cell suspension was then gently stirred at 60 rev./min at 37°C in 5% CO₂. The cells were harvested when the cell density reached 10^6 cells/ml, usually after 6–7 days.

Northern blot analysis was carried out essentially according to the method of Sambrook *et al.* (1989) using as a probe the *Xba*I–*Bam*HI fragment of the G21 sequence (Fargin *et al.*, 1988) labelled with ³²P by random priming. RNA was purified from control and recombinant CHO cells and separated by electrophoresis on a 1.5% agarose gel containing formaldehyde. The RNA was then transferred to a nylon membrane (Genescreen) and baked for 2 h at 80°C. Hybridization was carried out overnight using 20 ng of the labelled probe/ml at 37°C in medium containing 4×SSPE (1×SSPE = 0.15 M-NaCl/10 mM-NaH₂PO₄/1 mM-EDTA, pH 7.4), 1% SDS, 500 µg of salmon sperm DNA/ml, 250 µg of tRNA/ml and 50% formamide. Washing conditions for the final two 30 min washes were 0.1×SSC/0.1% SDS at 68°C (1×SSC = 0.15 M-NaCl/0.01 M-sodium citrate). The membrane was exposed to X-ray film for 24 h at –70°C with an intensifying screen.

Preparation of cell extracts

Cell homogenates were prepared by scraping the cells from the culture flasks with a rubber policeman, followed by Polytron homogenization at maximum speed for 10 s. For cells grown in suspension culture, cells were harvested by centrifugation (1700 g, 15 min, 4°C) and a homogenate was prepared as above. A crude cell membrane preparation was obtained by centrifugation of the cell homogenate at 300 g (10 min, 4°C), followed by centrifugation of the supernatant at 48 000 g (30 min, 4°C). The pellet was resuspended in assay buffer and stored at –80°C.

Bovine hippocampal membranes were prepared as described by Norman *et al.* (1985), except that a Potter-S Teflon/glass homogenizer was used to disrupt the tissue (15 passes, 850 rev./min). The homogenate was centrifuged at 300 g (10 min, 4°C) before centrifugation of the supernatant twice at 48 000 g (15 min, 4°C), the final pellet being resuspended in assay buffer and frozen at –80°C. Protein measurements were as described in Lowry *et al.* (1951).

Radioligand-binding assays

Crude membranes from CHO cells (20–40 µg) or bovine hippocampus (80–100 µg) were incubated with [³H]8-OH-DPAT (176 Ci/mmol) (0.5 nM for competition experiments; 0.1–7 nM for saturation analysis with competing drugs where appropriate at the concentrations indicated) at 25°C for 2.5 h in a 1 ml final volume of assay buffer (Hepes, 20 mM; MgSO₄, 5 mM, pH 7.4), in triplicate. Assays were determined by rapid harvesting and washing (15 ml buffer) on a Dynatech Automash Cell Harvester. Specific binding of [³H]8-OH-DPAT was defined as that binding inhibited by 10 µM-5-HT.

Ligand-binding data were analysed by non-linear least-squares curve fitting using the LIGAND computer package.

Adenylate cyclase assays

Adenylate cyclase was assayed using the method of Salomon *et al.* (1974). Briefly, 200 µg of CHO cell homogenate was incubated with [³²P]ATP (2 µCi), GTP (100 µM), phosphocreatine (5 mM), creatine kinase (250 µg/ml), cyclic AMP (cAMP; 1 mM), magnesium acetate (2 mM), pargyline (10 µM), ascorbic acid (0.6 mM), theophylline (4 mM), NaCl (100 mM), ATP (0.2 mM)

and Tris (84 mM), pH 7.4, in a total assay volume of 1 ml for 30 min. [³²P]cAMP was isolated after stopping the reaction by the addition of 250 µl of 1% (v/v) SDS by sequential chromatography on Dowex and alumina columns. A tracer quantity of [³H]cAMP was added to monitor recovery. Maximal adenylate cyclase stimulation was achieved by the addition of forskolin (in a volume of 100 µl, to a final concentration of 100 µM), and in control experiments basal and forskolin-stimulated (about 10-fold) accumulation of [³²P]cAMP was linear over the 30 min time period used for assays. For the effects of 5-HT agonists, the agonist was added at the start of the assay. To investigate the effect of spiperone, this antagonist was added together with the agonist.

RESULTS

Whereas the untransfected CHO cells lacking DHFR showed no [³H]8-OH-DPAT binding (results not shown), the mixed population of transfectants was found to express 50 fmol of [³H]8-OH-DPAT binding/mg of cell homogenate protein ($n = 2$). A total of 35 monoclonal cell lines were isolated from the mixed population and screened, and all except two showed significant levels of receptor expression (between 20 and 3000 fmol/mg). One clone (clone 7) was selected for further analysis as it expressed [³H]8-OH-DPAT binding at high levels in preliminary assays. A Northern blot using a full-length probe for the 5-HT_{1A} receptor revealed a single 2.2 kb species in mRNA purified from cells of clone 7, but there was no hybridization to mRNA from untransfected cells (Fig. 1).

Further analysis by saturation binding using [³H]8-OH-DPAT was carried out with clone 7 cells, and the expression level ($B_{\max}$) was determined to be 2.8 ± 0.7 pmol of receptor/mg of membrane protein. This compares with 0.45 ± 0.07 pmol/mg for membranes from bovine hippocampus. The saturation binding data fitted best to a single-binding-site model in both cases, with dissociation constants (K_d) of 1.23 ± 0.46 nM in clone 7 cells ($n = 3$) and 0.73 ± 0.26 nM in bovine hippocampus ($n = 4$). (Fig. 2). The level of expression in the clone 7 cells was shown to be stable, with no change in $B_{\max}$ after 19 passages. When saturation analysis with [³H]8-OH-DPAT was carried out in a different buffer system [Tris, 50 mM; MgSO₄, 5 mM, pH 7.4, similar to that used by Albert *et al.* (1990) and Fargin *et al.* (1988)] the equilibrium binding parameters were similar (clone 7: $B_{\max}$, 2.8 ± 0.2 pmol/mg, K_d 0.8 ± 0.11 nM, means $\pm$ S.E.M., $n = 3$; hippocampus: $B_{\max}$, 0.34 ± 0.17 pmol/mg, K_d 0.44 ± 0.13 nM, $n = 4$).

A comparison of the pharmacological profile of the expressed

Fig. 1. Northern blot analysis of 5-HT_{1A} receptor mRNA in CHO cells

Lanes 1–3, non-transfected CHO cells containing 5 µg, 10 µg and 20 µg of RNA respectively; lanes 4–7, clone 7 cells containing 10 µg, 20 µg, 50 µg and 100 µg of RNA respectively. See the Materials and methods section for details. The size of the hybridizing species is 2.2 kb.

Fig. 2. Saturation analysis of [³H]8-OH-DPAT binding to 5-HT_{1A} receptors in bovine hippocampus (△) and recombinant CHO cells (clone 7) (○)

Specific [³H]8-OH-DPAT binding was determined in the two tissues for a range of radioligand concentrations as described in the Materials and methods section. The data shown are from representative experiments replicated as described in the text, and the data points are the means of triplicate determinations. The curves shown are the best-fit curves to single-binding-site models (clone 7 cells: $B_{\max}$, 4200 fmol/mg; K_d 1.06 nM; hippocampus: $B_{\max}$, 357 fmol/mg; K_d 0.27 nM). The mean binding parameters are given in the text.

Table 1. Pharmacological characterization of [³H]8-OH-DPAT binding to recombinant CHO cells (clone 7) expressing 5-HT_{1A} receptors and to bovine hippocampus

[³H]8-OH-DPAT (0.5 nM) binding was determined as described in the Materials and methods section in the presence of different competing ligands. Results are means $\pm$ S.E.M. of three or more determinations.

Competing ligand	K_i (nM)	
	Clone 7	Hippocampus
5-Carboxamidotryptamine	0.35 $\pm$ 0.07	0.27 $\pm$ 0.08
Buspirone	19 $\pm$ 6	16 $\pm$ 5
8-OH-DPAT	3.4 $\pm$ 0.3	2.9 $\pm$ 0.1
Ketanserin	8900 $\pm$ 2100	4600 $\pm$ 1000
5-HT	0.79 $\pm$ 0.1	1.27 $\pm$ 0.32
Spiperone	210 $\pm$ 35	190 $\pm$ 54

receptors assayed by [³H]8-OH-DPAT binding in clone 7 cells and in bovine hippocampus was carried out (Table 1, Fig. 3). The two receptors had very similar affinities for the compounds tested. In all cases, the competition data fitted best to single-binding-site models.

Linkage of the expressed receptor to a G-protein was demonstrated by dose-dependent inhibition of [³H]8-OH-DPAT binding to membranes from clone 7 cells and bovine hippocampus in the

Fig. 3. Pharmacological characterization of [³H]8-OH-DPAT binding to 5-HT_{1A} receptors in bovine hippocampus and recombinant CHO cells (clone 7)

Specific [³H]8-OH-DPAT binding was determined in the presence of a range of concentrations of competing ligands (△, 5-carboxamidotryptamine; ○, 5-HT; ◆, 8-OH-DPAT; □, buspirone; ■, spiperone; ▽, ketanserin) in bovine hippocampus membranes (a) and clone 7 membranes (b). The curves are best-fit curves to a single-binding-site model; the data points are the means of triplicate determinations and the data are from a representative experiment replicated as in Table 1.

presence of GTP (Fig. 4) or guanosine 5'-[γ -imido]triphosphate (Gpp[NH]p), which both showed very similar potencies. IC₅₀ values for GTP were 6.9 $\pm$ 2.7 μ M (mean $\pm$ S.E.M., n = 4, clone 7) and 11.6 $\pm$ 2.3 μ M (n = 4, hippocampus), and for Gpp[NH]p were 12.5 $\pm$ 4.0 μ M (n = 3; clone 7) and 6.3 $\pm$ 2.1 μ M (n = 3, hippocampus).

To test the ability of the expressed receptor to produce an intracellular response in a second messenger system, adenylate cyclase activity was measured in homogenates of control and recombinant (clone 7) CHO cells with and without forskolin and 5-HT agonists (Fig. 5). Whereas 100 μ M-forskolin stimulated adenylate cyclase 10–20 fold in both sets of cells, only in clone 7 cells did 5-HT agonists significantly inhibit the forskolin stimu-

Fig. 4. Effect of GTP on [3 H]8-OH-DPAT binding to 5-HT $_{1A}$ receptors in bovine hippocampus (Δ) and recombinant CHO cells (clone 7) ($\circ$)

Specific [3 H]8-OH-DPAT binding was determined as described in the Materials and methods section in the presence of different concentrations of GTP. The data points are the means of triplicate determinations from a representative experiment replicated as described in the text.

lation (Table 2). The inhibition was dose-dependent, and the maximum inhibition of adenylate cyclase depended on the agonist used, with 5-carboxamidotryptamine producing the greatest maximal inhibition (about 40%). As well as variation in the maximal inhibition there was variation in the concentration of agonist required to give its half-maximal response (IC_{50}) (Table 3). The IC_{50} values were in the same rank order as the K_d values derived by ligand binding (Table 1). The inhibition of adenylate cyclase in clone 7 cells by 5-carboxamidotryptamine was virtually eliminated by pre-treatment of the cells for 24 h with 100 ng of pertussis toxin/ml. Under these conditions only $3.2 \pm 3.7\%$ (mean $\pm$ S.E.M., $n = 3$) inhibition of adenylate cyclase was seen. The addition of the antagonist spiperone ($1 \mu M$) shifted the 5-carboxamidotryptamine dose-response curve to the right in a parallel manner, as expected for a competitive antagonist (Fig. 5). From the shift in the dose-response curve a K_i value of 8.0 ± 2.6 nM (mean $\pm$ S.E.M., $n = 3$) was calculated.

Clone 7 cells were also grown in suspension culture, and the levels of receptor expression and their properties were maintained compared with cells grown in adherent culture ([3 H]8-OH-DPAT binding: B_{max} , 3.3 ± 1.1 pmol/mg, K_d 2.1 ± 0.3 nM, mean $\pm$ range, two experiments). Similarly, for cells grown in suspension culture for 6 days, the IC_{50} values for GTP inhibition of [3 H]8-OH-DPAT binding were similar to those seen in cells grown in adherent culture (IC_{50} for cell homogenate, $9.3 \pm 2.3 \mu M$, $n = 3$; for membrane preparation, $7.5 \pm 2.8 \mu M$, $n = 4$).

In contrast, for cells grown in suspension culture there was a gradual loss of the ability of 5-HT agonists to inhibit forskolin-stimulated adenylate cyclase (Fig. 6), with no inhibition observed after at least 25 days in suspension culture. This loss of inhibition was seen both as a decrease in the maximal inhibition achieved

Fig. 5. Inhibition of adenylate cyclase by 5-HT agonists in homogenates of CHO cells expressing 5-HT $_{1A}$ receptors (clone 7)

Adenylate cyclase activity (stimulated by $100 \mu M$ -forskolin) was determined as described in the Materials and methods section in the presence of different concentrations of 5-carboxamidotryptamine (Δ), 8-OH-DPAT ($\circ$) and buspirone ($\square$), or in the presence of $1 \mu M$ -spiperone and different concentrations of 5-carboxamidotryptamine ($\blacktriangle$). The data are the means of triplicate determinations from a representative experiment replicated as in Table 3. The adenylate cyclase activity shown is that portion of the activity inhibitable by high concentrations of 5-carboxamidotryptamine.

Table 2. Effect of 8-OH-DPAT on adenylate cyclase activity in non-transfected and clone 7 CHO cells

Adenylate cyclase activity was determined as described in the Materials and methods section under basal conditions, after stimulation by forskolin and in the presence of 8-OH-DPAT in both untransfected and clone 7 CHO cells. Data are expressed as means $\pm$ S.E.M., and are percentages of the uninhibited forskolin-stimulated adenylate cyclase activity from at least three determinations performed in triplicate.

	Adenylate cyclase activity (% of control)	
	Untransfected CHO cells	Clone 7 CHO cells
Basal activity	10.3 ± 1.9	6.3 ± 0.8
Basal activity + 8-OH-DPAT ($10 \mu M$)	8.7 ± 2.6	4.7 ± 0.7
Forskolin-stimulated activity + 8-OH-DPAT ($10 \mu M$)	96.5 ± 0.8	58.9 ± 9.5

by the agonist and as an increase in the concentration of agonist needed to achieve half-maximal inhibition.

DISCUSSION

In this paper, we describe the stable high-level expression of human 5-HT $_{1A}$ receptors in recombinant CHO cells transfected with the receptor gene. The study generated a panel of cell lines expressing the receptor at different levels, as assessed by [3 H]8-OH-DPAT binding, and we have characterized the expressed

Table 3. Characterization of 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in clone 7 cells

Adenylate cyclase activity was determined as in Table 2. The 'effect (%)' is the inhibition of forskolin-stimulated adenylate cyclase seen at high concentrations of the agonist, expressed as a percentage of the inhibition produced by high concentrations of 5-carboxamidotryptamine ($39 \pm 2\%$, mean $\pm$ S.E.M., $n = 5$, inhibition of the forskolin stimulation). Values are means $\pm$ S.E.M. of n determinations performed in triplicate.

Ligand	IC ₅₀ (nM)	Effect (%)	<i>n</i>
5-Carboxamidotryptamine	1.49 $\pm$ 0.46	100	5
8-OH-DPAT	16.7 $\pm$ 2.8	88.7 $\pm$ 1.3	4
Buspirone	74.6 $\pm$ 17.2	66.4 $\pm$ 0.2	4
5-Carboxamidotryptamine + spiperone (1 μ M)	232 $\pm$ 69	103.2 $\pm$ 1.7	3

Fig. 6. Inhibition of adenylate cyclase by 5-carboxamidotryptamine in homogenates of CHO cells expressing the 5-HT_{1A} receptor (clone 7) grown in adherent or suspension culture

Adenylate cyclase activity (stimulated by 100 μ M-forskolin) was determined as described in the Materials and methods section in the presence of different concentrations of 5-carboxamidotryptamine. The data are means of triplicate determinations and are for cells grown in adherent culture ($\blacktriangle$), or cells grown in suspension culture for 4 days ($\circ$), 5 days ($\square$) and 25 days ($\triangle$). The curves were obtained by non-linear regression using the computer program Genfit. IC₅₀ values for 5-carboxamidotryptamine were 1.49 ± 0.36 nM (mean $\pm$ S.E.M., $n = 5$) for adherent cultured cells, and for cells grown in suspension culture they were 3.5 nM ($n = 1$; 4 days), 30.9 nM ($n = 1$; 5 days) and > 1000 nM ($n = 3$; 25 days).

receptor in detail in one cell clone (clone 7). This clone shows saturable high-affinity binding of [³H]8-OH-DPAT, and in competition experiments a range of 5-HT_{1A} receptor-selective compounds competed with [³H]8-OH-DPAT binding with high affinity. Their affinities are also very similar to those seen for the 5-HT_{1A} receptor of bovine hippocampus determined in parallel assays. This shows that an authentic 5-HT_{1A} receptor has been expressed and that the bovine and human receptors show quite similar pharmacological properties. Northern blot analysis of RNA from the cells showed a single hybridizing species of 2.2 kb, consistent with previous reports on the size of the human 5-HT_{1A} receptor mRNA (Fargin *et al.*, 1988).

In all cases in saturation and competition experiments, the ligand binding data fit best to a model indicating a single class of sites in both the recombinant cell line and bovine hippocampus. Similar results have been reported for [³H]8-OH-DPAT binding to 5-HT_{1A} receptors from rat and pig brain, where a single class of binding sites was seen in saturation analyses (K_d 1.2–5 nM) and in competition studies (Gozlan *et al.*, 1983; Hall *et al.*, 1985; Hoyer *et al.*, 1985). However, when the human 5-HT_{1A} receptor was expressed stably in HeLa cells and the rat receptor was expressed in Ltk⁻ cells, saturation analysis of [³H]8-OH-DPAT binding indicated two affinity states (K_d values respectively 0.4 nM/1.89 nM and 0.78 nM/8.7 nM; Fargin *et al.*, 1989; Albert *et al.*, 1990). For other receptors, higher- and lower-affinity agonist binding sites have been attributed to interactions with G-proteins, with the higher-affinity binding sites being associated with receptors coupled to G-proteins and the lower-affinity binding sites associated with uncoupled receptors. In the present report, the expressed receptors in cells grown in adherent culture seemed to be in a single affinity state with high affinity for agonists. The coupling of this form to a G-protein is supported by the fact that essentially all the [³H]8-OH-DPAT binding could be eliminated by GTP or Gpp[NH]p. The IC₅₀ values for the guanine nucleotides are very close to those found in bovine hippocampus (present study) and rat hippocampus (Hall *et al.*, 1985). These IC₅₀ values are, however, substantially higher than values obtained with 5-HT_{1A} receptors expressed in other cell lines (see above; Fargin *et al.*, 1989; Albert *et al.*, 1990), suggesting that the coupling of receptor and G-protein may vary according to the cell type used for expressing the recombinant receptor.

The expressed 5-HT_{1A} receptor in clone 7 cells was also functional in that 5-HT agonists inhibited forskolin-stimulated adenylate cyclase, and the effect of a 5-HT agonist could be inhibited by an antagonist, spiperone. The inhibition of adenylate cyclase by a 5-HT agonist could also be prevented by pretreatment of the cells with pertussis toxin, so that a G_i- or G_o-type of G-protein must be involved. Although some studies in brain tissue report 5-HT_{1A} receptor inhibition of adenylate cyclase (De Vivo & Maayani, 1986), in other studies stimulation of the enzyme is reported (Shenker *et al.*, 1987). In the present study there was no evidence for stimulation, and the stimulation in brain homogenate may therefore reflect activation of another 5-HT receptor closely related to the 5-HT_{1A} receptor expressed here.

Three 5-HT_{1A} receptor agonists (5 carboxamidotryptamine, 8-OH-DPAT and buspirone) with differing abilities to inhibit adenylate cyclase in brain (De Vivo & Maayani, 1986; Dumuis *et al.*, 1987; Schoeffter & Hoyer, 1988) were tested on clone 7 cells in the present study. Each agonist was able to inhibit adenylate cyclase to different extents when used at a high concentration. Both the extent of the inhibition and the IC₅₀ values for inhibition were comparable with data obtained for brain (De Vivo & Maayani, 1986; Dumuis *et al.*, 1987; Schoeffter & Hoyer, 1988). The IC₅₀ values also were in the same rank order as the affinities of the agonists derived from ligand binding. Although we do not know the ratio of 5-HT_{1A} receptor to G-protein in clone 7 cells, the fact that the level of expression of the receptor expressed per mg of protein is about seven times higher than that in brain suggests that the receptor/G-protein ratio does not affect the relative abilities of agonists to inhibit adenylate cyclase. Thus an agonist such as buspirone, which behaves as a partial agonist in brain, does likewise in the highly expressing cell line.

Clone 7 cells could also be grown in suspension culture, where much greater amounts of cell protein may be obtained. In suspension culture the level of receptor expression is maintained, so this may provide a useful system for isolating large amounts

of receptor protein in the future. However, although receptor number was maintained, the ability of the receptor to inhibit adenylate cyclase was gradually lost in suspension culture. This did not reflect a change in receptor-G-protein coupling, as judged by the ability of GTP to inhibit [^3H]8-OH-DPAT binding. The most likely explanation seems to be a change at the level of the G-protein-adenylate cyclase interaction. This could in turn reflect effects on the cytoskeleton caused by the rather different requirements of cells for growth in suspension culture compared with adherent culture. Alternatively, the effects could result from an exhaustion of a factor in the culture medium which affects adenylate cyclase responses. For example, Johnson *et al.* (1990) have described effects of serum components on the activity of receptor-linked adenylate cyclase activities.

The cell line (clone 7) expressing human 5-HT_{1A} receptors, analysed in detail here, provides a very useful model system for studying the receptor. It must be remembered that the cell line is an artificial system which may contain interactions not normally found *in vivo*, such as between the recombinant receptor and 'incorrect' G-proteins. Nevertheless, the ligand-binding properties of the receptor are essentially identical to those of the receptor expressed in brain, and the receptor is coupled via a G-protein to a response typical of the 5-HT_{1A} receptor, i.e. inhibition of adenylate cyclase. The level of receptor expression (approx. 3 pmol/mg of protein) is about seven times that of brain tissue, and the use of the CHO cell as the cell host for expression should enable large-scale suspension culture of the cell line to be carried out under well-defined conditions (e.g. see Curling *et al.*, 1990). This recombinant cell line should therefore provide a very useful system for studies on the 5-HT_{1A} receptor protein, particularly for receptor isolation and purification.

We thank the SERC for financial support, Sue Davies for preparing the manuscript and Dave Murray and Rob Hazelwood for helpful advice.

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Received 16 October 1991/22 January 1992; accepted 20 February 1992