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Partial agonism at serotonin 5-HT_{1B} and dopamine D_{2L} receptors using a luciferase reporter gene assay

Daniel M. Kemp [a], Samantha E. George [b], Peter J. Bungay [c], Louise H. Naylor [a,*]

[a] *Department of Biosciences, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ, UK*
[b] *Merck, Sharpe and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2XR, UK*
[c] *Discovery Biology, Pfizer Central Research, Sandwich, Kent, CT13 9NJ, UK*

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Abstract

We have used a luciferase reporter gene assay to study the functional responses of two G-protein-coupled receptors in Chinese hamster ovary (CHO) cells. The rank order of potency of drugs for the endogenous 5-HT_{1B} receptor was 5-Hydroxytryptamine (5-HT) > zolmitriptan > dihydroergocristine > (−)lisuride (with no response to bromocriptine). However, only 5-HT and (−)lisuride produced a full functional response, with zolmitriptan and dihydroergocristine achieving 69 ± 2% and 50 ± 1% of the maximal response. In the same cells stably transfected with the rat dopamine D_{2L} receptor, dopamine and bromocriptine produced a full agonist functional response, whilst (−)lisuride produced a biphasic response curve, indicating activity at both the endogenous 5-HT_{1B} and exogenous dopamine D_{2L} receptors. Using the receptor specific antagonists, pindolol and (+)butaclamol, (−)lisuride was shown to produce 52% of the maximal response at the dopamine D_2 receptor relative to dopamine. In comparison to a cAMP accumulation assay, the rank orders of potency and intrinsic activity were the same for all compounds used. These results demonstrate that this reporter gene assay is capable of discriminating both potency and efficacy of drugs and can be used to characterise partial agonists at endogenously and heterologously expressed receptors in CHO cells.

Keywords: Luciferase; Partial agonism; 5-HT_{1B} receptor; Dopamine D_{2L} receptor; Reporter gene; CHO (Chinese hamster ovary) cell

1. Introduction

Many members of the superfamily of G-protein coupled receptors are linked to the adenylyl cyclase signal transduction pathway, and therefore either mediate stimulation or inhibition of cAMP accumulation within the cytosol. Functional responses to G-protein coupled receptor activation have traditionally been measured by direct quantitation of cAMP accumulation in the cell. However, the response signal does not terminate at the level of cAMP, but continues via protein kinase A and transcription factors to induce the expression of genes that are coupled to specific promoters containing cAMP response elements (Montminy, 1997). The advent of reporter gene technology has enabled direct observation of gene expression by monitoring easily detectable gene phenotypes expressed under the control of specific response elements. Several studies utilising this system to monitor agonist and antagonist mediated functional coupling of G-protein coupled receptors to the adenylyl cyclase signalling pathway have previously been published (Castanon and Spevac, 1994; Stratowa et al., 1995a,b; George et al., 1997a, 1998). In recent years however, areas of pharmaceutical research have focused more on specific intrinsic activities of compounds providing a requisite for screening tools capable of detecting partial agonists (agonists that bind with high affinity to a receptor but induce a fraction of the full agonist response). The advantages of using partial agonists for therapeutic treatment is well established in that they effectively block the overactivity at target receptors avoiding the effects of withdrawal symptoms or other side-effects associated with the use of antagonists due to total blockade of receptor function (Waller, 1990). This rationale has been addressed in testing D_2 receptor partial agonists in psychostimulant addiction (Pulvirenti and Koob, 1994).

5-HT_{1B} and dopamine D_{2L} receptors belong to the superfamily of G-protein coupled receptors and agonist

* Corresponding author. Tel.: +44-1227-823744; Fax: +44-1227-763912; E-mail: l.h.naylor@ukc.ac.uk

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stimulation of these receptors induces the activation of the G protein G*i*, leading to G$i\alpha$ subunit mediated inhibition of adenylyl cyclase activity (Missale et al., 1997). This negative coupling of 5-HT_{1B} and dopamine D_{2L} receptors to adenylyl cyclase allows for investigation of the agonistic properties of ligands by monitoring the G-protein coupled receptor mediated inhibition of forskolin stimulated reporter gene expression (George et al., 1997b).

Previously, we stably transfected the luciferase reporter gene (from the firefly, *Photinus pyralis*) in to a Chinese hamster ovary (CHO) cell line (CHO dhfr$^-$) under the transcriptional control of a promoter, rich in cAMP response elements (Himmler et al., 1993; George et al., 1997b). The level of gene expression was measured by monitoring the accumulation of the gene phenotype in the cytosol using bioluminescence (Benzakour et al., 1995; Roelant et al., 1996). In this study the endogenously expressed 5-HT_{1B} receptor (Berg et al., 1994; Giles et al., 1996; George et al., 1997a) and the heterologously expressed rat dopamine D_{2L} receptor (George et al., 1998) were both targeted for investigation of partial agonist functional responses in this reporter cell line. As well as demonstrating the flexibility and sensitivity of this technique for measuring differing levels of intrinsic activity, this work also highlights the ability to discriminate between responses induced by multiple receptor mediated activation of gene expression by selective antagonist treatment.

2. Materials and methods

2.1. Materials

The following drugs were obtained from the sources stated: BW311C90WH (311C90, Zomig, Zolmitriptan) was a gift from Zeneca Pharmaceuticals. (−)Lisuride, dopamine, dihydroergocristine methane sulphate, bromocriptine, (+)butaclamol and pindolol were obtained from RBI. 5-Hydroxytryptamine, forskolin, dimethyl sulfoxide (DMSO) and dithiothreitol were obtained from Sigma. All cell culture reagents were obtained from Sigma except foetal calf serum (Globepharm).

2.2. Cell culture

Chinese hamster ovary cells deficient in the enzyme dihydrofolate reductase (CHO dhfr$^-$) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) foetal calf serum, L-glutamine (2 mM), non-essential amino acids hypoxanthine and thymidine (HT supplement), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were grown at 37°C in a humidified atmosphere containing 5% CO_2 in air.

2.3. DNA transfection

2.3.1. CHO-luc cell line

The cAMP responsive reporter plasmid pADneo2-C6-BGL, containing six tandem cAMP response element repeats within the promoter (Himmler et al., 1993), was transfected into CHO dhfr$^-$ cells using the calcium phosphate precipitation technique, as previously described (George et al., 1997a). Briefly, cells were subcultured 24 h prior to transfection at a density of 1×10^5 cells/cm^2. Selective medium containing 0.4 mg/ml active geneticin (G-418) was applied to the cells 48 h after transfection. Cells demonstrating resistance to G-418 were screened for inducible luciferase expression in response to forskolin (20 μM). Following two rounds of dilution cloning, the positive clone displaying the greatest fold induction of luciferase in response to 20 μM forskolin was chosen as the reporter cell line of choice and was used in all subsequent experiments.

2.3.2. D2 CHO-luc cell line

The CHO-luc cell line was stably cotransfected with the rat D_{2L} dopamine receptor (D_2pSVL) and a vector containing the DHFR gene (pSV2 dhfr), again using the calcium phosphate precipitation technique (George et al., 1998). Cells were grown in selective medium containing G-418 (400 μg/ml) to select for the reporter plasmid, and dialysed foetal calf serum lacking HT supplement to select for the DHFR gene. Following two rounds of dilution cloning, the clonal cell line exhibiting the maximum inhibition of forskolin stimulated luciferase expression in the presence of dopamine (10 μM) was selected, and is hereafter referred to as the D_2CHO-luc cell line. The level of expression of D_{2L} receptors was approximately 100 fmol/mg (George et al., 1998). The endogenous 5-HT_{1B} receptor, though detectable by cAMP accumulation studies was undetectable by radioligand binding (Giles et al., 1996), hence the expression level is unknown.

2.4. Luciferase assay

Reporter cells were seeded at a density of 4×10^5 cells/well in a white (opaque) 24-well culture plate (Packard), 18–20 h prior to assay. Cells were washed twice with DMEM before adding the diluted test compounds (150 μl) and incubating at 37°C and 5% CO_2 for 4 h. Ligands were diluted in DMEM containing forskolin (0.5 μM), dithiothreitol (0.5 mM) and 1% DMSO. Cell washes and ligand dilutions were carried out using serum free DMEM which lacked phenol red.

Luciferase expression was determined using the LucLite™ kit (Packard), where 100 μl of the reagent was added to the wells at the end of the incubation time. Plates were then briefly shaken to ensure complete cell lysis and luminescence was measured using a microplate scintilla-

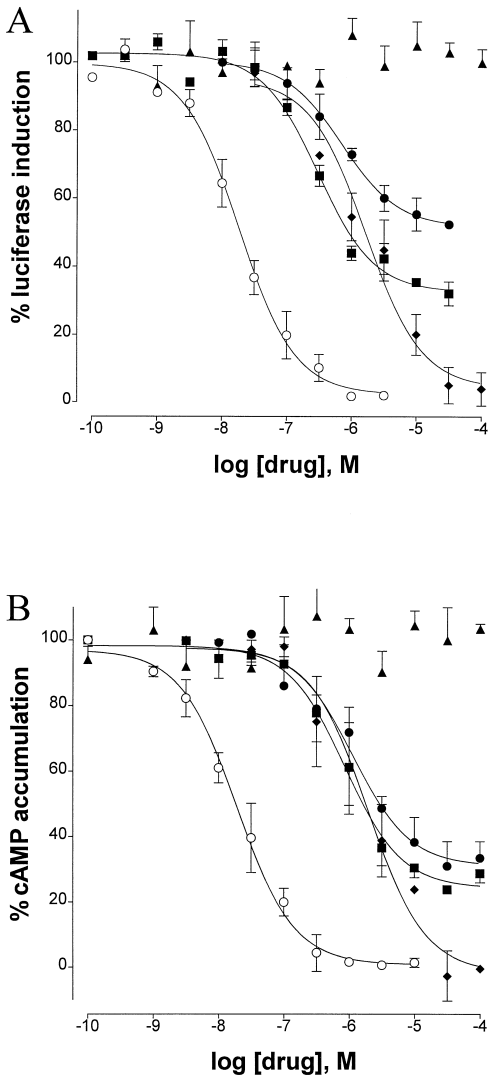

Fig. 1. Agonist concentration response curves for 5-HT (○), (−)lisuride (♦), dihydroergocristine (●), zolmitriptan (■) and bromocriptine (▲) at the endogenous 5-HT_{1B} receptor in CHO-luc cells. In (A), cells were stimulated with 0.5 μM forskolin and responses are expressed as % of luciferase induction in the presence of forskolin and the absence of agonist. In (B), 50 μM forskolin was used and cAMP accumulation expressed as % activation in the presence of forskolin and the absence of agonist. Data points are mean values (±S.E.M.) of at least three independent experiments carried out in triplicate (A) or duplicate (B).

tion and luminescence counter (Wallac MicroBeta Trilux 1450) in luminescence mode, with each well being counted for 1 s following a 15-min adaptation in the dark.

2.5. cAMP accumulation assays

Cells were seeded at a density of 4×10^5 cells/well in a 24-well culture plate, 18–20 h prior to assay. Cells were washed twice with DMEM before the addition of ligands in 300 μl DMEM, containing forskolin (50 μM), dithiothreitol (0.5 mM) and 1% DMSO. Cells were then incubated at 37°C and 5% CO_2 for 30 min before washing twice with DMEM. The culture plate, containing 500 μl serum-free DMEM and lacking phenol red, was then placed in a boiling water bath for 7 min. The samples were then spun at 2000 rpm for 5 min and the supernatant analysed for cAMP. cAMP accumulation was measured using the Biotrak cAMP SPA screening assay system (Amersham) according to the manufacturers instructions. Bound [^{125}I]-cAMP was quantitated using a Top Count™ 9912V (Packard).

2.6. Data analysis

Concentration response data generated using the luciferase assay or cAMP accumulation assay, following incubation of cells with receptor ligands, were analysed by subtracting basal levels (i.e., with diluent alone), then normalising values as a percentage of controls (for details see results). This data was analysed by nonlinear regression with variable slope, using the computer packages Graphpad Inplot 4 and Graphpad Prism. If slopes were found to be not significantly different to 1 at the 5% confidence level using the Student's *t*-test, then slopes were constrained to 1 for the purpose of estimating EC_{50} values. Biphasic curves were generated using a two sited nonlinear regression curve.

3. Results

3.1. Luciferase and cAMP accumulation mediated by endogenous 5-HT_{1B} receptors in CHO-luc cells

The cloned cell lines used in these experiments have been previously characterised (George et al., 1997a, 1998). Cells stably transfected with the luciferase gene only are referred to as CHO-luc cells.

Forskolin (0.5 μM) produced an increase in the level of luciferase expression in CHO-luc cells as a result of

Table 1

Drug	Luciferase pEC_{50}	% Efficacy	cAMP pEC_{50}	% Efficacy
5-HT	7.23 ± 0.06 (n = 12)	100	7.7 ± 0.08 (n = 6)	100
Zolmitriptan	6.52 ± 0.08 (n = 3)	69 ± 2	6.02 ± 0.11 (n = 3)	78 ± 4
Dihydroergocristine	6.15 ± 0.03 (n = 3)	50 ± 1	5.93 ± 0.15 (n = 3)	71 ± 2
Lisuride	5.78 ± 0.14 (n = 4)	97 ± 5	5.71 ± 0.14 (n = 3)	100 ± 5

Calculations of affinity (pEC_{50}) and efficacy relative to 5-HT (%) for agonists at the 5-HT_{1B} receptor, obtained by luciferase and cAMP accumulation assays in CHO-luc cells.

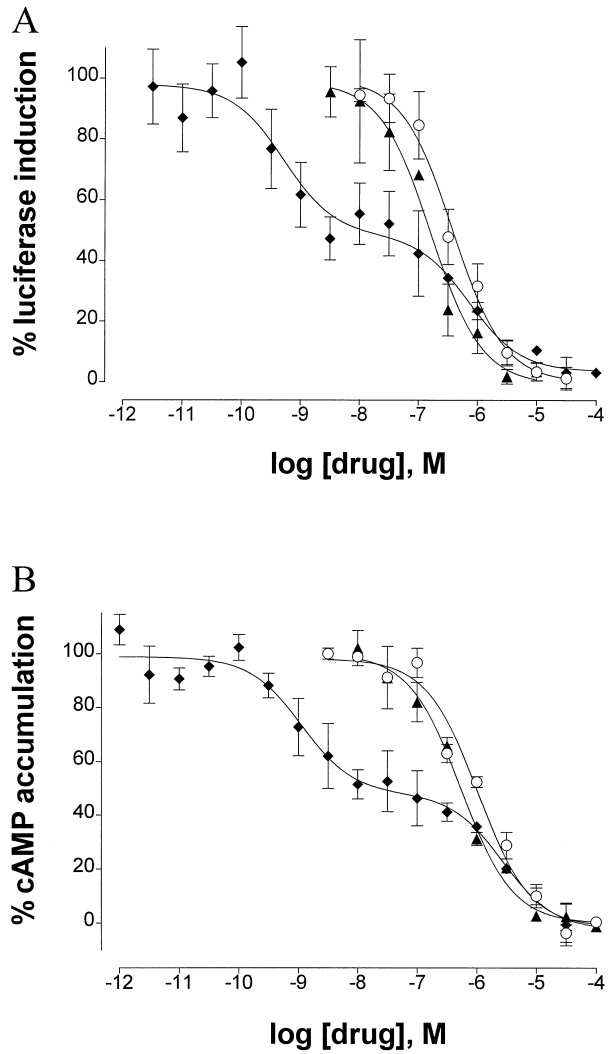

Fig. 2. Agonist concentration response curves for dopamine (○), (−)lisuride (◆) and bromocriptine (▲) in CHO-luc cells expressing dopamine D_{2L} receptors (D2 CHO-luc cells). In (A), cells were stimulated with 0.5 μM forskolin and responses are expressed as % of luciferase induction in the presence of forskolin and the absence of agonist. In (B), 50 μM forskolin was used and cAMP accumulation expressed as % activation in the presence of forskolin and the absence of agonist. Data points are mean values (±S.E.M.) of at least three independent experiments carried out in triplicate (A) or duplicate (B).

increased levels of cAMP accumulation and this forskolin stimulated level was normalised to 100%. Activation of the 5-HT_{1B} receptor by 5-HT caused an inhibition of the forskolin-induced luciferase expression level in a saturable, concentration-dependent manner ($pEC_{50} = 7.23 \pm 0.06$) (Fig. 1A). Full inhibition by 5-HT was normalised to 0%. All subsequent concentration responses were directly compared with that of 5-HT (within each experiment). Both zolmitriptan and dihydroergocristine were partial agonists at the 5-HT_{1B} receptor ($pEC_{50} = 6.52 \pm 0.08$, and 6.15 ± 0.03), producing 69 ± 2 and 50 ± 0.6% of the 5-HT response, respectively (Fig. 1A; Table 1). (−)Lisuride was a full agonist (97 ± 5% efficacy) at the 5-HT_{1B} receptor ($pEC_{50} = 5.78 \pm 0.14$). Thus, the compounds used demonstrated a potency rank order: 5-HT > zolmitriptan > dihydroergocristine > (−)lisuride. The rank order of intrinsic activity was 5-HT = (−)lisuride > zolmitriptan > dihydroergocristine. Bromocriptine produced no effect in this cell line at concentrations below 100 μM.

In the cAMP accumulation studies, 50 μM forskolin was used to produce a detectable increase in cAMP levels in CHO-luc cells. Upper and lower levels of activation were calculated by the same method as for luciferase expression and all concentration responses were then compared with that of 5-HT. Orders of both potency and intrinsic activity were the same as for the luciferase activity assays (Fig. 1B; Table 1). The intrinsic activities of zolmitriptan and dihydroergocristine were 78 ± 4 and 71 ± 2%, respectively, relative to 5-HT. These data appear to confirm the luciferase data in the previous experiments, as the rank orders were unaffected.

3.2. Luciferase and cAMP accumulation mediated by recombinant dopamine D2 receptors expressed in CHO-luc cells (D2 CHO-luc)

In D2 CHO-luc cells (cells stably transfected with both the luciferase gene and the rat dopamine D_{2L} receptor gene), dopamine inhibition of forskolin (0.5 μM) stimulated luciferase expression occurred in a concentration-dependent manner ($pEC_{50} = 6.42 \pm 0.09$, $n = 12$) (Fig. 2A; Table 2). Intrinsic activity was determined by the same method as before with respect to % inhibition of forskolin treated cells. The concentration response curve of the $5\text{-HT}_{1A/1B}$ receptor full agonist (−)lisuride was biphasic in this recombinant cell line ($pEC_{50} = 9.30$ and 6.14, $n = 4$) with intrinsic activities of 52% for the high affinity phase and 48% for the low affinity phase, relative to that of dopamine (Fig. 2A). In the presence of 10 μM (+)butaclamol, a dopamine D_2 receptor antagonist, the

Table 2

Drug	Luciferase pEC_{50}	% Efficacy	cAMP pEC_{50}	% Efficacy
Dopamine	6.42 ± 0.09 ($n = 12$)	100	5.97 ± 0.09 ($n = 7$)	100
Lisuride	9.30 ± 0.24	52	9.0 ± 0.19	56
	6.14 ± 0.25 ($n = 5$)	48	5.6 ± 0.23 ($n = 3$)	44
Bromocriptine	6.79 ± 0.10 ($n = 3$)	100 ± 4	6.3 ± 0.07 ($n = 3$)	100 ± 2

Calculations of affinity (pEC_{50}) and efficacy relative to dopamine (%) for agonists at the dopamine D_{2L} receptor, obtained by luciferase and cAMP accumulation assays in D2 CHO-luc cells.

concentration response curve of (−)lisuride was monophasic ($pEC_{50} = 6.26 \pm 0.06$) (Fig. 3; Table 3) with a relative intrinsic activity of 97 ± 2%, suggesting that the high affinity phase of the biphasic curve was indeed a result of activity at the dopamine D_{2L} receptor. Dopamine D_2 receptor antagonist treatment apparently exposed the full agonist concentration response of (−)lisuride at the 5-HT_{1B} receptor as shown in Fig. 1A. This response was shown to equal the E_{max} of 5-HT in this cell line (data not shown). To emphasise the partial agonist effect of (−)lisuride at the dopamine D_{2L} receptor, cells were treated with the 5-HT_{1B} receptor antagonist, pindolol (100 μM). This caused a rightward shift in the low affinity phase of the (−)lisuride concentration response curve ($pEC_{50} = 4.64 \pm 0.26$), exaggerating the partial agonist response of (−)lisuride at the dopamine D_2 receptor and confirming that the low affinity response was a result of activity at the 5-HT_{1B} receptor (Fig. 3; Table 3). The dopamine D_2 receptor agonist, bromocriptine, was a full agonist (100% efficacy) in this system ($pEC_{50} = 6.79 \pm 0.10$). The rank order of potency at the D_2 receptor was (−)lisuride»bromocriptine = dopamine. The rank order of intrinsic activity was dopamine = bromocriptine > (−)lisuride.

In the cAMP accumulation studies, 50 μM forskolin was used to produce a detectable increase in cAMP levels in D2 CHO-luc cells. Upper and lower levels of activation were calculated by the same method as for luciferase expression and all concentration responses were then compared with that of dopamine. Potencies and intrinsic activities of dopamine, (−)lisuride and bromocriptine were similar to those determined by the luciferase assay (Fig. 2B; Table 2). (−)Lisuride produced a biphasic concentration response ($pEC_{50} = 9.00 \pm 0.19$, and 5.60 ± 0.23) with % efficacy at the high and low affinity phases of 57 and 43% respectively, relative to dopamine, and bromocriptine displayed full agonist properties (100% efficacy). Again, this assay produced similar results to those using the luciferase assay, and the rank order of potency and intrinsic activity was preserved between the two types of assay.

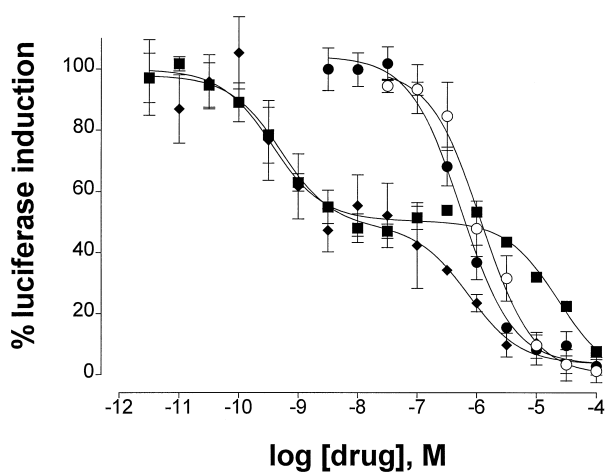

Fig. 3. Agonist and antagonist studies in D2 CHO-luc cells. Concentration response curves for dopamine (○), (−)lisuride (♦), (−)lisuride in the presence of 100 μM pindolol (■) and (−)lisuride in the presence of 10 μM (+)butaclamol (●). Cells were stimulated with 0.5 μM forskolin and responses are expressed as % of luciferase induction in the presence of forskolin and the absence of agonist. Data points are mean values (±S.E.M.) of at least three independent experiments carried out in triplicate.

Table 3

Drug	Luc. pEC_{50}	% Efficacy
Dopamine	5.92 ± 0.08 ($n = 3$)	100
Lisuride	9.32 ± 0.30	53
	6.20 ± 0.27 ($n = 3$)	47
Lisuride + pindolol (100 μM)	9.44 ± 0.10	49
	4.64 ± 0.26 ($n = 3$)	51
Lisuride + (+)butaclamol (10 μM)	6.26 ± 0.06 ($n = 3$)	97 ± 2

Calculations of affinity (pEC_{50}) and efficacy relative to dopamine (%) for antagonist effects on (−)lisuride at both the dopamine D_{2L} and 5-HT_{1B} receptors, obtained by luciferase assays in D2 CHO-luc cells.

4. Discussion

In the studies described here, we have demonstrated partial agonism at endogenously and heterologously expressed receptors in CHO cells using a luciferase reporter gene assay directly linked to the adenylyl cyclase pathway by means of a cAMP responsive promoter (Himmler et al., 1993; George et al., 1997a).

In cells stably expressing the luciferase gene (CHO-luc cells), the functional concentration response to zolmitriptan was partial at the 5-HT_{1B} receptor relative to the full agonist 5-HT. Zolmitriptan has been reported to be a novel 5-$HT_{1B/1D}$ receptor partial agonist (Martin et al., 1997), exhibiting a pEC_{50} of 6.8 and an intrinsic activity of 77% relative to 5-HT at the 5-HT_{1B}-like receptor expressed in CHO-K1 cells, using a cAMP accumulation assay. This correlates well with our studies using both luciferase ($pEC_{50} = 6.52 \pm 0.08$) and cAMP accumulation ($pEC_{50} = 6.02 \pm 0.11$) assays. The apparent decrease in the potency of zolmitriptan in our studies compared with Martin et al. (1997) may be due to variation in receptor density (endogenous levels in our case) or more likely as a result of treatment with the non-specific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) in their assay. In addition, the rank order of intrinsic activity of zolmitriptan and dihydroergocristine was consistent in both the luciferase and cAMP assays (Table 1) and the pEC_{50} values were observed to be not significantly different ($p > 0.05$) except for dihydroergocristine.

In CHO cells stably expressing both the luciferase gene and the dopamine D_{2L} receptor (D_2CHO-luc cells) at near physiological levels (100 fmol/mg protein), the

(−)lisuride concentration response in forskolin stimulated cells was different to the response to this drug in CHO-luc cells. The biphasic concentration response curve generated suggested that (−)lisuride was not only activating the 5-HT_{1B} receptor (Fig. 1) but also the dopamine D_{2L} receptor (Figs. 2 and 3). The lower affinity fraction of the concentration response curve produced a similar pEC_{50} in both the CHO-luc cell line (5.78 ± 0.14) and in D2 CHO-luc cells (6.14 ± 0.25), suggesting that this response maybe due to activation at the 5-HT_{1B} receptor. The higher affinity fraction of the concentration response ($pEC_{50} = 9.30 \pm 0.24$) appeared to be due to the presence, and hence, activation of the dopamine D_{2L} receptor, and this was confirmed by sensitivity to the D_2 antagonist, (+)butaclamol.

The (−)lisuride concentration response at the dopamine D_{2L} receptor however was only 52% of that of the full agonist dopamine, and this was highlighted by antagonising the 5-HT_{1B} response with pindolol. Bromocriptine however, was a full agonist at the dopamine D_{2L} receptor (Fig. 2). The rank order of potency of these drugs at the dopamine D_{2L} receptor were (−)lisuride»bromocriptine ≥ dopamine. The rank order of intrinsic activity was dopamine = bromocriptine > (−)lisuride.

As with the partial agonist results at the $5HT_{1B}$ receptor, it was shown that cAMP accumulation studies reflected the results shown with the luciferase assay. pEC_{50} values and levels of intrinsic activity were not significantly different ($p > 0.05$), hence the rank orders of potency and intrinsic activity were the same. These data confirm that in the case of negatively coupled G-protein coupled receptor activation, cAMP accumulation levels are consistent with the levels of expression of genes linked directly to the cAMP signal transduction pathway. It has been shown that this assay is more sensitive to receptor functional activity than cAMP accumulation assays (George et al., 1997b) presumably due to signal amplification along the pathway. This amplification may in turn explain the observed decrease in functional intrinsic activity of partial agonists in luciferase expression assays vs. cAMP accumulation assays, though this trend was not statistically significant except for dihydroergocristine.

To date, there have been no published reports addressing the variable levels of intrinsic activity of receptor: ligand interactions (partial agonism) using the reporter gene assay. Brauner-Osborne et al. (1996) have shown functional partial agonism by receptor selection and amplification technology (R-SAT), which utilises the β-galactosidase reporter gene to monitor NIH 3T3 cell proliferation after long term incubation with agonists. They established partial agonism by administering a cocktail of full agonists and antagonists. Partial agonism has however been frequently reported using cAMP accumulation assays (Adham et al., 1992; MacEwan et al., 1995; Martin et al., 1997) and GTPγS binding studies (Davidson et al., 1997; Lamothe et al., 1997; Pauwels et al., 1997).

The luciferase assay also offers the possibility of investigating the mechanisms of partial agonism. Potential reasons for partial agonist functional responses are (i) intrinsic receptor partial agonism, obviated by receptor density studies, (ii) receptor promiscuity (multiple G-protein activation), or (iii) multiple receptor activation (Jasper and Insel, 1992; Hoyer and Boddeke, 1993; Rovati and Nicosia, 1994). Up regulation of dopamine D_{2L} receptors by prolonged antagonist activation (Filtz et al., 1994), or by prolonged forskolin incubation (Johansson and Westlind-Danielsson, 1994) would, by augmenting receptor reserve, increase the functional response of a partial agonist in classes (i) and (iii) toward that of a full agonist. However, preliminary studies in our laboratory suggest that, even though receptor number may be increased, prolonged forskolin treatment (> 16 h) induces desensitisation of adenylyl cyclase mediated luciferase activity, which severely limits the sensitivity of the assay with regard to monitoring intrinsic activities (unpublished observation).

Variable receptor expression levels between tissues can alter the efficacy of a drug; thus, partial agonists can be used to specifically target tissues based on efficacy of a drug in a particular tissue. Recently, the use of dopamine D_2 receptor partial agonists for the treatment of schizophrenia (caused by hyperactive dopaminergic neurons) has gained merit in that these compounds activate presynaptic dopamine autoreceptors, leading to downregulation of dopamine neurotransmission, while antagonising activation of the postsynaptic receptors due to their low intrinsic activity (Wustrow et al., 1997). Higher receptor reserve at presynaptic dopamine D_2 receptors (Meller et al., 1988) increases the sensitivity to agonists and partial agonists, complimenting the desired effect. 5-$HT_{1B/1D}$ receptors mediate vasoconstriction of blood vessels in the vascular periphery (Ferrari and Saxena, 1993) and are also involved in the inhibition of protein extravasation (Moscowitz, 1992), two mechanisms linked to the effects of migraine. Zolmitriptan is currently being developed as a therapeutic agent for the treatment of migraine (Lipton and Stewart, 1997). 5-$HT_{1B/1D}$ receptors are mainly located presynaptically and stimulation inhibits release of 5-HT (Boschert et al., 1994). This suggests a target for the treatment of depression, thought to be caused by too low levels of 5-HT (Glennon, 1990; Murphy, 1990).

In conclusion, this assay offers marked advantages for G-protein coupled receptor functional activity investigations over the traditional methods (cAMP accumulation and GTPγS binding), being a simpler, non-invasive technique which does not involve the use of radioactivity. As a functional assay, it determines the effects on target gene expression rather than the activity of upstream mediators (e.g., cAMP), thus conveying more information about the physiological effects of G-protein coupled receptor activation within whole cells. The assay also accounts for the effects of transcription factors, offering a potentially more accurate view of functional responses and also facilitating

the therapeutic targeting of these specific mediators of gene transcription. Our studies clearly highlight the ability to discriminate between receptors that couple to the same signal transduction pathway by virtue of the differing affinities and intrinsic activities of drugs at these receptors. However, since cross-talk between different signal transduction pathways at the transcription factor level is also possible (Hill, 1998), an awareness of these signalling factors is important when analysing functional responses using reporter gene expression. Furthermore, the ability to obtain reproducible functional data using physiological levels of receptor expression (due to signal amplification), without the need to up-regulate receptor numbers, would hopefully reflect a more realistic representation of receptor function in living organisms. As a pharmaceutical screen for drug research, it is as rapid as traditional screening methods and has the capacity to detect varying levels of intrinsic activity at both physiological and upregulated levels of receptor expression.

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