ELSEVIER

European Journal of Pharmacology 396 (2000) 1–8

ejp

www.elsevier.nl/locate/ejphar

Antagonist activity of meta-chlorophenylpiperazine and partial agonist activity of 8-OH-DPAT at the 5-HT_7 receptor

Martyn Wood *, Mark Chaubey, Peter Atkinson, David R. Thomas

Neuroscience Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK

Received 24 January 2000; received in revised form 3 March 2000; accepted 10 March 2000

Abstract

This study compared the use of adapter G-proteins to link G_s coupled G-protein receptors to a Ca^{2+} signal, enabling high throughput functional studies using a fluorescent imaging plate reader (FLIPR, Molecular Devices).The pharmacological profile of the human 5-hydroxytryptamine (5-HT_7) receptor was studied using the adapter G-proteins $G_{\alpha 16}$ and G_{qs5} and compared to previously published adenylyl cyclase and receptor binding data. Human embryonic kidney (HEK) 293 cells stably expressing the human 5-$HT_{7(a)}$ receptor were transiently transfected with the adapter G-proteins. Changes in intracellular Ca^{2+} were monitored using the fluorescent Ca^{2+}-indicator Fluo-4. 5-Carboxamidotryptamine (5-CT) induced an increase in fluorescence in transfected cells only, which was attenuated by *N*-ethylmalaeimide and abolished by thapsigargin, consistent with a G-protein mediated mobilisation of intracellular Ca^{2+}. The pharmacological profile of agonists at the 5-HT_7 receptor was similar using either adapter G-protein. Agonist potency estimates were similar to that reported in binding studies but were greater than that seen in adenylyl cyclase studies. 8-Hydroxy-*N,N*-dipropylaminotetralin (8-OH-DPAT) and tryptamine acted as partial agonists using the adapter G-proteins, but were full agonists in recombinant systems using adenylyl cyclase. meta-Chlorophenylpiperazine (mCPP) and trifluoro-methylphenyl piperazine (TFMPP) were antagonists on intracellular Ca^{2+}. Antagonist pharmacological profiles were similar between adapter G-proteins, receptor binding, and adenylyl cyclase studies. These results show that adapter G-proteins can be used to study G_s-linked receptors using the high throughput FLIPR system measuring changes in intracellular Ca^{2+} and provide novel information on mCPP and 8-OH-DPAT.

Keywords: 5-HT (5-Hydroxytryptamine serotonin); 5-HT receptor; FLIPR (flourescent imaging plate reader); Fluorescence; Meta-chlorophenylpiperazine; 8-OH-DPAT (8-hydroxy-*N,N*-dipropylaminotetralin)

1. Introduction

5-Hydroxytryptamine (5-HT) receptor subtypes have been classified based on structural, operational, and transductional criteria into seven major groups (Hoyer et al., 1994), including the human 5-HT_7 receptor which has been cloned (Bard et al., 1993). 5-HT_7 receptor mRNA is found in the greatest abundance in the brain where it is discretely localised within the thalamus, hypothalamus, and various limbic and cortical regions (To et al., 1995; Gustafson et al., 1996). The 5-HT_7 receptor is also found in smooth muscle cells from blood vessels and in the gastro-intestinal tract (Bard et al., 1993; Schoeffter et al., 1996). 5-HT_7 receptors have been implicated with circadian rhythmicity, through pharmacological and receptor localisation studies, which indicate that 5-HT_7 receptors may be involved in phase shifts of neuronal activity in the suprachiasmatic nucleus of rat hypothalamus (Lovenberg et al., 1993; Tsou et al., 1994). Based on such brain localisation, it has been suggested that 5-HT_7 receptors may be linked to psychiatric disorders including depression (Sleight et al., 1995) and schizophrenia, the latter being based on evidence that atypical antipsychotics such as clozapine exhibit moderate to high affinity for the 5-HT_7 receptor (Roth et al., 1994). The 5-HT_7 receptor is a member of the G-protein coupled receptor superfamily and has been shown to be positively coupled to adenylyl cyclase (Bard et al., 1993; Adham et al., 1998). A number of receptor splice variants have been shown to exist in man; a truncated splice variant, 5-$HT_{7(b)}$ short and the long form, 5-$HT_{7(a)}$, both of which display similar pharmacological and functional characteristics (Jasper et al., 1997).

* Corresponding author. Tel.: +44-1279-622247; fax: +44-1279-622230.

E-mail address: martyn_wood-1@sbphrd.com (M. Wood).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00213-2

The fluorescent imaging plate reader (FLIPR, Molecular Devices, CA, USA) allows high throughput functional assays to be carried out in intact cells (Schroeder and Neagle, 1996). In particular, it has been widely used in the study of both ligand-gated ion channels (Wood et al.,2000) and G-protein-coupled receptors (Porter et al., 1999) where receptor activation changes intracellular Ca^{2+} levels, which is measured using the fluorescent Ca^{2+} sensitive dye, Fluo-3. For G-protein-coupled receptors, the normal signal transduction pathway would be initiated by receptor mediated activation of a member of the G_q protein family leading to phospholipase C activation (see Clapham, 1995). As the 5-HT_7 receptor normally couples through G_s to stimulate adenylyl cyclase (Thomas et al., 1999), receptor activation would not normally give rise to a change in intracellular Ca^{2+} levels. Two methods have been described which allow the functional coupling of non-G_q coupled receptors to phospholipase C. Milligan et al. (1996) described the use of the promiscuous G-protein $G_{\alpha 16}$ as a universal G protein adapter which would allow coupling of a wide range of G-protein-coupled receptors to phospholipase C and Conklin et al. (1993) have shown that carboxy terminal mutations of the G_α subunit may alter the specificity of the G-protein. Thus in the latter study, replacement of the five c-terminal amino acids of G_q with those of G_s, produced the chimeric G-protein G_{qs5} which coupled receptors normally linked to the stimulation of adenylyl cyclase to stimulation of phospholipase C. However, the α_{1D}- and α_{1A}-adrenoceptors failed to stimulate $G_{\alpha 16}$ (see Milligan et al., 1996) and the β_2-adrenoceptor failed to couple to G_{qs5} (Conklin et al., 1996) suggesting that any one adapter G-protein may not be universal.

We have therefore used these adapter G-proteins in order to assess their utility in high throughput screening and in structure–activity relationship studies, using the FLIPR. We have utilised the recently described Ca^{2+}-sensitive dye Fluo-4, which gives a greater light output than Fluo-3 (Molecular Probes Handbook). Using a transient expression system, we have compared the adapter G-proteins $G_{\alpha 16}$ and G_{qs5} using the human 5-$HT_{7(a)}$ receptor as a characterised example of a G_s-coupled receptor (Thomas et al., 1998). In particular, we have compared the pharmacologically profile of the human 5-$HT_{7(a)}$ receptor obtained with these two adapter G-proteins and further compared the profile to that obtained in cyclase and binding studies in non-transfected human embryonic kidney (HEK) 293 cells stably expressing the 5-HT_7 receptor, i.e. without a transfected adapter G-protein.

2. Materials and methods

2.1. Materials

For culture and sub-cultivation of HEK 293 cells stably expressing the human 5-$HT_{7(a)}$ receptor (293/5-HT_7), materials were obtained from GIBCO/BRL except G418 from Calbiochem and Accutase from Innovative Cell Technologies. For transfections: Optimem I serum free media and LipofectAMINE PLUS™ were obtained from GIBCO/BRL. Non-replicating chimeric vectors containing cDNAs for $G_{\alpha 16}$ and G_{qs5} were obtained from Molecular Devices. Fluo-4 was obtained as the acetoxymethyl ester from Molecular Probes. 5-Carboxamidotryptamine (5-CT), R(+)-lisuride, risperidone, methiothepin, mesulergine, ketanserin were from RBI. Trifluoro-methylphenyl piperazine (TFMPP) and thapsigargin were from Tocris. 5-HT, 8-hydroxy-*N*,*N*-dipropylaminotetralin (8-OH-DPAT), 5-methoxy-*N*,*N*-di-methyltryptamine (5-MeO-NDMT), tryptamine, meta-chlorophenylpiperazine (mCPP), amitriptyline, *N*-ethylmalaeimide and clozapine were from Sigma. Olanzapine was from SmithKline Beecham, Department of Medicinal Chemistry. HEK 293 cells expressing the 5-HT_7 receptor were described in Thomas et al. (1998).

Black-walled clear-bottomed microplates were obtained from Corning-Costar. NaCl, KCl, HEPES, Glucose, $MgCl_2$, $CaCl_2$, probenecid, and dimethyl sulphoxide (DMSO) were from Sigma.

2.2. Cell culture and transfection

293/5-HT_7 cells were grown in Minimum Essential Media (MEM), with 10% dialysed Fetal Bovine Serum, 1% non-essential amino acids, and the antibiotic G418 at 400 μg/ml. The cells were maintained at 37°C, 5% CO_2, 95% O_2 in a humidified incubator. Cells were subcultured with the cell detachment agent Accutase. Cells were plated into 96-well black-walled clear-bottomed microplate at a density of 40,000 cells per well The microplates were coated with poly-D-lysine prior to cell plating, to allow adherence of the cells. All cells used were less than passage 40, at which stage new cells were brought up from cryopreservation and used after passage 8.

Cells were split so that they were sub-confluent on the morning of the experiment.

Cells were then transiently transfected with $G_{\alpha 16}$ and G_{qs5} cDNAs using LipofectAMINE™ PLUS and the manufacturer recommended procedure. Typically, 10 μg of cDNA was used for one T80 flask of 293/5-HT_7 cells (c10^7 cells). Following transfection, normal serum containing growth medium was added to the cells and incubated overnight. Cells were then harvested and plated in 96 well microplates as described and incubated overnight. For sham transfections, distilled water was substituted for DNA, but all other reagents and procedures remained the same.

2.3. FLIPR assay

Agonists were prepared typically at 1 mM in Tyrode's buffer (composition (mM) KCl: 2.5; NaCl: 145; HEPES: 10; glucose: 10; $MgCl_2$: 1.2; $CaCl_2$: 1.5; probenecid: 2.5,

and pH was 7.4) except R(+)-lisuride which required 100% DMSO. For antagonists, solutions were made at 10 mM in 100% DMSO and were diluted in Tyrode's buffer. The final concentration of DMSO in the assay was less than 0.3%. Drug dilutions were carried out in 96 well blocks using a Biomek 2000 (Beckman). Fluo-4 (1 mg) was dissolved in DMSO (440 μl) and pluronic acid (20% in DMSO, 440 μl) and an aliquot diluted in media to give 20 μM.

The cells were incubated for 1 h at 37°C, 5% CO_2 and 95% O_2 with Fluo-4 (final concentration 4 μM) in the presence of probenecid (0.8 mM final). Cells were then washed thoroughly using a Denley cell wash system to remove extracellular dye and a residual volume of 125 μl of Tyrode's buffer was left in each well. After washing, 25 μl of antagonists or buffer was added to each well and incubated for 30 min at 37°C, 5% CO_2, 95% O_2. The cell plate was loaded into the FLIPR and a signal test was taken and laser power adjusted to obtain a basal level of ~10,000 Fluorescence Intensity Units (FIU). The cells were then excited at 488 nm using the FLIPR laser and fluorescence emission determined used a CCD camera with a bandpass interference filter (510–560 nm). Fluorescence readings were taken at 1 s intervals for 60 s and a further 24 readings were taken at 5 s intervals. Compounds (50 μl) were added after 20 s using the FLIPR. Raw fluorescence data was exported for each well and tabulated versus time within an ASC II file. Data was then imported into Excel and the peak response over basal determined.

2.4. Radioligand binding

Radioligand binding assays using [^{3}H]-5-CT were carried out in washed membranes as described in Thomas et al. (1998). Membranes were prepared from non-transfected and G_{qs5} transfected 293/5-HT_7 cells.

2.5. Data analysis

Concentration–effect data was analysed by non-linear regression fitted to the four-parameter logistic equation to provide EC_{50} and maximal response (E_{max}; Bowen and Jerman, 1995). Each 96-well plate contained at least one 5-CT concentration–effect curve to provide EC_{50} and E_{max} values. Antagonist data (IC_{50}) were analysed using the same method from inhibition curves using an EC_{80} concentration of 5-CT (10 nM) which gave a large, reproducible sub-maximal response. Antagonist affinity was defined as apparent pK_B according to Craig (1993). Results are quoted as mean ± S.E.M. from (n) separate experiments. Statistical significance between data from different experiments was determined using Student's *t*-test. Statistical differences in curve-fitted results within an experiment were determined using the *F*-test, e.g. was the fitted maximal response different.

3. Results

3.1. Agonist studies

5-CT and 5-HT (10^{-10}–10^{-6} M) both increased fluorescence in G_{qs5}- and $G_{\alpha 16}$-transfected cells and had no significant effect in sham-transfected cells. The maximal increase in fluorescent units was typically 12,000 for G_{qs5} and 8000 with $G_{\alpha 16}$ and varied usually by less than 10% between experiments but could vary by up to 20%. This probably reflects variations in transfection efficiency and cell viability following the transfection procedure. Peak

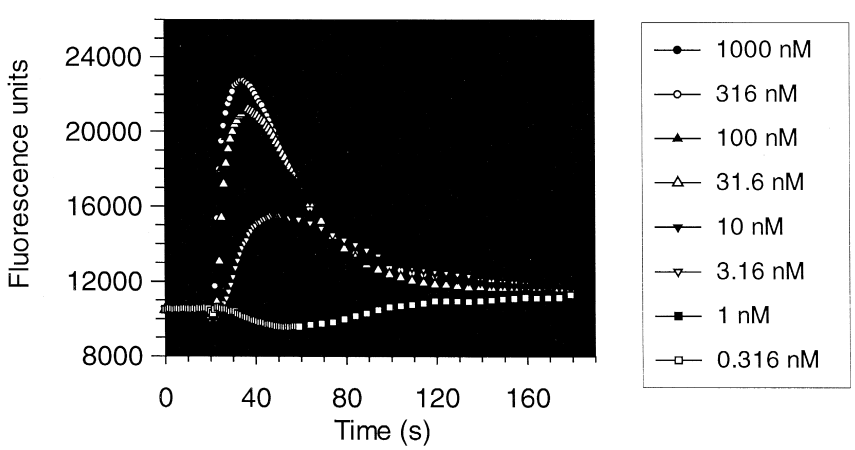

Fig. 1. Time-course for 5-CT-induced increase in intracellular Ca^{2+} in $G_{\alpha 16}$ transfected 5-HT_7/HEK 293 cells. Results are the fluorescent measurements taken from a single 96 well plate where cells were incubated with varying concentrations of 5-CT. Data is from a single experiment, repeated twice with similar results.

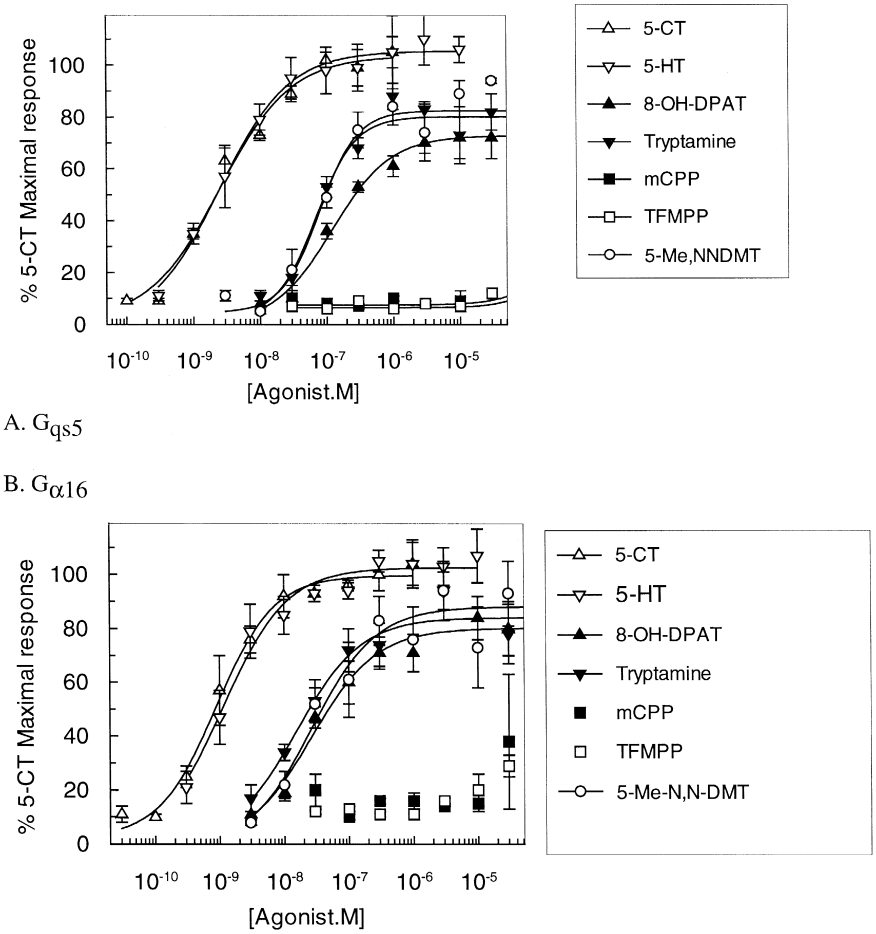

Fig. 2. Concentration–response curves at 5-HT_7 receptors using the adapter G-proteins G_{qs5} (A) and $G_{\alpha 16}$ (B). Data points represent the peak increase in fluorescence obtained at each concentration and are the means from three to six separate experiments. Data are shown as percentage of the fitted maximal response to 5-CT.

height was typically reached within 10–15 s after agonist addition and returned to baseline within 2 min (shown for $G_{\alpha 16}$ in Fig. 1, with similar results observed with G_{qs5}). Carbachol typically induced a response of 40,000 fluorescent units mediated through an endogenous muscarinic receptor. Basal levels were typically 10,000 fluorescent units. Concentration–effect curves were constructed to a range of putative agonists in both G_{qs5}- and $G_{\alpha 16}$-transfected 5-HT_7 cells. Results are shown in Fig. 2 and summarised in Table 1, with results presented as a percentage of the maximal response seen to 5-CT in order to normalise results between experiments and correct for variables such as differences in cell density, Fluo-4 loading, and particularly transfection efficiency, which would be expected to vary between experiments. The results obtained above are compared with binding and cyclase functional data in Table 2. There was a tendency for compounds to be more potent in the $G_{\alpha 16}$ system than with G_{qs5} and this was significant for 5-HT, 8-OH-DPAT, and tryptamine (see Table 1). The maximal response seen with 8-OH-DPAT, and tryptamine was less than that seen with 5-CT and this was significant for both compounds with G_{qs5} but was only significant for 8-OH-DPAT with $G_{\alpha 16}$

Table 1
Pharmacological profile of a series of putative 5-HT_7 receptor agonists in HEK 293/5-HT_7 cells transfected with G_{qs5} or $G_{\alpha 16}$
Data are means from $n = 11$ separate experiments for 5-CT and from $n = 3$ for other agonists, ± S.E.M. Efficacy was defined as the fraction of the fitted maximal response relative to the fitted maximal response to 5-CT (defined as 1).

Agonist	$G_{\alpha 16}$		G_{qs5}	
	pEC_{50}	Efficacy	pEC_{50}	Efficacy
5-CT	8.59 ± 0.14	1.00	8.33 ± 0.15	1.00
5-HT	8.93 ± 0.13	1.02 ± 0.05	8.60 ± 0.02[a]	1.04 ± 0.10
8-OH-DPAT	7.44 ± 0.14	0.87 ± 0.04[b]	6.90 ± 0.06[a]	0.73 ± 0.06[b]
Tryptamine	7.70 ± 0.27	0.91 ± 0.13	7.17 ± 0.08[a]	0.82 ± 0.03[b]
mCPP	< 5	–	< 5	–
TFMPP	< 5	–	< 5	–
5-MeNNDMT	7.46 ± 0.04	0.93 ± 0.06	7.08 ± 0.02	0.89 ± 0.06[b]

[a] $P < 0.05$ pEC_{50} between $G_{\alpha 16}$ and G_{qs5}, Student's paired *t*-test.
[b] $P < 0.05$ efficacy compared to 5-CT, $P < 0.05$ *F*-test.

Table 2
Comparison of agonist functional potency and binding affinity at the human 5-HT_7 receptor

Agonist	Ca_I^{2+} G_{qs5}, pEC_{50}	Binding, pK_I	Cyclase (cAMP), pEC_{50}
5-CT	8.33 ± 0.15	9.10 ± 0.10[a]	7.70 ± 0.10[a]
5-HT	8.60 ± 0.02	8.20 ± 0.10[a]	6.80 ± 0.10[a]
8-OH-DPAT	6.90 ± 0.06	6.60 ± 0.10[a]	6.10 ± 0.10[a]
Tryptamine	7.17 ± 0.08	–	5.62 ± 0.36[b]

[a]Radioligand binding and cyclase (cAMP) assays taken from Thomas et al. (1998).
[b]Radioligand binding and cyclase (cAMP) assays taken from Adham et al. (1998).

Table 4
Radioligand binding affinities of compounds in G_{qs5}-transfected and non-transfected 293/5HT_7 cells
Data are means ± S.E.M. from three separate experiments with data from non-transfected cells taken from Thomas et al. (1998).

Compound	G_{qs5}-transfected cells (pK_i)	Non-transfected cells (pK_i)
Risperidone	8.55 ± 0.25	8.30 ± 0.10
5-CT	9.78 ± 0.28	9.10 ± 0.10[a]
5-HT	8.84 ± 0.11	8.20 ± 0.10[a]

[a]$P < 0.05$, Student's *t*-test.

(see Table 1). Both mCPP and TFMPP lacked apparent agonist-like effects with either $G_{\alpha 16}$ or G_{qs5} on intracellular Ca^{2+} at the human 5-HT_7 receptor in HEK 293 cells at concentrations up to 10^{-5} M. At higher concentrations, TFMPP and mCPP produced agonist-like effects in $G_{\alpha 16}$ or G_{qs5} transfected 5-HT_7 cells as well as in non-transfected 5-HT_7 expressing HEK 293 cells (not shown).

3.2. Antagonist studies

For antagonist studies, 5-HT_7/HEK 293 cells transfected with either $G_{\alpha 16}$ or G_{qs5} were stimulated with a sub-maximal concentration (10 nM) of 5-CT. Results are shown in Table 3 where they are compared to publish receptor binding values and functional data from cAMP assay in the 293/5-HT_7 cells. There was excellent agreement in affinity estimates for antagonists between the two adapter G proteins. There was also a good correlation between these affinity estimates and those obtained from published binding and cAMP studies (slope 0.8–0.9, r 0.8–0.9) although antagonist affinities were higher in the FLIPR assay systems ($P < 0.05$ for clozapine, risperidone, mesulergine, olanzapine, Student's unpaired *t*-test, two-tail).

As mCPP and TFMPP lacked agonist activity at the 5-HT_7 receptor, their ability to inhibit functional responses to 5-CT was examined. The top concentration of mCPP and TFMPP used was 10^{-5} M to limit interference from presumed activation of endogenous receptor (see above). mCPP antagonised 5-CT induced increases in intracellular Ca^{2+} at the 5-HT_7 receptor expressed in HEK 293 cells with apparent pK_B values of 6.25 ± 0.23 and 6.71 ± 0.24 (mean ± S.E.M., $n = 3$, for the $G_{\alpha 16}$ and G_{qs5} systems, respectively). Similarly, TFMPP had apparent pK_B values of 5.60 ± 0.24 and 6.12 ± 0.21 (for $G_{\alpha 16}$ and G_{qs5}, respectively).

3.3. Radioligand binding studies

The inhibition of binding of [^{3}H]-5-CT to G_{qs5} transfected 5-HT_7 expressing HEK 293 cells is shown in Table 4 and compared to that seen in non-transfected 5-HT_7 cells. There was a significant increase in affinity estimates for the agonists 5-CT and 5-HT in G_{qs5} transfected cells compared to non-transfected cells (see Table 4). There was no difference in affinity estimate for the antagonist risperidone.

3.4. Signaling studies

Pre-treatment of G_{qs5} transfected cells with thapsigargin for 30 min, inhibited the 5-CT-induced increase in fluorescence with a calculated pK_B of 8.60 ± 0.10 ($n = 3$). Similarly, pre-treatment with *N*-ethylmalaeimide also inhibited the response to 5-CT. *N*-ethylmalaeimide (30 μM) significantly ($P < 0.05$, *F*-test) reduced the maximal response to

Table 3
Antagonist affinities at the human 5-HT_7 receptor as determined using $G_{\alpha 16}$ and G_{qs5} in FLIPR compared with radioligand binding and cyclase (cAMP) assays
All data are the mean of three to five independent experiments ± S.E.M. Cyclase and binding data taken from Thomas et al. (1998). ND = not determined.

Antagonist	$G_{\alpha 16}$, apparent pK_B	G_{qs5}, apparent pK_B	Binding, pK_I	cAMP, pK_B
Risperidone	9.15 ± 0.28	9.68 ± 0.26	8.30	8.30
Methiothepin	8.21 ± 0.16	8.12 ± 0.21	8.50	8.1
Mesulergine	8.51 ± 0.12	8.74 ± 0.23	7.50	7.50
Clozapine	8.40 ± 0.14	8.78 ± 0.59	7.20	7.20
Olanzapine	7.15 ± 0.09	7.00 ± 0.32	6.50	6.30
Ketanserin	6.56 ± 0.10	7.57 ± 0.18	6.10	6.30
Amitriptyline	7.05 ± 0.22	6.81 ± 0.13	ND	ND

5-CT to 75% ± 11 for $G_{\alpha 16}$ transfected cells and to 48% ± 6 for G_{qs5} transfected cells ($n = 3$), compared to non-treated cells. In both groups of cells, *N*-ethylmalaeimide (30 μM) had no significant effect on the functional potency of 5-CT. *N*-ethylmalaeimide had no effect on [^{3}H]-5-CT binding to the 5-HT_7 receptor at concentrations up to 10 μM.

4. Discussion

These results show that the adapter G-proteins $G_{\alpha 16}$ and G_{qs5} can be used to link G_s-coupled receptors to a Ca^{2+} signal, allowing high throughput functional assays using non-G_q coupled seven-transmembrane receptors to be performed on the FLIPR. Although previous studies have shown that $G_{\alpha 16}$ can be used to link G_s-coupled receptors to a Ca^{2+} signal (adenosine A_{2A}; Stables et al., 1997), no detailed pharmacological study has been reported, nor indeed have the adapter proteins G_{qs5} and $G_{\alpha 16}$ been compared.

Using the 5-HT_7 receptor as an example of a G_s coupled seven-transmembrane receptor, we have found that the pharmacological profile of the 5-HT_7 receptor was similar when coupled to either G_{qs5} or $G_{\alpha 16}$. There was evidence that 8-OH-DPAT and tryptamine showed partial agonist activity using G_{qs5} whereas only 8-OH-DPAT was a partial agonist using $G_{\alpha 16}$. The potency of agonists tended to be lower with G_{qs5} compared to $G_{\alpha 16}$, being significantly different for 5-CT and tryptamine. This suggests that there may be a lower coupling efficiency of the G_{qs5} protein with the 5-HT_7 receptor than seen with $G_{\alpha 16}$. There were no significant differences in antagonist affinities between to two adapter G-proteins.

Although there was little difference in pharmacological profile between adapter G-proteins, some differences were observed compared to what is assumed to be the native signal transduction system, adenylyl cyclase activity. In guinea-pig hippocampus, 5-HT_7 receptor-mediated stimulation of adenylyl cyclase has been demonstrated (Thomas et al., 1999). The agonists 5-CT, 5-HT, and 8-OH-DPAT are all less potent in cyclase studies as compared to their binding affinity (Thomas et al., 1998), whereas their functional potency on the FLIPR using the adapter G-proteins are similar to their binding affinities. Other differences included 5-HT was of similar or greater functional potency than 5-CT and 5-CT was less potent in the FLIPR than on binding. In many published radioligand and adenylyl cyclase studies, 5-CT is more potent than 5-HT at the 5-HT_7 receptor (Adham et al., 1998; Thomas et al., 1998) although this difference is not seen in some studies with 5-HT and 5-CT having similar receptor binding affinities (Jasper et al., 1997; Tsou et al., 1994). This difference in the rank order of potency between 5-HT and 5-CT was not due to a change in radioligand binding properties as a consequence of the transfection procedure or of coupling to a different G-protein. Indeed, the affinity of 5-CT for the 5-HT_7 receptor was increased following the transfection procedure. This may reflect a kinetic difference between the intracellular Ca^{2+} assay, where the response is seen seconds after addition of the agonist, compared to radioligand binding and adenylyl cyclase studies, which involve long incubations.

In functional studies using recombinant systems associated with high expression levels, the large receptor reserve is often linked to high functional potency (see Porter et al., 1999). The low functional potency seen in 5-HT_7 cyclase studies suggests that either the active form of the receptor is the low affinity state or that the 5-HT_7 receptor may be desensitised following the prolonged incubation times used in cyclase studies (typically > 10 min) compared to the rapid response in intracellular Ca^{2+} assays (typically < 10 seconds). Indeed, agonist-induced desensitisation has been reported in adenylyl cyclase experiments mediated by 5-HT_7 receptors (Schimizu et al., 1998). Interestingly, there may be differences in desensitisation-mechanisms between cell lines which may be responsible for the high functional potency of 5-HT and 5-CT (pEC_{50} 8.8 for 5-CT) seen in other studies in Chinese hamster ovary cells (Tsou et al., 1994).

Although there was a good correlation between antagonist affinity measured in functional studies using either G_{qs5} or $G_{\alpha 16}$ and receptor binding affinity, many antagonists were significantly more potent in intracellular Ca^{2+} studies. This may be due to various factors. Firstly, the use of the modified Cheng–Prusoff correction (Craig, 1993) assumes that compounds are competitive inhibitors and false estimates of antagonist potency may arise if compounds are non-competitive. Secondly, under the conditions employed in the FLIPR, where the antagonist is pre-incubated with the receptor and the response is measured within seconds of agonist addition, non-equilibrium kinetics may be observed which could lead to false estimates of antagonist affinity.

The present study also suggested that mCPP and TFMPP display antagonist properties at the human 5-HT_7 receptor. These compounds are usually associated with agonist activity at 5-HT receptors (see Kennett, 1993) although mCPP may display partial agonist and antagonist properties at the human 5-HT_{2B} receptor where it has low intrinsic activity (Thomas et al., 1996; Porter et al., 1999). There is literature data available on the binding affinity of mCPP and TFMPP at the 5-HT_7 receptor (e.g. Shen et al., 1993) but there is no literature data available on their functional activity at this receptor. Both mCPP and TFMPP at high concentrations affected intracellular Ca^{2+} mobilisation through a presumed action on an endogenous receptor. The nature of this is not known and it is presumed at these high concentrations that mCPP and TFMPP are non-selective.

The 5-HT_7-mediated intracellular Ca^{2+} response was not observed in cells not transfected with the adapter

G-protein. In transfected cells, the Ca^{2+} response was attenuated by *N*-ethylmalaeimide, which alkylates α-subunits (Jackisch et al., 1994). Importantly, *N*-ethylmalaeimide treatment did not affect radioligand binding affinity suggesting that the effects of *N*-ethylmalaeimide were mediated through the G-protein rather than a direct effect on the receptor. That the intracellular Ca^{2+} response was blocked by thapsigargin, which inhibits Ca^{2+} pumps in the endoplasmic reticulum (see Clapham, 1995), suggests that the Ca^{2+} was released from intracellular stores. This is consistent with the observed response being mediated by the transfected G-proteins.

In conclusion, we have shown that the adapter G-proteins can be used to study G_s-linked receptors using a high-throughput system measuring changes in intracellular Ca^{2+}. In the present study, 8-OH-DPAT and tryptamine appear as partial agonists compared to 5-CT whereas they appear as full agonists in other functional studies in recombinant systems. In high expression recombinant systems, low efficacy agonists often appear as full agonists due to the presence of receptor reserve (see Kenakin, 1987). It is worth noting that in a native cell preparation where there is assumed to be a low receptor reserve, 8-OH-DPAT displayed partial-agonist-like properties (Schimizu et al., 1998). We also provide the first evidence for antagonist properties mCPP at the 5-HT_7 receptor.

Acknowledgements

The authors would like to thank Dr. Frank Brown for helpful discussions, Professor Derek Middlemiss for comments on the manuscript, Shabina Nasir for technical advise with the transfections and Vicky Holland for 5-HT_7 radioligand binding studies.

References

Adham, N., Zgombick, J.M., Bard, J., Branchek, T.A., 1998. Functional characterization of the recombinant human 5-hydroxytryptamine$_{7(a)}$ receptor isoform coupled to adenylate cyclase stimulation. J. Pharmacol. Exp. Ther. 287, 508–514.

Bard, L.A., Zgombick, L., Adham, N., Vaysse, P., Branchek, T.A., Weinshank, R.L., 1993. Cloning of a novel human serotonin receptor (5-HT_7) positively linked to adenylate cyclase. J. Biol. Chem. 268, 23422–23426.

Bowen, W.P., Jerman, J.C., 1995. Nonlinear regression using spreadsheets. Trends Pharmacol. Sci. 16, 413–417.

Clapham, D.E., 1995. Calcium signalling. Cell 80, 259–268.

Conklin, B.R., Farfel, Z., Lustig, K.D., Julius, D., Bourne, H.R., 1993. Substitution of three amino acids switches receptor specificity of $G_q\alpha$ to that of $G_i\alpha$. Nature 363, 274–276.

Conklin, B.R., Herzmark, P., Ishida, S., Voynoyasenetskaya, T.A., Sun, Y., Farfel, Z., Bourne, H.R., 1996. Carboxyl-terminal mutations of G(Q-alpha) and G(S-alpha) that alter the fidelity of receptor activation. Mol. Pharmacol. 50, 885–890.

Craig, D.A., 1993. The Cheng–Prusoff relationship: something lost in the translation. Trends Pharamcol. Sci. 14, 89–91.

Gustafson, E.L., Durkin, M.M., Bard, J.A., Zgombick, J., Branchek, T.A., 1996. A receptor autoradiographic and in situ hybridization analysis of the distribution of the 5-$HT_{(7)}$ receptor in rat brain. Br. J. Pharmacol. 117, 657–666.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–243.

Jackisch, R., Hotz, H., Allgaier, C., Hertting, G., 1994. Presynaptic opioid receptors on dopaminergic nerves in the rabbit caudate nucleus-coupling to pertussis toxin-sensitive G-proteins and interaction with D-2 autoreceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 349, 250–258.

Jasper, J.R., Kosaka, A., To, Z.P., Chang, D.J., Eglen, R.M., 1997. Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT_7 receptor (5-$HT_{7(b)}$). Br. J. Pharmacol. 122, 126–132.

Kenakin, T.P., 1987. Pharmacologic Analysis of Drug–Receptor Interaction. Raven Press, New York.

Kennett, G.A., 1993. 5-HT_{1C} receptors and their therapeutic interest. Curr. Opin. Invest. Drugs 2, 317–362.

Lovenberg, T.W., Baron, B., De Lecea, L., Miller, J.D., Prosser, R.A., Rea, M.A., Foye, P.E., Racke, M., Slone, A.L., Siegel, B.W., Danielson, P.E., Sutcliffe, J.G., Erlander, M.G., 1993. A novel adenylyl cyclase-activating serotonin receptor (5-HT_7) implicated in the regulation of mammalian circadian rhythms. Neuron 11, 449–458.

Milligan, G., Marshall, F., Rees, S., 1996. G_{16} as a universal adapter: implications for agonist screening strategies. Trends Pharmacol. Sci. 17, 235–237.

Porter, R.H.P., Benwell, K.R., Lamb, H., Malcolm, C.S., Allen, N.H., Revell, D.F., Adams, D.R., Sheardown, M.J., 1999. Functional characterisation of agonists at recombinant human 5-HT2A, 5-HT_{2B} and 5-HT_{2C} receptors in CHO-K1 cells. Br. J. Pharmacol. 128, 13–20.

Roth, B.L., Craigo, S.C., Choudhary, M.S., Uluer, A., Monsma, F.J. Jr., Shen, Y., Meltzer, H.Y., Sibley, D.R., 1994. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J. Pharmacol. Exp. Ther. 268, 1403–1410.

Schimizu, M., Nishida, A., Zensho, H., Miyata, M., Yamawaki, S., 1998. Agonist-induced desensitization of adenylyl cyclase mediated by 5-hydroxytryptamine$_7$ receptors in rat frontocortical astrocytes. Brain Res. 784, 57–62.

Schoeffter, P., Ullmer, C., Bobirnac, I., Gabbiani, G., Lubbert, H., 1996. Functional, endogenously expressed 5-hydroxytryptamine 5-HT_7 receptors in human vascular smooth muscle cells. Br. J. Pharmacol. 117, 993–994.

Schroeder, K.S., Neagle, B.D., 1996. FLIPR: a new instrument for accurate, high throughput optical screening. J. Biomol. Screening 2, 75–80.

Shen, Y., Monsma, F.J., Metcalf, M.A., Jose, P.A., Hamblin, M.W., Sibley, D.R., 1993. Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. J. Biol. Chem. 24, 18200–18204.

Sleight, A.J., Carolo, C., Petit, N., Zwingelstein, C., Bourson, A., 1995. Identification of 5-HT_7 receptor binding sites in rat hypothalamus. Mol. Pharmacol. 47, 93–103.

Stables, J., Green, A., Marshall, F., Fraser, N., Knight, E., Sautel, M., Milligan, G., Lee, M., Rees, S., 1997. A bioluminescent assay for agonist activity at potentially any G-protein-coupled receptor. Anal. Biochem. 252, 115–126.

Thomas, D.R., Gager, T.L., Holland, V., Brown, A.M., Wood, M.D., 1996. m-Chlorophenylpiperazine (mCPP) is an antagonist at the cloned human 5-HT_{2B} receptor. NeuroReport 7, 1457–1460.

Thomas, D.R., Gittins, S.A., Collin, L.L., Middlemiss, D.N., Riley, G., Hagan, J., Gloger, I., Ellis, C.E., Forbes, I.T., Brown, A.M., 1998. Functional characterization of the human cloned 5-HT_7 receptor (long form); antagonist profile of SB-258719. Br. J. Pharmacol. 124, 1300–1306.

Thomas, D.R., Middlemiss, D.N., Taylor, S.G., Nelson, P., Brown, A.M., 1999. 5-CT stimulation of adenylyl cyclase activity in guinea-pig hippocampus: evidence for involvement of 5-HT_7 and 5-HT_{1A} receptors. Br. J. Pharmacol. 128, 58–164.

To, Z.P., Bonhaus, D.W., Eglen, R.M., Jakeman, L.B., 1995. Characterization and distribution of putative 5-HT_7 receptors in guinea-pig brain. Br. J. Pharmacol. 115, 107–116.

Tsou, A.-P., Kosaka, A., Bach, C., Zuppan, P., Yee, C., Tom, L., Alvarez, R., Ramsey, S., Bonhaus, D.W., Stefanich, E., Jakeman, L., Eglen, R.M., Chan, H.W., 1994. Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase. J. Neurochem. 63, 456–464.

Wood, M.D., Murkitt, K., Gager, T.L., Ranson, J., Sanger, G., in press. Pharmacological characterisation of the human $P2X_4$ receptor using the FLIPR. Pharmacol. Rev. Commun.