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Pharmacological characterisation of human 5-HT_2 receptor subtypes

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Abstract

Prompted by conflicting literature, this study compared the pharmacology of human 5-hydroxytryptamine$_2$ (5-HT_2) receptors expressed in SH-SY5Y cells using a fluorometric imaging plate reader (FLIPR) based Ca^{2+} assay. 5-Hydroxytryptamine (5-HT) increased intracellular calcium concentration ($[Ca^{2+}]_i$) at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors ($pEC_{50} = 7.73 \pm 0.03$, 8.86 ± 0.04 and 7.99 ± 0.04, respectively) and these responses were inhibited by mesulergine ($pK_B = 7.42 \pm 0.06$, 8.77 ± 0.10 and 9.52 ± 0.11). A range of selective agonists and antagonists displayed the expected pharmacology at each receptor subtype.

Sodium butyrate pretreatment increased receptor expression in SH-SY5Y/5-HT_{2B} (15-fold) and SH-SY5Y/5-HT_{2C} cells (7-fold) and increased agonist potencies and relative efficacies. In contrast, sodium butyrate pretreatment of SH-SY5Y/5-HT_{2A} cells did not affect receptor expression. The present study provides a direct comparison of agonist and antagonist pharmacology at 5-HT_2 receptor subtypes in a homogenous system and confirms that agonist potency and efficacy varies with the level of receptor expression.

Keywords: 5-HT_2 receptor; FLIPR (fluorometric imaging plate reader); Ca^{2+}; SH-SY5Y; Sodium butyrate; Anxiety; Depression

1. Introduction

The 5-hydroxytryptamine$_2$ (5-HT_2) receptor family comprises of three types, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (Hoyer et al., 1994), which have been reported to play a major role in a range of central nervous system functions including anxiety, depression, migraine, sleep, satiety and schizophrenia (Baxter et al., 1995). The relative efficacy of compounds with affinity for 5-HT_2 receptors in man and in animal models further suggests that these receptors may be involved in the aetiology of various disease states (Baxter et al., 1995; Akiyoshi et al., 1996). However, the lack of selective ligands, in particular agonists, has made identification of the receptor subtype(s) involved in these disease states difficult. Predominantly, antagonists have been used to discriminate the role of the 5-HT_2 receptor subtypes and both selective and non-selective agents have been marketed for clinical indications including depression (mianserin), migraine (methysergide) and schizophrenia (risperidone) (Baxter et al., 1995).

The 5-HT_2 receptors are linked to the G_q family of G-proteins and subsequent activation of phospholipase C, induction of phosphoinositide metabolism and an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) (Martin and Humphrey, 1994; Porter et al., 1999). Although these receptors have been characterised in both radioligand binding (Hoyer, 1989; Bonhaus et al., 1995) and functional studies (Baxter et al., 1994; Newton et al., 1996; Wood et al., 1997), few studies have directly compared all three receptor subtypes. The only study published to date that has partially characterised the functional pharmacology of all three receptor subtypes in the same assay system, contained no data relating to antagonists (Porter et al., 1999). For these reasons, direct pharmacological comparisons between studies cannot easily be made. Moreover, the use of such diverse functional assays, including measurement of [^{3}H]inositol phosphate formation (Wood et al., 1997), $[Ca^{2+}]_i$ (Ullmer et al., 1996; Porter et al., 1999), electrophysiology (Newton et al., 1996), organ bath experiments (Watts et al., 1995) and isolated tissue preparations (Baxter et al., 1994) has made comparisons of agonist potency and relative efficacy even more difficult. The differences in potencies and relative efficacies reported in the literature (Baxter et al., 1994; Newton et al., 1996;

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Wood et al., 1997; Porter et al., 1999) may be due, at least in part, to the different levels of receptor expression, the degree of amplification and/or the cellular environment of the receptor, in the respective assay systems (Newton et al., 1996; Wood et al., 1997).

The present study was carried out to address these issues, using a fluorometric imaging plate reader (FLIPR). Direct comparisons of the pharmacology of the three 5-HT_2 receptor subtypes expressed in the same cellular environment, the neuronal-like SH-SY5Y cell line, were conducted using a standardised methodology. The level of receptor expression in recombinant systems can be modulated biochemically using sodium butyrate, which has been shown to induce cell cycle arrest in early G1 phase (Barnard and Warwick, 1993) and increase the expression of cell surface proteins including some receptors (Duman et al., 1994). To investigate the effects of such changes in receptor density on the potency and intrinsic activity of agonists, studies were performed with and without sodium butyrate pretreatment.

2. Materials and methods

2.1. Cell culture

Adherent SH-SY5Y cells stably expressing the recombinant human 5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C} (unedited isoform) receptors (Newton et al., 1996) were obtained from the SmithKline Beecham Centre for Applied Neuropsychobiology, Oxford and maintained in culture at 37°C (under 95%:5% O_2:CO_2) in Dulbecco's Modification of Eagle's Medium supplemented with 5–10% dialysed foetal calf serum and 400 μg geneticin. Sodium butyrate pretreated cells were cultured with medium containing 5 mM sodium butyrate 24 h prior to seeding into 96-well plates.

2.2. Measurement of $[Ca^{2+}]_i$ using the FLIPR

SH-SY5Y cells, separately expressing 5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C} receptors, were seeded into black walled clear-base 96-well plates (Costar, UK) at a density of 20,000 cells/well and cultured overnight. Cells were then incubated with medium containing the cytoplasmic calcium indicator, Fluo-3 in the acetylmethyl form (4 μM; Teflabs, TX, USA) and 2.5 mM probenecid at 37°C for 60 min. The loaded cells were washed four times with, and finally resuspended in, Tyrode's medium (in mM; NaCl 145, KCl 2.5, HEPES 10, Glucose 10, $MgCl_2$ 1.2, $CaCl_2$ 1.5) containing 2.5 mM probenecid, before being incubated for 30 min at 37°C with either buffer or antagonist. The plates were then placed into a FLIPR (Molecular Devices, UK) to monitor cell fluorescence (λ_{ex} = 488 nm, λ_{EM} = 540 nm) (Sullivan et al., 1999) before and after the addition of various agonists.

2.3. Radioligand binding

Methodologies for membrane preparations and radioligand binding studies were essentially those of Newton et al. (1996). Saturation binding assays were performed on membranes prepared from SH-SY5Y/5-HT_{2A} cells using [^{3}H]ketanserin and on SH-SY5Y/5-HT_{2B} or SH-SY5Y/5-HT_{2C} membranes using [^{3}H]mesulergine. Membranes (100–150 μg protein) were incubated with six concentrations of radioligand (0.5–50 nM) for 60 min at 37°C, in the presence and absence of mianserin (10 μM), to define non-specific and total binding, respectively. Binding reac-

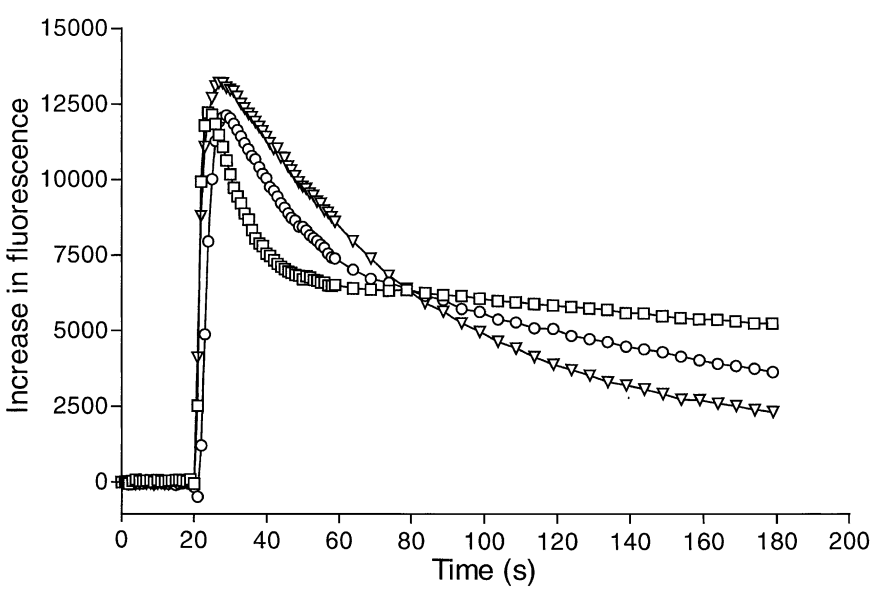

Fig. 1. 5-HT (EC_{80}) stimulated increase in $[Ca^{2+}]_i$ in SH-SY5Y cells expressing human 5-HT_{2A} (□), human 5-HT_{2B} (○) or human 5-HT_{2C} (▽) receptor subtypes. Data shown are typical traces from a single representative experiment.

tions were terminated by rapid filtration onto Whatman GF/B glass fibre filters with five 1-ml washes using Tris–HCl buffer at 4°C (50 mM Trizma (Sigma, UK) pH 7.7 at 25°C, 5 mM $MgCl_2$). Radioactivity was determined by liquid scintillation spectrometry and binding parameters (capacity and affinity) estimated by computer assisted non-linear regression analysis using Ligand (Biosoft, UK). Final protein content was assayed according to the method of Bradford (1976).

2.4. Data analysis

Functional responses were measured as peak fluorescence intensity minus basal. Iterative curve-fitting and parameter estimations were carried out using a four-parameter logistic model and Microsoft Excel (Bowen and Jerman, 1995). For agonists, relative efficacy (E_{max}) values were calculated as fitted maximum of each concentration effect curve expressed as a percentage of fitted maximum of a 5-HT curve from the same plate. Antagonist potency values (IC_{50}) were converted to apparent pK_B values using a modified Cheng–Prusoff correction (Cheng and Prusoff, 1973).

$$\text{Apparent } pK_B = -\log \frac{(IC_{50})}{1 + \frac{[\text{agonist}]}{(EC_{50})}}.$$

Statistical tests for significance were conducted using an unpaired Student's *t*-test comparing control and sodium butyrate treatments.

Data are expressed as mean ± S.E.M. unless otherwise stated.

2.5. Materials

5-Methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydrolpyrrolo[2,3-f]indole) (SB-206553) and *N*-(1-methyl-5-indolyl)-*N*′-(3-methyl-5-isothiazolyl) urea (SB-204741) were synthesised in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, Harlow, UK. Yohimbine, tryptamine, sodium butyrate and all bulk reagents were obtained from Sigma (Poole, Dorset, UK).

1-{5-(2-Thienyl-methoxy)-1*H*-3-indoly}propan-2-amine (BW723C86), 1-(3-chlorophenyl)piperazine (*m*-CPP), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridiny)-1*H*-indole (RU24969), *R*-(+)-7-chloro-8-hyroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro(1*H*)-3-benzapine (SCH23390), *N*-(3-trifluoromethyl-phenyl)piperazine (TFMPP) and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) were obtained from Tocris Cookson, Bristol, UK. 5-Hydroxytryptamine (5-HT), 2-methyl-5-hydroxytryptamine (2-Me-5-HT), 6-chloro-2-(1-piperaxinyl)pyrazine (MK212), 5-carboxyamidotryptamine (5-CT), α-methyl-5-hydroxy tryptamine (α-Me-5-HT), 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6A,7,8,9,10,10A-octahyroindolo[4,3-FG]quinolone (LY53857) and all other agents used were obtained from Research Biochemicals International, Poole, UK. All tissue culture reagents were obtained from Life Technologies (UK). [^{3}H]ketanserin (63 Ci/mmol) was obtained from NEN-Du Pont, UK and [^{3}H]mesulergine (78 Ci/mmol) was obtained from Amersham International, UK.

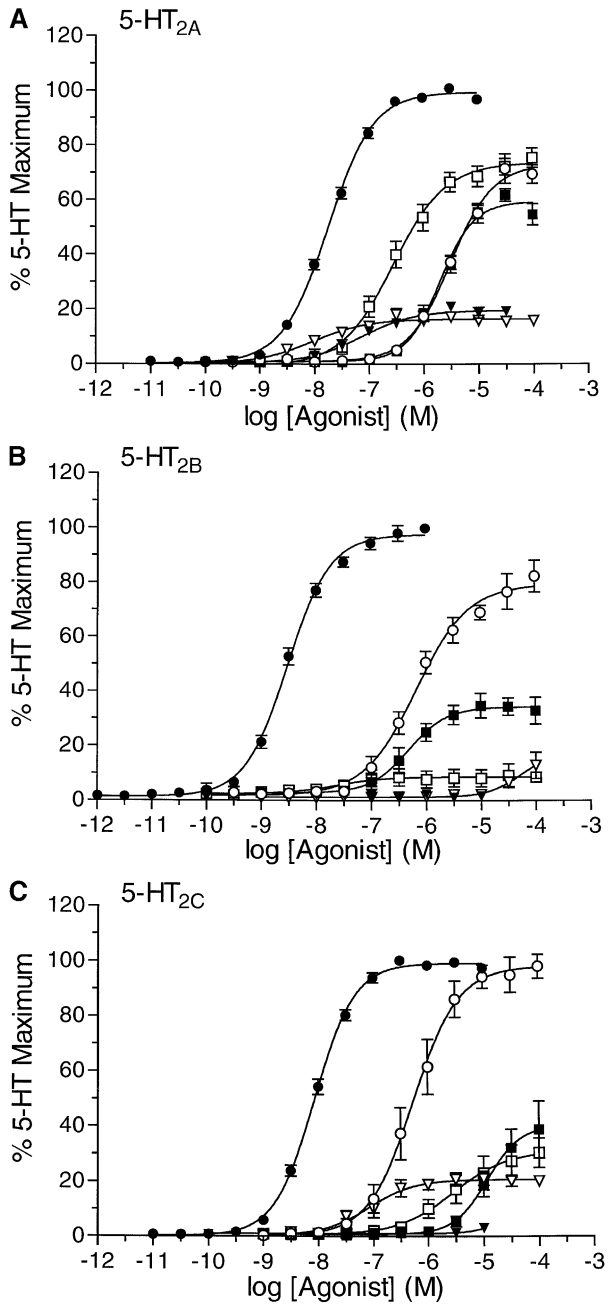

Fig. 2. Agonist stimulation of SH-SY5Y cells expressing 5-HT_{2A} receptors (A), 5-HT_{2B} receptors (B) and 5-HT_{2C} receptors (C) with 5-HT (●), MK212 (○), Lisuride (▼), SCH23390 (▽), 2-Methly-5-HT (■) and RU24969 (□). Data are mean (±S.E.M.) from at least three independent experiments.

3. Results

3.1. Effects of agonists at 5-HT$_2$ receptors expressed in SH-SY5Y cells

Responses to 5-HT in all three cell lines were typified by a rapid initial peak in fluorescence (maximal 5–9 s after agonist addition) followed by a gradual decline towards baseline values (Fig. 1). The rate at which the response returned to basal levels varied between the three cell lines. The response to 5-HT in the SH-SY5Y/5-HT$_{2A}$ cell line was maintained for longer than that in the SH-SY5Y/5-HT$_{2B}$ or the SH-SY5Y/5-HT$_{2C}$ cells (Fig. 1). However, the magnitude of the initial peak was comparable between cell lines (Fig. 1). None of the compounds tested elicited a response in non-transfected SH-SY5Y cells (data not shown).

Table 1
Agonist potencies and relative efficacies at 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$ receptor subtypes

Compound	Control		Sodium butyrate	
	pEC$_{50}$	E_{max}	pEC$_{50}$	E_{max}
(a) 5-HT$_{2A}$				
5-HT	7.73 (±0.03)	99.5 (±0.8)	7.51 (±0.06)	99.6 (±0.4)
BW723C86	6.16 (±0.14)	61.5 (±5.0)	5.71 (±0.07)	39.3 (±4.3)
m-CPP	6.72 (±0.06)	32.1 (±2.2)	6.37 (±0.09)	18.7 (±4.5)
DOI	8.24 (±0.08)	86.9 (±3.7)	8.21 (±0.05)	68.0 (±1.8)
RU24969	6.55 (±0.09)	71.5 (±3.7)	6.14 (±0.05)	55.0 (±8.1)
SCH23390	7.83 (±0.10)	17.1 (±1.3)	IA	
2-Me-5-HT	5.60 (±0.07)	65.5 (±2.8)	5.37 (±0.15)	40.2 (±6.4)
TFMPP	6.58 (±0.08)	28.0 (±2.5)	6.37 (±0.16)	17.7 (±4.2)
8-OH-DPAT	5.28 (±0.07)	33.0 (±2.2)	5.51(±0.12)	15.2 (±9.1)
MK212	5.65 (±0.09)	63.4 (±4.6)	ND	
5-CT	6.45 (±0.08)	81.4 (±3.9)	5.84 (±0.06)	74.4 (±15.3)
Lisuride	7.27 (±0.04)	18.2 (±2.2)	ND	
Tryptamine	6.80 (±0.05)	94.3 (±2.2)	6.42 (±0.05)	75.2 (±0.5)
α-Me-5-HT	6.80 (±0.05)	102.3 (±5.4)	6.92 (±0.03)	96.2 (±6.5)
(b) 5-HT$_{2B}$				
5-HT	8.86 (±0.04)	98.7 (±0.8)	8.65 (±0.05)	99.4 (±0.4)
BW723C86	7.40 (±0.11)	85.5 (±6.0)	7.45 (±0.08)	91.7 (±4.3)
m-CPP	IA		7.10 (±0.04)	33.1 (±4.0)
DOI	7.48 (±0.10)	80.9 (±3.3)	7.72 (±0.09)	91.0 (±2.7)
RU24969	IA		6.42 (±0.19)	41.8 (±16.1)
SCH23390	4.56 (±0.14)	13.8 (±5.6)	6.32 (±0.12)	44.5 (±9.6)
2-Me-5-HT	6.28 (±0.07)	35.4 (±3.1)	6.58 (±0.04)	62.9 (±1.9)
TFMPP	IA		6.69 (±0.05)	32.1 (±5.2)
8-OH-DPAT	5.36 (±0.09)	32.0 (±3.5)	5.72 (±0.09)	55.0 (±4.2)
MK212	6.21 (±0.11)	48.8 (±4.2)	6.55 (±0.08)	70.0 (±4.7)
5-CT	7.31 (±0.08)	101.2 (±5.2)	7.43 (±0.16)	93.3 (±0.5)
Lisuride	IA		IA	
Tryptamine	7.37 (±0.07)	92.5 (±4.6)	7.84 (±0.16)	88.6 (±4.2)
α-Me-5-HT	7.91 (±0.06)	106.4 (±3.0)	8.59 (±0.02)	93.5 (±8.7)
(c) 5-HT$_{2C}$				
5-HT	7.99 (±0.04)	99.9 (±0.1)	8.85 (±0.06)	101.26 (±1.3)
BW723C86	5.89 (±0.18)	45.0 (±5.1)	6.57 (±0.14)	99.5 (±5.2)
m-CPP	6.97 (±0.05)	69.2 (±5.5)	7.52 (±0.11)	100.8 (±7.1)
DOI	7.00 (±0.13)	53.8 (±7.1)	8.13 (±0.09)	108.6 (±6.6)
RU24969	5.50 (±0.16)	30.0 (±4.8)	6.97 (±0.29)	94.5 (±11.5)
SCH23390	7.04 (±0.13)	20.9 (±1.8)	7.48 (±0.38)	101.2 (±1.5)
2-Me-5-HT	4.88 (±0.12)	39.5 (±6.5)	6.07 (±0.22)	90.6 (±4.0)
TFMPP	6.69 (±0.11)	57.7 (±4.0)	7.40 (±0.09)	108.7 (±6.6)
8-OH-DPAT	IA		IA	
MK212	6.36 (±0.13)	99.5 (±3.8)	7.70 (±0.26)	99.1 (±2.7)
5-CT	5.82 (±0.10)	101.0 (±5.3)	ND	
Lisuride	IA		IA	
Tryptamine	7.00 (±0.07)	93.2 (±5.1)	8.10 (±0.17)	98.9 (±10.0)
α-Me-5-HT	7.18 (±0.08)	100.7 (±8.8)	8.59 (±0.22)	107.5 (±3.2)

IA denotes inactive (10 μM). ND denotes not determined. Data are mean (±S.E.M.) from at least three independent experiments.

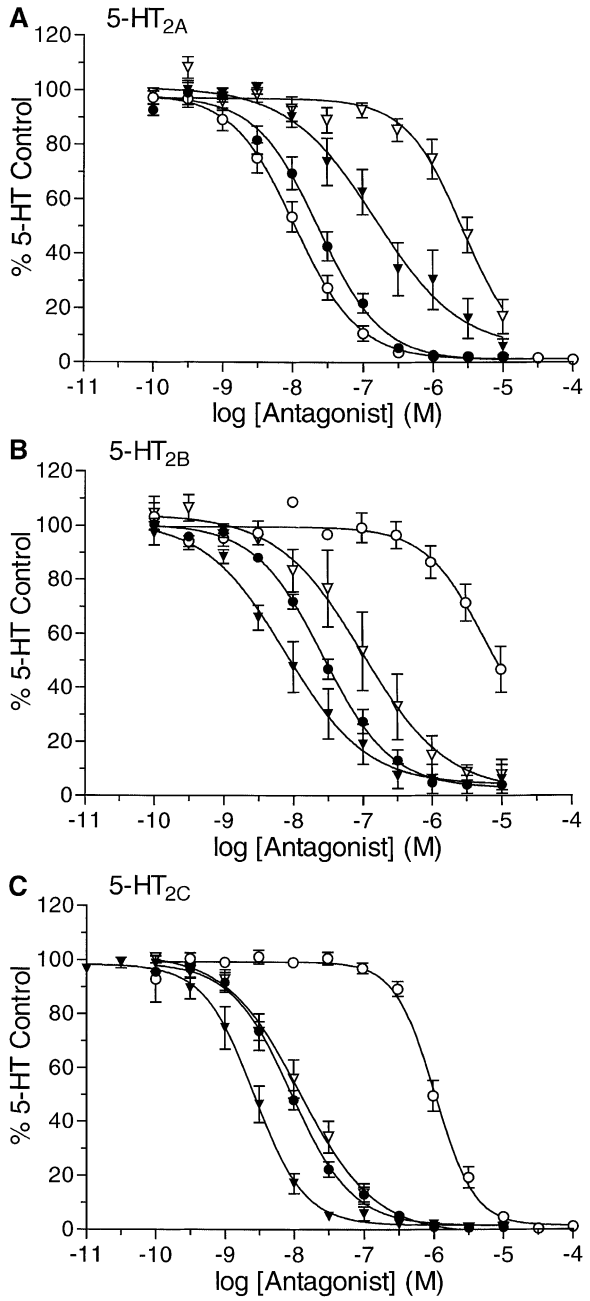

Fig. 3. Inhibition of 5-HT (EC_{80}) induced stimulation of SH-SY5Y cells expressing 5-HT_{2A} receptors (A), 5-HT_{2B} receptors (B) and 5-HT_{2C} receptors (C) with Mianserin (●), Ketanserin (○), Mesulergine (▼) and SB-206553 (▽). Data are mean (±S.E.M.) from at least three independent experiments.

In each cell line, 5-HT caused a concentration-dependent increase in $[Ca^{2+}]_i$ (Fig. 2A, B and C), with pEC_{50} values of 7.73 ± 0.03, 8.86 ± 0.04 and 7.99 ± 0.04 at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, respectively. Slope values were not significantly different from unity. The activity of various other 5-HT_2 receptor agonists, exhibiting a range of potencies and relative efficacies, are summarised in Table 1a, b and c. The rank order of potency for agonists was DOI > SCH23390 = 5-HT > Lisuride > α-Me-5-HT = Tryptamine = *m*-CPP = TFMPP = RU24969 = 5-CT > BW723C86 > MK212 = 2-Me-5-HT > 8-OH-DPAT for 5-HT_{2A} receptors, 5-HT > α-Me-5-HT > DOI = BW723C86 = Tryptamine = 5-CT > 2-Me-5-HT = MK212 > 8-OH-DPAT > SCH23390 for 5-HT_{2B} receptors and 5-HT > α-Me-5-HT = SCH23390 = DOI = Tryptamine = *m*-CPP > TFMPP > MK212 > BW723C86 = 5-CT > RU24969 > 2-Me-5-HT for 5-HT_{2C} receptors, respectively. The rank order of potency obtained for each receptor was generally consistent with the established pharmacology (Table 1b,c).

The relative efficacies of partial agonists tended to be higher in the sodium butyrate-treated SH-SY5Y/5-HT_{2B} cells than those in control cells (Table 1b). Most compounds also displayed a moderate increase in potency when tested in sodium butyrate-treated SH-SY5Y/5-HT_{2B} cells (Table 1b) though SCH23990 displayed a more pronounced increase relative to other agonists tested (Table 1b). A similar pattern was seen in the sodium butyrate-treated SH-SY5Y/5-HT_{2C} cells, though in this system a marked increase in both potency and relative efficacy was seen following sodium butyrate pretreatment (Table 1c). In the sodium butyrate-treated SH-SY5Y/5-HT_{2A} cells, potencies were unaffected, though relative efficacies were generally lower than those in control cells (Table 1a).

3.2. Effects of antagonists at 5-HT_2 receptors expressed in SH-SY5Y cells

In antagonist studies, mianserin caused a concentration dependant inhibition of 5-HT-induced increases in $[Ca^{2+}]_i$ (Fig. 3A, B and C). The affinities of mianserin and other antagonists are summarised in Table 2. The rank order of antagonist affinity was methiothepin = spiperone > ketanserin > LY53857 = mianserin > mesulergine > SB-206553 > yohimbine > SB-204741, at 5-HT_{2A} receptors, LY53857 = methiothepin > mesulergine > mianserin > SB-206553 > yohimbine = SB-204741 > spiperone > ketanserin at 5-HT_{2B} receptors and mesulergine = methiothepin = LY53857 > mianserin > SB-206553 > ketan-

Table 2
Antagonist affinities (pK_B) at human 5-HT_2 receptor subtypes

Compound	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
SB-206553	6.27 (±0.08)	7.53 (±0.20)	8.38 (±0.08)
SB-204741	5.82 (±0.09)	6.70 (±0.21)	6.23 (±0.09)
Mianserin	8.25 (±0.10)	8.21 (±0.09)	9.04 (±0.08)
Ketanserin	8.68 (±0.12)	6.00 (±0.12)	7.00 (±0.08)
Mesulergine	7.42 (±0.06)	8.77 (±0.10)	9.52 (±0.11)
Yohimbine	6.05 (±0.11)	6.95 (±0.15)	< 5
Spiperone	8.98 (±0.15)	6.45 (±0.02)	6.88 (±0.27)
Methiothepin	9.05 (±0.05)	9.20 (±0.06)	9.35 (±0.44)
LY53857	8.31 (±0.02)	9.47 (±0.05)	9.30 (±0.09)

Data are mean (±S.E.M.) from at least three independent experiments.

Table 3
Binding affinities and capacities in control and sodium butyrate-pretreated cells

	Treatment	K_d (nM)	B_{max} (pmol/mg protein)
5-HT_{2A}	Control	4.17 (±1.3)	0.38 (±0.02)
	Sodium butyrate	2.59 (±0.6)	0.27 (±0.04)
5-HT_{2B}	Control	5.08 (±1.9)	0.46 (±0.03)
	Sodium butyrate	8.77 (±0.3)[a]	6.69 (±0.15)[b]
5-HT_{2C}	Control	4.12 (±1.6)	0.89 (±0.09)
	Sodium butyrate	6.14 (±0.3)[a]	6.27 (±0.04)[b]

Data are mean (±S.E.M.) from at least three independent experiments.
[a]Significantly different from control, $P < 0.05$.
[b]Significantly different from control, $P < 0.01$.

serin > spiperone > SB-204741 > yohimbine at 5-HT_{2C} receptors. None of the antagonists tested increased $[Ca^{2+}]_i$ in any of the cell types (data not shown).

3.3. Effects of sodium butyrate treatment on 5-HT_2 receptor binding in SH-SY5Y cells

In radioligand binding studies, sodium butyrate-pretreated SH-SY5Y/5-HT_{2B} and SH-SY5Y/5-HT_{2C} cells were shown to have significantly higher receptor expression, 15-fold and seven-fold, respectively, compared to control cells (Table 3). The affinity of [^{3}H]mesulergine was also significantly decreased, though by less than two-fold, at 5-HT_{2B} and 5-HT_{2C} receptors following sodium butyrate treatment (Table 3). In contrast, neither receptor expression nor the affinity of [^{3}H]ketanserin were significantly altered by sodium butyrate in SH-SY5Y/5-HT_{2A} cells (Table 3).

4. Discussion

5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes (Hoyer et al., 1994) have been implicated in a range of functions of the central nervous system including anxiety, depression, migraine, sleep, schizophrenia and control of feeding (Baxter et al., 1995). Direct pharmacological comparison of the 5-HT_2 receptor subtypes has been difficult because few studies have included all three receptors (Newton et al., 1996; Wood et al., 1997) or have data from a diverse range of assay systems (Baxter et al., 1995). Furthermore, the only published study to date which has pharmacologically characterised each receptor subtype using the same assay conditions and parental cell line, contained no data relating to antagonists (Porter et al., 1999). The present study provides comparable pharmacological data, relating to both agonists and antagonists, generated in a common neuronal-like cellular environment, at all three receptor subtypes. In addition, the effects of increasing receptor expression using sodium butyrate pretreatment have been investigated.

The responses to 5-HT in all three cell lines containing recombinant human 5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C} receptors displayed similar kinetics, though the rate at which responses returned to basal levels varied between cell lines.

It is possible that such differences in the temporal profile of the response to 5-HT in each cell line, though relatively small, reflect differences in the efficiency of coupling to phospholipase C, release of Ca^{2+} from different intracellular stores or possibly the involvement of other second messenger systems. Indeed, such differences in G-protein coupling between 5-HT_{2A} and 5-HT_{2C} receptors have been reported (Berg et al., 1994), as has the ability of 5-HT_2 receptors to couple to both phospholipase C and phospholipase A_2 (Berg et al., 1998). However, in the absence of definitive studies investigating signal transduction in SH-SY5Y cells expressing 5-HT_2 receptor subtypes, the reason for the small differences in response to 5-HT remain unclear.

Some receptor agonists exhibited selectivity for one receptor subtype. DOI was a selective agonist at 5-HT_{2A} vs. 5-HT_{2B} and 5-HT_{2C} in agreement with published data (Newton et al., 1996; Porter et al., 1999) and BW723C86 and α-Me-5HT displayed selectivity for 5-HT_{2B} vs. 5-HT_{2A} and 5-HT_{2C} receptors as previously reported (Baxter et al., 1995; Porter et al., 1999). Some antagonists also displayed selectivity for one receptor subtype. Ketanserin and spiperone were 40-fold and over 100-fold selective for 5-HT_{2A} receptors over 5-HT_{2B} and 5-HT_{2C}, respectively, whereas SB-204741 and yohimbine displayed some selectivity for 5-HT_{2B} receptors and SB-206553 and mesulergine selectivity for 5-HT_{2C} receptors. There is good correspondence between antagonist affinity estimations in the present study and those generated in radioligand binding studies using membranes prepared from SH-SY5Y cells expressing 5-HT_{2A} receptors (Newton et al., 1996). However, in the present study, pK_B values in SH-SY5Y/5-HT_{2C} cells tended to be higher than pK_i values previously reported from radioligand binding studies (Newton et al., 1996), though the rank order was maintained. Similarly, the pK_B values from the 5-HT_{2B} receptor expressing cells corresponded with the pK_i values from previous radioligand binding studies and the rank order of affinity was maintained (Wood et al., 1997).

Although there is a good correlation between antagonist affinities in the present study and those reported in radioligand binding studies (Newton et al., 1996), the same cannot be said for all three receptor subtypes when considering agonist potency and relative efficacy in functional studies. In the SH-SY5Y/5-HT_{2B} cells, compounds previously reported as weak partial agonists had no apparent intrinsic activity. For example, *m*-CPP, a weak partial agonist at 5-HT_{2B} receptors (Baxter et al., 1994; Newton et al., 1996; Porter et al., 1999), RU24969 and TFMPP failed to invoke Ca^{2+} responses in these cells. However, all of these compounds acted as partial agonists when tested in the sodium butyrate-pretreated cells. The fact that pretreat-

ment of the SH-SY5Y/5-HT_{2B} cells with sodium butyrate increased the relative efficacy of these partial agonists is probably explained by the observation that such pretreatment also increases receptor expression by 15-fold. This is consistent with the reported effects of sodium butyrate (Duman et al., 1994) on receptor expression and it is likely that such an increase will affect the level of receptor reserve in the system. An increase in the relative efficacy of partial agonists and a moderate increase in potency in general are consistent with accepted receptor theory and suggests an increase in receptor reserve. Since these low efficacy partial agonists display intrinsic activity in native systems, such as rat fundus preparations (Baxter et al., 1994) and these data are in excellent agreement with intrinsic activities determined using sodium butyrate-treated SH-SY5Y/5-HT_{2B} cells, it is probable that a high degree of receptor reserve and/or very efficient coupling of 5-HT_{2B} receptors exists in the fundic strip. This further highlights the need for diligence when comparing functional responses across tissues.

Similarly, the observed seven-fold increase in receptor expression following sodium butyrate pretreatment of SH-SY5Y/5-HT_{2C} cells is consistent with the marked increase in both the relative efficacy and potency of receptor agonists observed. The presence of receptor reserve in this system is also inferred by the discrepancy between the affinity of 5-HT in binding studies using these cells (Newton et al., 1996) and the potency of 5-HT the FLIPR system; pK_i of 6.80 and pEC_{50} of 7.99, respectively. It is noteworthy that the largest effects on both potency and relative efficacy following sodium butyrate pretreatment were seen in the SH-SY5Y/5HT_{2C} cells despite the fact that sodium butyrate pretreatment increased receptor expression to a greater extend in the SH-SY5Y/5HT_{2B} cells. This might be explained, at least in part, by the suggestion that the magnitude of increase in both potency and relative efficacy of agonists is dependent on the relative level of receptor reserve prior to sodium butyrate pretreatment. However, in the absence of affinity estimations from comparable radioligand binding studies, it is difficult to quantify the level of receptor reserve, if any, in untreated SH-SY5Y/5-HT_{2B} and SH-SY5Y/5-HT_{2C} cells.

The fact that sodium butyrate pretreatment did not significantly affect affinity or binding capacity in the SH-SY5Y/5HT_{2A} cells is consistent with the observations that neither potency nor efficacy are increased in functional studies. If anything, in the sodium butyrate-pretreated SH-SY5Y/5HT_{2A} cells, relative efficacies tended to be lower than those in control cells. This observation is not easily explained but it is likely that the reported effects of sodium butyrate to increase overall protein expression (Duman et al., 1994) would have very many cellular consequences that could ultimately affect the relative efficacies of compounds as determined in functional studies. Moreover, sodium butyrate itself has been reported to affect the efficiency of coupling to G-proteins (Kassis et al., 1984) and signal transduction differences between 5-HT_{2A} and 5-HT_{2C} receptors have also been reported (Berg et al., 1994). In these studies 5-HT_{2C} receptors were shown to couple to additional effector mechanisms, other than the phospholipase C-mediated induction phosphoinositide hydrolysis, that 5-HT_{2A} receptors do not (Berg et al., 1994). It is therefore possible that 5-HT_{2A} and 5-HT_{2C} receptors couple to different G-protein subtypes and that these subtypes are differentially affected by sodium butyrate pretreatment. This might explain, at least in part, the differential effects of sodium butyrate on the 5-HT_2 receptor subtype expressing cells.

In conclusion, the present study has extended the pharmacological profile of the human 5-HT_{2A} and 5-HT_{2C} receptor subtypes expressed in SH-SY5Y cells (Newton et al., 1996) to include the 5-HT_{2B} receptor subtype and to encompass both agonist and antagonist profiles at each receptor. The present study also demonstrates the utility of the FLIPR as a device for rapidly assessing the activity of a wide range of agonists and antagonists at multiple receptors using comparable methodologies. We have provided compelling evidence that sodium butyrate pretreatment of SH-SY5Y/5-HT_{2B} and SH-SY5Y/5-HT_{2C} cells increases 5-HT_{2B} and 5-HT_{2C} receptor expression. The apparent differences in agonist potency and relative efficacy in control and sodium butyrate cells are suggestive of an increase in receptor reserve. In contrast, sodium butyrate pretreatment of SH-SY5Y/5-HT_{2A} cells did not increase 5-HT_{2A} receptor expression. The present study has highlighted some of the problems encountered when using high expression recombinant cell lines to investigate agonist pharmacology and how caution should be observed when extrapolating to the in vivo situation.

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