Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 472 (2003) 33–38
www.elsevier.com/locate/ejphar

Characterization of the 5-HT_6 receptor coupled to Ca^{2+} signaling using an enabling chimeric G-protein

Jean Y. Zhang, Stanley Nawoschik, Dianne Kowal, Deborah Smith, Taylor Spangler, Rafal Ochalski, Lee Schechter, John Dunlop*

Neuroscience Discovery Research, Wyeth Research, CN-8000, Princeton, NJ 08543-8000, USA

Received 13 May 2003; accepted 15 May 2003

Abstract

We examined the feasibility of coupling the 5-HT_6 receptor to a Ca^{2+} signaling read-out using a chimeric G-protein, comprising of $G_{\alpha q}$ with the C-terminal five amino acids from $G_{\alpha s}$, to facilitate assays on the fluorometric imaging plate reader (FLIPR). Using a transient transfection assay in human embryonic kidney (HEK) cells, Ca^{2+} signaling in response to serotonin (5-HT) was facilitated by co-transfection of the 5-HT_6 receptor with the $G_{\alpha q}/G_{\alpha s}$ chimera, but not with the 5-HT_6 receptor alone or with a similar chimera incorporating the C-terminal five amino acids of $G_{\alpha i3}$. A series of agonist concentration–response curves were constructed using the 5-HT_6-$G_{\alpha q}/G_{\alpha s}$ signaling assay generating the following rank order of agonist potency; 5-methoxytryptamine (EC_{50}, 9 nM) = 5-HT (12 nM) = 2-methyl 5-HT (13 nM)>tryptamine (86 nM) = 5-carboxamidotryptamine (5-CT) (119 nM) ≫ lisuride (>1 μM). In comparison, essentially identical EC_{50} values were observed for the stimulation of cAMP accumulation with the same compounds; 5-methoxytryptamine (EC_{50}, 6 nM) = 5-HT (6 nM) = 2-methyl 5-HT (15 nM)>tryptamine (91 nM) = 5-CT (153 nM)>lisuride (>350 nM). Clozapine and SB 271046 both produced a concentration-dependent antagonism of the 5-HT-stimulated Ca^{2+} response with IC_{50} values of 45 and 11 nM, respectively. In contrast, aripiprazole, a recently launched atypical anti-psychotic with a novel mechanism of action described as a dopamine/serotonin stabilizer, was essentially devoid of 5-HT_6 receptor antagonist activity. Our results demonstrate that a FLIPR-based Ca^{2+} signaling assay is a feasible approach to the functional characterization of 5-HT_6 receptor ligands. Moreover, the equivalent coupling efficiency, as indexed by agonist potency, observed using this system compared with the native coupling assay to cAMP suggests that the C-terminal five amino acids of $G_{\alpha s}$ are the major determinant for the receptor/G-protein interaction of the 5-HT_6 receptor subtype.

Keywords: Fluorometric imaging plate reader; FLIPR; 5-HT_6 receptor; Chimeric G-protein; Ca^{2+} signaling

1. Introduction

The fluorometric imaging plate reader (FLIPR) has greatly improved our capacity to perform functional characterization of receptors by virtue of its high-throughput capabilities. In particular, the characterization of G-protein coupled receptors signaling through the $G_{\alpha q}$-phospholipase C cascade, leading to increases in intracellular Ca^{2+}, has been facilitated by the introduction of this technology. To date, a number of $G_{\alpha q}$-coupled receptors have been profiled in some detail with respect to both agonist and antagonist pharmacology including the serotonin 5-HT_2 receptor subfamily (Porter et al., 1999; Jerman et al., 2001), the orexin receptors (Smart et al., 1999, 2000b, 2001b), the histamine H_1 subtype (Miller et al., 1999), bradykinin B_1 and B_2 receptors (Simpson et al., 2000), the bombesin BB2 receptor (Brough et al., 2000) and the P2Y purinoceptors (Patel et al., 2001). In addition, the vanilloid VR1 receptor, a receptor-gated cation channel highly permeable to Ca^{2+} ions, has also been extensively studied using this approach (Smart et al., 2000a, 2001a).

In addition to the $G_{\alpha q}$-coupled receptors, it has been possible to study $G_{\alpha s}$ and $G_{\alpha i}$ coupled receptors using the FLIPR assay platform by taking advantage of the promiscuous G-protein, G15/16, or using chimeric G-proteins comprising $G_{\alpha q}$ with the C-terminal five amino acids replaced with those from $G_{\alpha s}$ or one of the $G_{\alpha i}$ family members (Coward et al., 1999). This latter approach is based on studies which have identified the C terminus of

* Corresponding author. Tel.: +1-732-274-4193; fax: +1-732-274-4755.
E-mail address: Dunlopj@wyeth.com (J. Dunlop).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01855-7

G-alpha G-proteins as a critical determinant for receptor/G-protein coupling and signal transduction (Conklin et al., 1993). For example, substitution of the C-terminal amino acids of $G_{\alpha q}$ with those from $G_{\alpha i2}$ permits coupling of the normally exclusively $G_{\alpha i}$-coupled dopamine D2 and adenosine A_1 receptors to the activation of phospholipase C (Conklin et al., 1993). Similarly, a chimera incorporating the C-terminal five amino acids of $G_{\alpha s}$ fused with $G_{\alpha q}$ allowed coupling of the $G_{\alpha s}$-coupled vasopressin V2 receptor to the stimulation of phospholipase C (Conklin et al., 1996). By taking advantage of this approach, both the $G_{\alpha s}$-coupled 5-HT_7 receptor (Wood et al., 2000a) and the $G_{\alpha i}$-coupled $GABA_B$ receptor (Wood et al., 2000b) have been profiled pharmacologically. In addition, we have recently used this approach, combined with site-directed mutagenesis, to probe the agonist binding pocket of the $G_{\alpha i}$-coupled histamine H_3 receptor (Uvegas et al., 2002).

In this study, we have examined the feasibility of using the chimeric G-protein approach to couple the $G_{\alpha s}$-coupled 5-HT_6 receptor to a Ca^{2+} signaling readout to facilitate characterization on the FLIPR platform. Multiple serotonin receptor subtypes have been identified, and many of them have been targets for drug development based on their potential role(s) in central nervous system (CNS) disorders. Cloning of the rat 5-HT_6 receptor (Monsma et al., 1993) identified a new subfamily of $G_{\alpha s}$-coupled 5-HT receptors linked to the stimulation of adenylyl cyclase activity. The pharmacology of the 5-HT_6 receptor subtype has received considerable attention due to the high-affinity for the receptor of a wide range of drugs used in psychiatry (Roth et al., 1994), and its exclusive localization to the CNS (Gerard et al., 1996). In particular, the high affinity for this receptor exhibited by both typical and atypical anti-psychotic agents has led to speculation that the 5-HT_6 receptor represents a therapeutic target for schizophrenia (Roth et al., 1994). Several other lines of evidence have implicated the 5-HT_6 receptor in the control of cholinergic transmission leading to the proposal that the receptor might be a target for restoring cognitive function (Bourson et al., 1995; Sleight et al., 1998; Bentley et al., 1999).

In this report, we describe the successful application of the chimeric G-protein strategy to the functional characterization of 5-HT_6 receptor ligands, providing an assay compatible with high-throughput FLIPR evaluation. Our results demonstrate that the 5-HT_6 receptor can be coupled to Ca^{2+} signaling using a $G_{\alpha q}/G_{\alpha s}$ chimera and, based on agonist potencies, the coupling efficiency was found to be similar to that observed when native coupling to $G_{\alpha s}$ was evaluated in cAMP accumulation studies. Finally, using this assay, we provide novel information on aripiprazole, a recently launched atypical anti-psychotic with a novel mechanism of action described as a dopamine/serotonin stabilizer (Jordan et al., 2002). In contrast to a number of both typical and atypical anti-psychotic agents, aripiprazole was found to be essentially devoid of 5-HT_6 receptor antagonist activity.

2. Materials and methods

2.1. Cell culture

Human embryonic kidney cells (HEK) seeded at a density of 3×10^6 in 10 cm^2 dishes were transfected with the human 5-HT_6 receptor in the presence or absence of vectors encoding chimeric G-proteins comprising $G_{\alpha q}$ with the C-terminal five amino acids substituted from $G_{\alpha s}$ or $G_{\alpha i3}$. In the presence of chimeric G-proteins, 2 μg of the receptor and 2 μg of G-protein vectors were used for transfection. In the absence of G-proteins, total DNA for each transfection was kept constant at 4 μg/DNA/plate by including pcDNA3.1 vector. The transfection was performed using Lipofectamine Plus combining 30 μl Lipofectamine and 20 μl of Plus reagent, according to the manufacturers (Invitrogen) instructions. Twenty-four hours following transfection, cells were seeded into 96-well black wall clear bottom plates at a density of 60–80,000 cells/well and were used the next day for FLIPR assays. Transfection efficiency was determined to be approximately 60–70% by visualizing a GFP-tagged receptor construct serving as a transfection control. For cAMP studies, a stable HeLa cell line expressing the 5-HT_6 receptor was utilized with cells seeded in 24-well plates the day prior to the assay.

2.2. FLIPR studies

5-HT_6 receptor coupling to increases in intracellular free Ca^{2+} in the presence of chimeric $G_{\alpha q}/G_{\alpha s}$ was measured using the fluorometric imaging plate reader (FLIPR, Molecular Devices, Sunnyvale, CA). Cells were loaded with the Ca^{2+} indicator dye Fluo-3-AM (1-(2-amino-5-[2,7-dichloro-6-hydroxy-3-oxy-9-xanthenyl]phenoxy)-2-(2-amino-5-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid-acetoxymethyl ester) in Hank's buffered saline (HBS) for 60 min at 37 °C. Cells were washed with HBS at room temperature and transferred to FLIPR for acquisition of Ca^{2+} signals. Excitation at 488 nm was achieved with an Argon ion laser and a 515-nm emission filter was used. In each experiment, baseline fluorescence after dye loading was adjusted to 10,000 fluorescence counts by adjusting the strength of the laser. Fluorescence images and relative intensities were captured at 1-s intervals for the first 55 s followed by 6-s intervals for the remainder of the run and cells were stimulated by addition of agonist after 10 baseline measurements, equivalent to 10 s into the run. An increase in fluorescence counts corresponds to an increase in intracellular Ca^{2+}.

2.3. cAMP studies

Cells were washed with Krebs buffer and incubated at 37 °C in Krebs supplemented with 500 μM 3-isobutly-1-methylxanthine (IBMX) for 5 min at 37 °C. Cells were then stimulated with agonists in the concentration range 0.1–

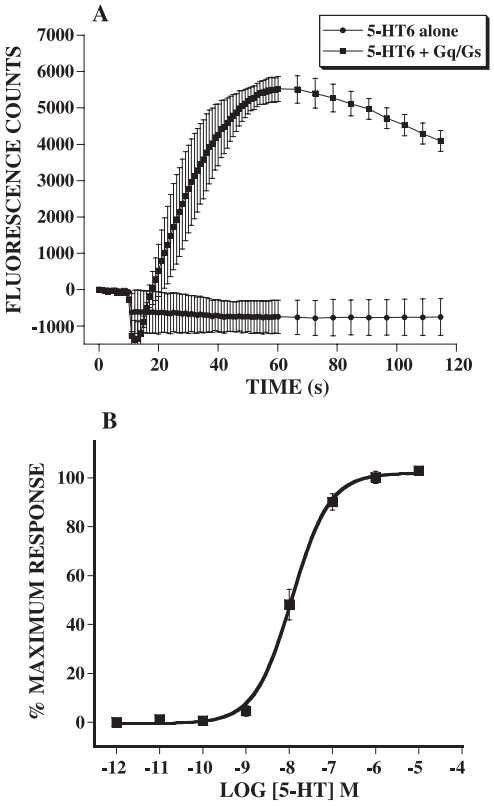

Fig. 1. (A) Ca^{2+} response in HEK cells co-expressing the 5-HT_6 receptor and a $G_{\alpha q}/G_{\alpha s}$ chimeric G-protein following stimulation with 10 μM 5-HT. Cells were loaded with the Ca^{2+} indicator dye fluo-3, transferred to FLIPR and stimulated after the collection of 10 baseline samples. A representative experiment is shown with each data point representing the mean value ± S.E.M. from 6 wells. (B) Concentration–response relationship for the 5-HT-stimulated Ca^{2+} response in HEK cells expressing the 5-HT_6 receptor and $G_{\alpha q}/G_{\alpha s}$ chimera. Data are the mean ± S.E.M. from three independent experiments.

10,000 nM for an additional 10 min at 37 °C. The assay was terminated with the addition of 0.5 M perchloric and intracellular cAMP levels were determined by radioimmunoassay with the cAMP scintillation proximity assay system.

2.4. Drugs

5-HT, 5-methoxytryptamine, 2-methyl-5-HT, tryptamine, 5-carboxamidotryptamine, lisuride, clozapine and IBMX were obtained from Sigma (St. Louis, MO), and Fluo-3-AM from Molecular Probes (Eugene, OR). The Medicinal Chemistry group at Wyeth synthesized SB 271046 (5-chloro-3-metyl-benzo[b]thiophene-2-sulphonic acid (4-methoxy-3-piperazin-1-yl-phenyl)-amide) and aripiprazole (OPC-14597; 7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl]-butyloxy-3,4-dihydro-2-(1H)-quinolinone)).

2.5. Data analysis

For the evaluation of agonist pharmacology on FLIPR, the Ca^{2+} changes in response to different concentrations of agonist were determined using a maximum minus minimum calculation of the raw fluorescence count data. Ca^{2+} changes were then expressed as a percentage of the response observed with a maximally effective concentration of 5-HT (10 μM) and EC_{50} values were estimated by non-linear regression curve fitting using the log-concentration % maximum 5-HT response curves.

3. Results

3.1. Coupling of 5-HT_6 receptor activation to Ca^{2+} signaling

Transient expression of the 5-HT_6 receptor in HEK cells did not result in Ca^{2+} coupling in response to stimulation with 10 μM 5-HT, but Ca^{2+} signaling was facilitated by co-expression of the receptor with a $G_{\alpha q}/G_{\alpha s}$ chimeric G-protein (Fig. 1A). Coupling was not facilitated when a $G_{\alpha q}/G_{\alpha i3}$ chimera was used and no Ca^{2+} signal in response to 5-HT was observed when cells were transfected with the $G_{\alpha q}/G_{\alpha s}$ chimera alone (data not shown). The effect of 5-HT on Ca^{2+} signaling in the 5-HT_6/$G_{\alpha q}$/$G_{\alpha s}$ expressing cells was concentration dependent, as depicted in Fig. 1B, with an estimated EC_{50} value of 12 nM. 5-HT_6 receptor expression levels up to 3000 fmol/mg protein were routinely observed (unpublished observations), indicating that the transient transfection was very robust.

3.2. Pharmacology of 5-HT_6 response on FLIPR

Further characterization of the 5-HT_6-coupled Ca^{2+} signal facilitated by the $G_{\alpha q}/G_{\alpha s}$ chimera was undertaken using established receptor agonists and antagonists. The log-

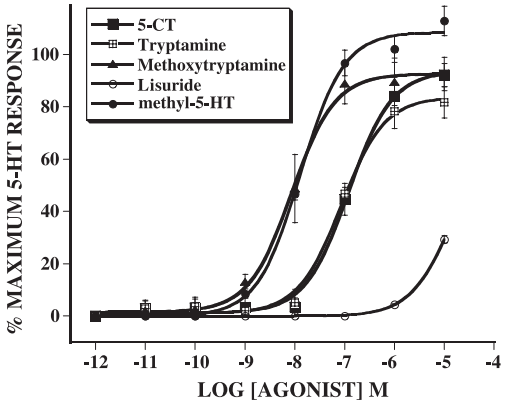

Fig. 2. Pharmacological profile of agonist-stimulated Ca^{2+} signaling in HEK cells co-expressing the 5-HT_6 receptor and a $G_{\alpha q}/G_{\alpha s}$ chimeric G-protein. Cells loaded with the Ca^{2+} indicator dye fluo-3 were stimulated with increasing concentrations of ligand and the Ca^{2+} responses (increase in fluorescence counts) were measured on FLIPR. Agonist responses were expressed as a percentage of the response observed with a maximally effective concentration of 5-HT. Data are the mean ± S.E.M. from three independent experiments.

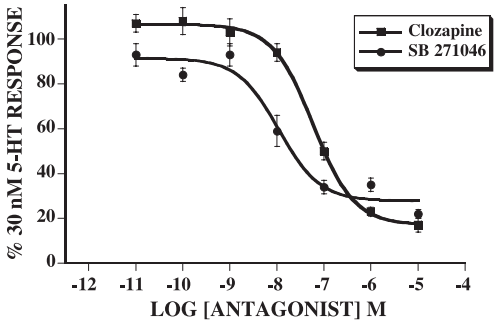

Fig. 3. Antagonism of 5-HT-stimulated Ca^{2+} signaling in HEK cells co-expressing the 5-HT_6 receptor and a $G_{\alpha q}/G_{\alpha s}$ chimeric G-protein. Cells loaded with the Ca^{2+} indicator dye fluo-3 were stimulated with 30 nM 5-HT in the absence or presence of increasing concentrations of clozapine or SB 271046. Antagonists were equilibrated with cells for 45 min before stimulation with 5-HT and preliminary experiments confirmed that both clozapine and SB 271046 were devoid of agonist activity. Data represent mean values ± S.E.M. from 12 replicates obtained within a single experiment that was repeated twice with similar results.

concentration–response curves generated for a series of agonists are illustrated in Fig. 2. All of the agonists tested, with the exception of lisuride, were highly efficacious, eliciting maximum Ca^{2+} responses similar to those observed with 5-HT. The rank order of agonist potency determined for the compounds examined was 5-methoxytryptamine (EC_{50}, 9 nM) = 5-HT (12 nM) = 2-methyl 5-HT (13 nM)>tryptamine (86 nM) = 5-CT (119 nM) ≫ lisuride (>1 μM).

Clozapine, an atypical anti-psychotic drug with high-affinity for the 5-HT_6 receptor, and SB 271046, a recently developed selective antagonist of the 5-HT_6 receptor subtype, both produced a concentration-dependent antagonism of the Ca^{2+} response to 5-HT observed in cells expressing the 5-HT_6 receptor and the $G_{\alpha q}/G_{\alpha s}$ chimera (Fig. 3). Estimated IC_{50} values for the inhibitory effects of clozapine and SB 271046 were 45 and 11 nM, respectively. Aripiprazole, a recently launched atypical anti-psychotic with a novel mechanism of action described as a dopamine/serotonin stabilizer (Jordan et al., 2002), failed to antagonize the 5-HT-stimulated Ca^{2+} response at concentrations up to 1 μM, while at the highest concentration tested (10 μM), the response to 30 nM 5-HT was inhibited by 69% (Fig. 4).

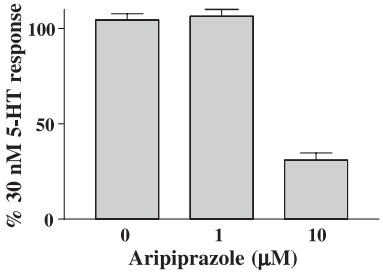

Fig. 4. Effect of aripiprazole on the 5-HT-stimulated Ca^{2+} signaling in HEK cells co-expressing the 5-HT_6 receptor and a $G_{\alpha q}/G_{\alpha s}$ chimeric G-protein. Cells loaded with the Ca^{2+} indicator dye fluo-3 were stimulated with 30 nM 5-HT in the absence or presence of aripiprazole at the indicated concentrations. Aripiprazole was equilibrated with cells for 45 min before stimulation with 5-HT and preliminary experiments confirmed that the compound was devoid of agonist activity. Data are the mean ± S.E.M. from three independent experiments.

Table 1
Estimated EC_{50} values for 5-HT_6 receptor stimulated Ca^{2+} signaling, in the presence of a $G_{\alpha q}/G_{\alpha s}$ chimera, compared with native coupling to adenylyl cyclase activation

	FLIPR		cAMP		K_i
	EC_{50}	E_{max}	EC_{50}	E_{max}	
5-methoxytryptamine	9 ± 3	93 ± 2	6	100	10 ± 2
5-HT	12 ± 2	100	6	100	16 ± 1
2-methyl-5-HT	13 ± 4	105 ± 4	15	100	7 ± 1
Tryptamine	86 ± 10	84 ± 2	91	100	71 ± 13
5-CT	119 ± 13	94 ± 2	153	100	136 ± 10
Lisuride	>1000	29 ± 2	350	71	8 ± 3

EC_{50} determinations (nM) represent mean values ± S.E.M. from three independent experiments for FLIPR and average values from two independent assays for cAMP studies. Similarly, E_{max} values, representing the relative maximum response to 10 μM 5-HT, were obtained from three independent assays for FLIPR (shown as mean ± S.E.M.) and two experiments for cAMP. FLIPR data were derived from cells co-expressing the 5-HT_6 receptor and the chimeric $G_{\alpha q}/G_{\alpha s}$ G-protein, while cAMP data were derived from cells expressing only the 5-HT_6 receptor. A significant correlation was obtained between the functional activity determined by FLIPR and cAMP analysis; $R^2 = 0.98$ determined by linear regression correlation analysis. 5-HT_6 receptor ligand affinities (K_i, nM) were determined using [^{3}H]5-HT displacement studies. With the exception of lisuride, a significant correlation was observed between binding affinity and functional activity by FLIPR ($R^2 = 0.94$) or cAMP ($R^2 = 0.98$) as determined by linear regression correlation analysis.

3.3. 5-HT_6 functional response by cAMP measurement

Comparative data for the stimulation of adenylyl cyclase following 5-HT_6 receptor activation were generated for the same agonist ligands profiled in the FLIPR assay. Results are presented in Table 1, indicating that the agonists were equipotent in the cyclase assay compared with the FLIPR assay, and correspondingly, the rank order of agonist potency was identical in the two assays. Lisuride was an exception, acting as a more potent agonist in the cAMP assay compared with the Ca^{2+} signaling response.

4. Discussion

The FLIPR platform for high-throughput Ca^{2+} signaling assays is now well-established as an efficient system for the characterization of G-protein-coupled receptors signaling via $G_{\alpha q}$ to the mobilization of intracellular Ca^{2+}, and the potential exists for the evaluation of non-$G_{\alpha q}$-coupled receptors using chimeric and/or promiscuous G-proteins. This study expands the repertoire of receptors demonstrated to be amenable to functional evaluation on FLIPR by facilitated coupling with a chimeric G-protein. Our data demonstrate that the $G_{\alpha s}$-coupled 5-HT_6 receptor can be functionally linked to the $G_{\alpha q}$ Ca^{2+} signaling pathway using

a G-protein chimera comprising $G_{\alpha q}$ with the C-terminal five amino acids of $G_{\alpha s}$. The C-terminal five amino acids of $G_{\alpha s}$ were sufficient to enable appropriate $G_{\alpha s}$ coupling specificity of the 5-HT_6 receptor as evidenced by the failure of a similar $G_{\alpha q}$ chimera incorporating the C-terminal five amino acids of $G_{\alpha i3}$ to facilitate coupling to the $G_{\alpha q}$ Ca^{2+} signaling pathway. Validation of apposite coupling specificity of the same $G_{\alpha q}/G_{\alpha i3}$ chimera has been achieved with its use to couple the $G_{\alpha i}$-coupled histamine H_3 receptor to a Ca^{2+} response (Uvegas et al., 2002).

5-HT_6 receptor coupling efficiency achieved using the chimeric G-protein strategy was compared to the native coupling of the receptor to $G_{\alpha s}$ and subsequent activation of adenylyl cyclase leading to cAMP accumulation. Our data indicate an extremely good correlation between the potency of 5-HT_6 receptor agonists to stimulate cAMP accumulation and to couple to Ca^{2+} signaling via the chimeric G-protein. The exception was lisuride, where maximal efficacy was not defined in the FLIPR assay due to a lack of plateau in the concentration–response curve. This has a likely significant impact on the discrepancy observed between the two assays. Given that the other agonist potencies determined in the two assays were essentially identical indicate that the C terminus of $G_{\alpha s}$ is the major determinant for the interaction of the 5-HT_6 receptor with the $G_{\alpha s}$ subunit, and is sufficient to ensure tight coupling. This observation is in contrast to our findings with the $G_{\alpha i}$-coupled histamine H_3 receptor (Uvegas et al., 2002), where agonist potencies determined in a FLIPR assay, using $G_{\alpha q}/G_{\alpha i3}$, were lower than those observed in cAMP assays. These data imply a less efficient interaction between the histamine H_3 receptor and the chimeric G-protein compared with the native $G_{\alpha i}$ subunit, and suggest the participation of additional motifs in the receptor/G-protein complex. Consistent with this hypothesis, both internal G-protein peptide motifs and the N terminus have been implicated in the receptor/G-protein interaction for certain Gα subunits (Conklin and Bourne, 1993; Kostenis et al., 1997; Ho and Wong, 2000).

In addition to observing good agreement between the functional activities measured in our hands, our data also correspond well with that described by other investigators. For example, a similar rank order of potency for the tryptamine-like agonists has been reported using a stable HEK cell line in combination with cAMP measurements (Boess et al., 1997), although the absolute agonist potencies were weaker than those observed in our hands. Furthermore, the same study documented a Kb of 22 nM for clozapine antagonism of the 5-HT-stimulated cAMP response as compared to the IC_{50} value of 45 nM (corresponding to a Kb of 13 nM) observed in the FLIPR assay, and an apparent K_i of 35 nM has also been calculated for clozapine based on a rightward shift in the 5-HT concentration–response curve for cAMP measured in a stable HeLa/5-HT_6 cell line (Kohen et al., 1996). In the case of SB 271046, this compound is reported to be a high-affinity ligand for the cloned 5-HT_6 receptor with a pK_i value of 8.92 for the displacement of [^{3}H]-lysergic acid diethylamide binding, and a functional antagonist with a pA_2 value of 8.7 for the inhibition of 5-HT stimulated cAMP response (Routledge et al., 2000). The potent activity of this compound in the FLIPR functional assay is in good agreement with the affinity and potency documented for SB 271046. In the present study, the antagonism observed for SB 271046 did not appear to be complete, with some residual agonist-stimulated response observed even at the highest concentrations of SB 271046. Despite this, we were unable to detect any intrinsic activity of SB 271046 as a means of accounting for the incomplete block.

Finally, we evaluated the 5-HT_6 receptor antagonist activity of aripiprazole, a recently launched atypical anti-psychotic with a novel mechanism of action described as a dopamine/serotonin stabilizer (Jordan et al., 2002), using the facilitated Ca^{2+} assay. In contrast to the potent antagonism demonstrated with the atypical anti-psychotic clozapine and the selective 5-HT_6 receptor antagonist SB 271046, aripiprazole failed to antagonize the Ca^{2+} response elicited by 5-HT in cells co-expressing the 5-HT_6 receptor and the chimeric $G_{\alpha q}/G_{\alpha s}$ G-protein except at a concentration of 10 μM. As eluded earlier, the 5-HT_6 receptor has become an interesting target from a drug discovery perspective by virtue of the high-affinity exhibited by a number of typical and atypical anti-psychotic drugs for the receptor. The lack of potent 5-HT_6 receptor antagonism demonstrated here for aripiprazole, taken in combination with the demonstrated efficacy of aripiprazole in treating schizophrenic patients (Taylor, 2003), indicates that the 5-HT_6 receptor antagonist activity is not an obligatory requirement for an anti-psychotic agent.

In summary, the use of a chimeric $G_{\alpha q}/G_{\alpha s}$ G-protein co-expressed with the $G_{\alpha s}$-coupled 5-HT_6 receptor provides a functional assay, amenable to Ca^{2+} studies on FLIPR, with appropriate pharmacology for 5-HT_6 receptor ligands. This approach enhances our capacity to characterize 5-HT_6 receptor ligands and complements the existing cAMP assay formats. In addition, the significant correlation in agonist potencies observed between the facilitated coupling assay using FLIPR compared to native G-protein coupling, indexed by cAMP formation, suggests that the C-terminal five amino acids of $G_{\alpha s}$ are a major determinant of 5-HT_6 receptor/effector coupling efficiency.

References

Bentley, J.C., Bourson, A., Boess, F.G., Fone, K.C., Marsden, C.A., Petit, N., Sleight, A.J., 1999. Investigation of stretching behaviour induced by the selective 5-HT6 receptor antagonist Ro 04-6790 in rats. Br. J. Pharmacol. 126, 1537–1542.

Boess, F.G., Monsma, F.J., Caralo, C., Meyer, V., Rudler, A., Zwingelstein, C., Sleight, A.J., 1997. Functional and radioligand binding characterization of rat 5-HT6 receptors stably expressed in HEK-293 cells. Neuropharmacology 36, 713–720.

Bourson, A., Borroni, E., Austin, R.H., Monsma, F.J., Sleight, A.J., 1995. Determination of the role of the 5-HT6 receptor in the rat brain: a

study using antisense oligonucleotides. J. Pharmacol. Exp. Ther. 274, 173–180.

Brough, S.J., Jerman, J.C., Jewitt, F., Smart, D., 2000. Characterization of an endogenous bombesin receptor in CHO/DG44 cells. Eur. J. Pharmacol. 409, 259–263.

Conklin, B.R., Bourne, H.R., 1993. Structural elements of G alpha subunits that interact with G beta gamma, receptors, and effectors. Cell 73, 631–641.

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

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

Coward, P., Chan, S.D.H., Wada, H.G., Humphries, G.M., Conklin, B.R., 1999. Chimeric G proteins allow a high-throughput signaling assay of $G_{\alpha i}$-coupled receptors. Anal. Biochem. 270, 242–248.

Gerard, C., Mestikawy, S.E., Lebrand, C., Adrien, J., Ruat, M., Traiffort, E., Hamon, M., Martres, M.-P., 1996. Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytrptamine treated rats. Synapse 23, 164–173.

Ho, M.K.C., Wong, Y.H., 2000. The amino terminus of $G_{\alpha z}$ is required for receptor recognition, whereas its alpha 4/beta 6 loop is essential for inhibition of adenylyl cyclase. Mol. Pharmacol. 58, 993–1000.

Jerman, J.C., Brough, S.J., Gager, T., Wood, M., Coldwell, M.C., Smart, D., Middlemiss, D.N., 2001. Pharmacological characterization of human 5-HT_2 receptor subtypes. Eur. J. Pharmacol. 414, 23–30.

Jordan, J., Koprivica, V., Chen, R., Tottori, K., Kikuchi, T., Altar, C.A., 2002. The antipsychotic aripiprazole is a potent, partial agonist at the human 5-HT1A receptor. Eur. J. Pharmacol. 441, 137–140.

Kohen, R., Metcalf, M.A., Khan, N., Druck, T., Huebner, K., Lachowicz, J.E., Meltzer, H.Y., Sibley, D.R., Roth, B.L., Hamblin, M.W., 1996. Cloning, characterization and chromosomal localization of a human 5-HT6 serotonin receptor. J. Neurochem. 66, 47–56.

Kostenis, E., Degtyarev, M.Y., Conklin, B.R., Wess, J., 1997. The N-terminal extension of $G_{\alpha q}$ is critical for constraining the selectivity of receptor coupling. J. Biol. Chem. 272, 19107–19110.

Miller, T.R., Witte, D.G., Ireland, L.M., Kang, C.H., Roch, J.M., Masters, J.N., Esbenshade, T.A., Hancock, A.A., 1999. Analysis of apparent noncompetitive responses to competitive H_1 histamine receptor antagonists in fluorescent imaging plate reader based Ca^{2+} assays. J. Biomol. Screen. 4, 249–258.

Monsma, F.J., Shen, Y., Ward, R.P., Hamblin, M.W., Sibley, D.R., 1993. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol. Pharmacol. 43, 320–327.

Patel, K., Barnes, A., Camacho, J., Paterson, C., Boughtflower, R., Cousens, D., Marshall, F., 2001. Activity of diadenosine polyphosphates at P2Y receptors stably expressed in 1321N1 cells. Eur. J. Pharmacol. 430, 203–210.

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 characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C 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., Shen, Y., Meltzer, H.Y., Sibley, D.R., 1994. Binding of typical and atypical antipsychotic agents to 5-hydroxytrptamine-6 and 5-hydroxytryptamine-7 receptors. J. Pharmacol. Exp. Ther. 268, 1403–1410.

Routledge, C., Bromidge, S.M., Moss, S.F., Price, G.W., Hirst, W., Newman, H., Riley, G., Gager, T., Stean, T., Upton, N., Clarke, S.E., Brown, A.M., Middlemiss, D.N., 2000. Characterization of SB-271046: a potent, selective and orally active 5-HT6 receptor antagonist. Br. J. Pharmacol. 130, 1606–1612.

Simpson, P.B., Woollacott, A.J., Hill, R.G., Seabrook, G.R., 2000. Functional characterization of bradykinin analogs on recombinant human bradykinin B-1 and B-2 receptors. Eur. J. Pharmacol. 392, 1–9.

Sleight, A.J., Boess, F.G., Bos, M., Levet-Trafit, B., Riemer, C., Bourson, A., 1998. Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT6 receptors. Br. J. Pharmacol. 124, 556–562.

Smart, D., Jerman, J.C., Brough, S.J., Rushton, S.L., Murdock, P.R., Jewitt, F., Elshourbagy, N.A., Ellis, C.E., Middlemiss, D.N., Brown, F., 1999. Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell line using FLIPR. Br. J. Pharmacol. 128, 1–3.

Smart, D., Gunthorpe, M.J., Jerman, J.C., Nasir, S., Gray, J., Muir, A.I., Chambers, J.K., Randall, A.D., Davis, J.B., 2000a. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). Br. J. Pharmacol. 129, 227–230.

Smart, D., Jerman, J.C., Brough, S.J., Neville, W.A., Jewitt, F., Porter, R.A., 2000b. The hypocretins are weak agonists at recombinant human orexin-1 and orexin-2 receptors. Br. J. Pharmacol. 129, 1289–1291.

Smart, D., Jerman, J.C., Gunthorpe, M.J., Brough, S.J., Ranson, J., Cairns, W., Hayes, P.D., Randall, A.D., Davis, J.B., 2001a. Characterization using FLIPR of human vanilloid receptor pharmacology. Eur. J. Pharmacol. 417, 51–58.

Smart, D., Sabido-David, C., Brough, S.J., Jewitt, F., Johns, A., Porter, R.A., Jerman, J.C., 2001b. SB-334867-A: the first selective orexin-1 receptor antagonist. Br. J. Pharmacol. 132, 1179–1182.

Taylor, D.M., 2003. Aripiprazole: a review of its pharmacology and clinical use. Int. J. Clin. Pract. 57, 49–54.

Uvegas, A.J., Kowal, D., Zhang, Y., Spangler, T.B., Dunlop, J., Semus, S., Jones, P.G., 2002. The role of transmembrane helix 5 in agonist binding to the human H_3 receptor. J. Pharmacol. Exp. Ther. 301, 451–458.

Wood, M., Chaubey, M., Atkinson, P., Thomas, D.R., 2000a. Antagonist activity of meta-chlorophenylpiperazine and partial agonist activity of 8-OH-DPAT at the 5-HT_7 receptor. Eur. J. Pharmacol. 396, 1–8.

Wood, M.D., Murkitt, K.L., Rice, S.Q., Testa, T., Punia, P.K., Stammers, M., Jenkins, O., Elshourbagy, N.A., Shabo, U., Taylor, S.J., Gager, T.L., Minton, J., Hirst, W.D., Price, G.W., Pangalos, M., 2000b. The human $GABA_{(B1b)}$ and $GABA_{(B2)}$ heterodimeric recombinant receptor shows low sensitivity to phaclofen and saclofen. Br. J. Pharmacol. 131, 1050–1054.