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Functional characterization of agonists at recombinant human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in CHO-K1 cells

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1 The goal of this study was to characterize the agonist pharmacology of human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} (VSV) receptors expressed in CHO-K1 (Chinese hamster ovary) cells.

2 We used a fluorometric imaging plate reader (FLIPR) which allows rapid detection of rises in intracellular calcium levels upon the addition of agonists.

3 Stimulation of all three receptors by 5-HT caused a robust concentration dependent increase in intracellular calcium levels. No such effect was observed from non-transfected control CHO-K1 cells.

4 The rank order of potency of agonists at the different receptor subtypes varied. Tryptamines, BW-723C86, d-norfenfluramine, Ro 60-0175 and LSD exhibited the following rank order of potency; 5-HT_{2B} > 5-HT_{2C} > 5-HT_{2A}. Piperazines such as m-Chlorophenylpiperazine (mCPP), ORG-12962, MK-212 and also ORG-37684 exhibited a rank order of potency of 5-HT_{2C} > 5-HT_{2B} > 5-HT_{2A}. The phenylisopropylamines DOI and DOB had a rank order of 5-HT_{2A} > 5-HT_{2B} > 5-HT_{2C}.

5 Many agonists tested had partial agonist actions when compared to 5-HT, and a wide range of relative efficacies were exhibited, which was cell line dependent. For example, mCPP had a relative efficacy of 65% at 5-HT_{2C} receptors but <25% at either 5-HT_{2A} or 5-HT_{2B} receptors.

6 Interpretation of literature values of functional assays using different cell lines, different receptor expression levels and different receptor isoforms, is complex. Species differences and the previous use of antagonist radioligands to characterize agonist potency in binding assays emphasizes the importance of studying agonists in the same experiment using the same assay conditions and parental cell lines.

Keywords: Serotonin; receptors; 5-HT_{2A}; 5-HT_{2B}; 5-HT_{2C}; FLIPR; calcium; fluorescence; Ro 60-0175

Abbreviations: CHO, Chinese hamster ovary; CNS, central nervous system; 5-CT, 5-carboxamidotryptamine; DOB, 2,5-dimethoxy-4-bromoamphetamine hydrochloride; DOI, 2,5-dimethoxy-4-iodoamphetamine hydrobromide; FBS, Foetal bovine serum; FLIPR, fluorometric imaging plate reader; LSD, lysergic acid diethylamide; 2-Me-5-HT, 2-methyl-5-hydroxytryptamine; α-Me-5-HT, α-methyl-5-hydroxytryptamine; mCPP, m-Chlorophenylpiperazine; PCR, polymerase chain reaction; PI, phosphatidyl inositol; PLA_2, phospholipase A_2; PLC, phospholipase C; RNA, ribonucleic acid; RT–PCR, reverse transcription–polymerase chain reaction

Introduction

Serotonin mediates its actions through several different receptor subtypes in the brain of which 5-HT_2 receptors form a closely related subgroup. The 5-HT_2 receptor family consists of 3 subtypes termed 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} (formerly known as 5-HT_2, 5-HT_{2F} and 5-HT_{1C} respectively). 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors were initially identified and characterized by pharmacological means, but subsequently classified by their close structural homology as revealed by molecular cloning and biochemical similarities (Humphrey *et al*., 1993; Hoyer *et al*., 1994; Martin & Humphrey, 1994). Hence the 5-HT_2 receptors share significant sequence homology at the amino acid level (up to 80% in their transmembrane region) and couple to the G_q family of G-proteins (Berg *et al*., 1998; Roth *et al*., 1998). Although there are regional differences in the distribution of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, they are all expressed in the brain with extensive pharmacological and functional similarities (Pompeiano *et al*., 1994; Bonhaus *et al*., 1995; Duxon *et al*., 1997; Roth *et al*., 1998). It is important therefore to determine specificity of drug actions at individual receptor subtypes. This need is further highlighted by the recent increased interest in the therapeutic utility of selective 5-HT_2 ligands to treat a variety of disorders including schizophrenia, anxiety, depression and obesity (Kennett, 1993; Dourish, 1995; Martin *et al*., 1998; Leysen & Kelder, 1998). However, to date there has been no comprehensive pharmacological study to characterize the effects of different compounds at all three receptor subtypes measuring the same second messenger response in cells of the same genetic background. This means it is often difficult to ascribe particular drug functions to a receptor subtype.

The potency, and selectivity of agonists is difficult to infer from radioligand binding studies as agonists, antagonists and inverse agonists have differing relative affinities for the coupled or uncoupled states of G-protein coupled receptors (Westphal & Sanders-Bush, 1994). Additionally, the observation that compounds can exhibit partial agonist actions, with varying degrees of intrinsic efficacy suggests that essential information is not always obtained from binding studies which do not measure a functional response (Conn & Sanders-Bush, 1987; Berg *et al*., 1998; Egan *et al*., 1998; Roth *et al*., 1998). A further complication is the recent finding that the 5-HT_{2C} receptor undergoes RNA editing (Burns *et al*., 1997). It has been suggested that this may affect receptor G-protein coupling efficiency, and hence the potency and efficacy of agonists may vary depending on the isoform being studied. This has made

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interpretation of the literature difficult, as in previous studies the identity of the 5-HT$_{2C}$ receptor isoform being studied has not been known. To date there have been no reports of RNA editing for the 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors.

Previous reports of species differences in the pharmacology of 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors suggest that cross-referencing human to rat data may be flawed, and that human receptors should be used when possible (Johnson *et al*., 1993; Nelson *et al*., 1993; Wainscott *et al*., 1996; Baxter, 1996). The present study was therefore undertaken to investigate the pharmacology of human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in the same cell lines with the same genetic background. Although 5-HT$_2$ receptors can couple to both phospholipase C (PLC) and phospholipase A$_2$ (PLA$_2$) (Berg *et al*., 1998), for the purpose of this study we characterized the calcium signal obtained as a consequence of PLC activation and subsequent IP$_3$ production. We used a fluorometric imaging plate reader (FLIPR) that integrates drug addition and calcium fluorescence measurements, allowing rapid detection of calcium following receptor activation (Schroeder & Neagle, 1996). This method is especially useful when studying receptors such as the 5-HT$_{2C}$ receptor, that undergo rapid desensitization as it circumvents the need for long agonist incubation times (Akiyoshi *et al*., 1995).

Methods

Molecular biology and cell culture

Human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptor cDNAs were purchased from Invitrogen (5-HT$_{2A}$ subcloned in to pCR3.1)

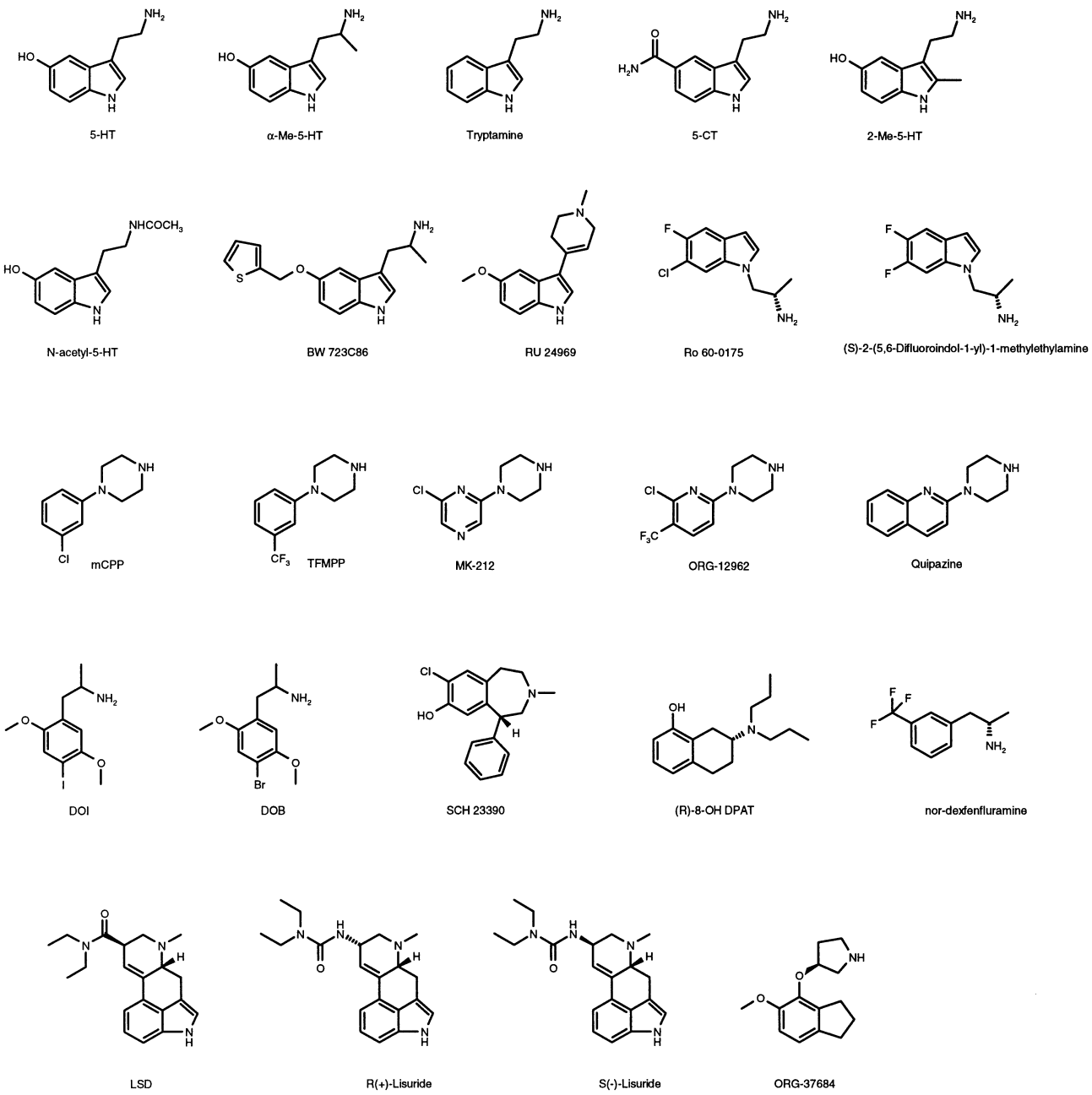

Figure 1 Structures of the compounds studied. Abbreviations: 5-CT, 5-carboxamidotryptamine; α-Me-5-HT, α-methyl-5-hydroxytryptamine; 2-Me-5-HT, 2-methyl-5-HT; LSD, lysergic acid diethylamide; DOI, 2,5-dimethoxy-4-iodoamphetamine hydrobromide; DOB, 2,5-dimethoxy-4-bromoamphetamine hydrochloride.

and Euroscreen (5-HT$_{2B}$ and 5-HT$_{2C}$ subcloned in to pCDNA3.1) (accession numbers EMBL: X57830, X77307 and GB: U49516 respectively). CHO-K1 (ECACC) cells were stably transfected with each receptor transcript using the calcium phosphate method. Stably transfected cell lines were selected using G-418, and clonal cell lines developed by limit dilution. The cell lines were screened for receptor expression by functional analysis using FLIPR. 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ cell lines were cultured in Dulbecco's modified Eagle Medium (DMEM) containing 10% heat inactivated dialyzed foetal bovine serum (FBS), 1% penicillin-streptomycin, 1% L-glutamine and 1% non-essential amino acids. Dialyzed FBS was used to prevent receptor desensitization as non-dialyzed FBS contains appreciable levels of serotonin. All cell lines were grown in selection of 800 μg ml^{-1} G-418.

Assay preparation

On the afternoon before the assay, cells were plated at a density of 30,000 cells/well into black 96 well plates with clear bottoms to allow cell inspection and fluorescence measurements from the bottom of each well. This density of cells was sufficient to yield a confluent monolayer the next day. Hanks balanced salt solution without phenol red containing 20 mM HEPES and 2.5 mM probenecid was prepared fresh on the day of assay, and used as the assay buffer. For insoluble compounds, DMSO was used to aid solubilization. However the final concentration of DMSO exposed to the cells never exceeded 0.3%, which had no effect alone (data not shown). The dye-loading buffer consisted of a final concentration of 4 μM Fluo-3-AM (dissolved in DMSO and pluronic acid) in serum free cell culture maintenance media containing 20 mM HEPES and 2.5 mM probenecid. The cells were dye loaded for approximately 90 min at 37°C in a 5% CO_2 incubator at 95% humidity by removing the existing maintenance media and adding 100 μl of the dye loading buffer to each well. Once dye loaded, the cells were washed thoroughly on a Denley cell washer with the assay buffer to remove any unincorporated dye. Exactly 100 μl assay buffer was left in each well.

Assay conditions

Each 96 well plate containing dye loaded cells were placed into the FLIPR drawer and the lazer intensity set to a suitable level (to obtain basal values of approximately 10,000 fluorescence units). The variation in basal fluorescence units across the plate was less than 10%. Fluorescence readings were obtained every 1 s for the first 60 s, and every 5 s for the next 30 s. Fluorescent measurements were captured by a cooled CCD camera and integrated to an on-line PC. Compound additions were made 10 s into the fluorescent measurements from an on-board 96 well pipettor loaded with black tips. 50 μl of compound was added to each well at a rate of 70 μl per second to ensure rapid equilibrium of compound with the cell receptor environment. The final assay volume was therefore 150 μl/well. The drug addition was made from a height of 125 μl ensuring that no compound could leach out of the tips to affect basal measurements. However, at the end of compound addition the black tips were not removed to minimize interference with fluorescence measurements. The maximum fluorescent signal obtained was recorded and normalized to a positive control of 10 μM 5-HT performed in quadruplicate on every plate. The maximum fluorescent signal was typically greater than 20,000 fluorescent units and obtained within 15 s of drug addition. The exact amount of fluorescence obtained did vary as a function of how long the cells had been dye loaded. Cells exposed to a slightly longer dye loading procedure presumably incorporated more dye as they elicited a slightly larger signal. However these small variations were not reflected by any alteration in pharmacology.

RT–PCR assays

Total RNA was isolated from 1.0×10^6 cells, using a standard RNA extraction procedure (Qiagen Ltd, Germany). 3 μl total RNA was reverse transcribed using the Access–RT/PCR kit (Promega, U.K.) and subjected to 30 cycles of PCR, as directed in the procedure, using receptor specific oligonucleotides. The forward and reverse oligonucleotides for the 5-HT$_{2A}$ receptor corresponded to nucleotides 515–535 and 895–875

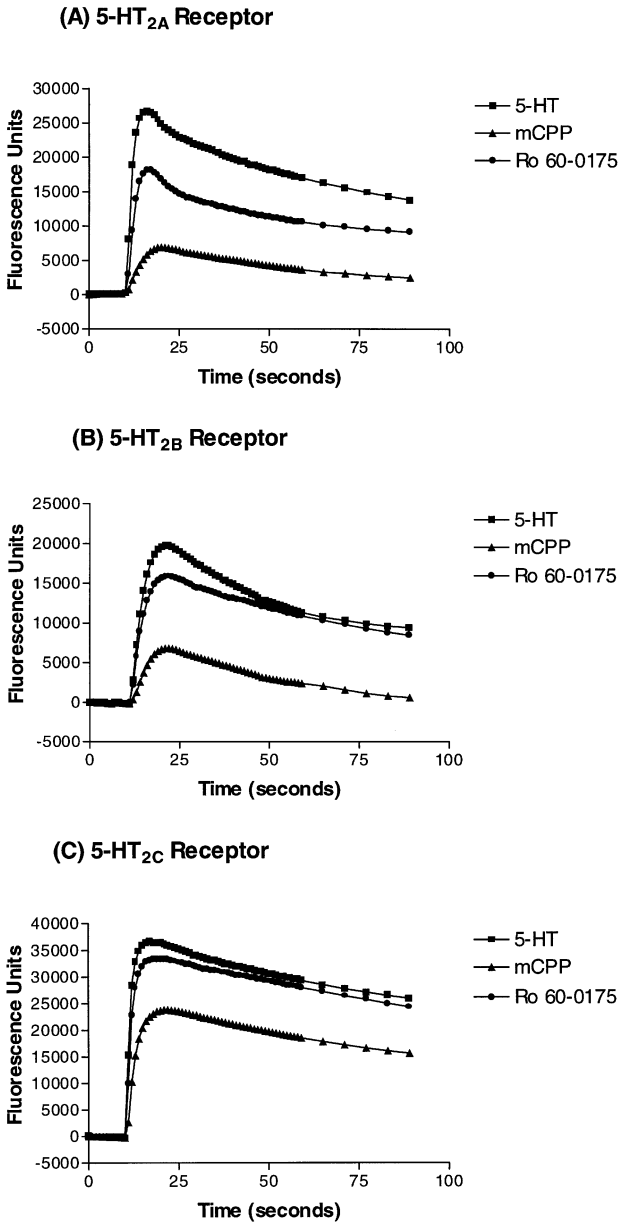

Figure 2 Time course of calcium signal following compound addition on cells expressing (A) 5-HT$_{2A}$ receptors, (B) 5-HT$_{2B}$ receptors and (C) 5-HT$_{2C}$ receptors. Results are the fluorescence measurements taken from a single 96 well plate where 10 μM compound is added after 10 s. The data is from a single representative experiment that was repeated at least three times. Each data point depicting the average fluorescence value was performed in duplicate.

respectively. Similarly the 5-HT$_{2B}$ primer pair corresponded to nucleotides 416–439 and 736–715 and the 5-HT$_{2C}$ receptor primers corresponded to nucleotides 1277–1298 and 1994–1975.

Drugs

All compounds were obtained from Sigma-RBI (Poole, U.K.) or Tocris (Bristol, U.K.) with the exceptions of ORG-37684, ORG-12962, Ro 60-0175, d-norfenfluramine and BW-723C86 which were synthesized by the Chemistry Department at Cerebrus Ltd. Stock solutions were prepared in assay buffer. For insoluble compounds DMSO was used to aid solubilization. Fluo-3-AM and pluronic acid were purchased from Molecular Probes (U.S.A.) or TefLabs (U.S.A.). Oligonucleotides were synthesized to order by OSWEL (Southampton, U.K.)

All cell culture reagents were purchased from Sigma (Poole, U.K.) or Gibco (Paisley, U.K.). Cell culture plastic ware was purchased from Falcon or Corning Costar.

Determination of agonist potencies and relative efficacies

Each 96 well plate contained four wells dedicated to a positive control defined as 10 μM 5-HT and four wells as a negative control defined as assay buffer alone. For pharmacological characterization, all data were normalized to the positive control wells, which were expressed as 100% signal. Each agonist dose response curve was constructed using a four parameter logistic equation from GraphPad Prism as follows: $Y = \text{bottom} + (\text{Top} - \text{Bottom})/1 + 10^{(\text{LogEC}_{50}\text{-X})\text{nH}}$. The efficacy of the compound was determined from the Top value, which is the maximum value of the Y plateau. The concentration of agonist that produced a half-maximal response is represented by the EC$_{50}$ value.

Results

No signal to 5-HT was obtained from non-transfected CHO-K1 cells that underwent an identical assay protocol. CHO-K1 cells were not found to endogenously express any of the 5-HT$_2$ receptor subtypes when examined by reverse transcription, PCR (RT–PCR) or binding (data not shown). CHO cells transfected with the 5-HT$_{2C}$ receptor gave the expected product when screened with 5-HT$_{2C}$ specific primers, but no product was obtained from non-transfected cells, or from 5-HT$_{2A}$ or 5-HT$_{2B}$ cell lines. The receptor specific primers for the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors exhibited the same degree of specificity only yielding the expected band size when tested. RT–PCR was routinely performed to confirm the specificity of receptor expression. Hence all responses to specific agonists obtained in the above study are believed to be due to the presence of the transfected receptor.

The structures of all the compounds studied are represented in Figure 1. Following the addition of agonist to the cells, a rapid rise in intracellular calcium was obtained. Maximally effective concentrations of each agonist caused a rise in calcium that was maximal approximately 15 s following drug addition (Figure 2). It was the peak of this rapidly obtained maximum rise in intracellular calcium over basal levels that was recorded, and expressed as a percentage of that obtained by 10 μM 5-HT. Although all the data was normalized for analysis of pEC$_{50}$ and relative efficacy values, a rise in fluorescence units of greater than 20,000 was typical for 5-HT at each cell line (Figure 2). Given that the CCD camera saturates at 65,000 fluorescence units, the measurements were well within the linear range of fluorescent measurements. The relative efficacy of all compounds was expressed as a fraction of that obtained by 10 μM 5-HT.

The rank order of potency of the compounds varied depending on the receptor studied (Table 1, Figure 3A–F).

Table 1 Functional characterization of compounds at cloned human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors expressed in CHO-K1 cells

	5-HT$_{2A\ 3-9}$		*5-HT$_{2B\ 23-9}$*		*5-HT$_{2C\ 20-28}$*	
	pEC$_{50}$	*Rel eff.*	*pEC$_{50}$*	*Rel eff.*	*pEC$_{50}$*	*Rel eff.*
5-HT	7.51±0.06	1.02	8.68±0.09	1.06	8.24±0.06	0.97
α-methyl-5-HT	7.39±0.04	0.97	8.70±0.04	0.77	7.94±0.05	0.92
Tryptamine	6.59±0.05	0.71	7.53±0.06	0.92	7.34±0.06	0.85
5-CT	6.26±0.05	0.65	7.81±0.05	0.90	6.39±0.08	0.88
20Me-5-HT	5.62±0.11	0.55	6.77±0.03	0.72	5.92±0.14	0.70
N-acetyl-5-HT	NA	<0.2	NA	<0.1	NA	<0.10
BW-723C86	6.66±0.13	0.43	8.97±0.14	0.83	7.03±0.07	0.51
mCPP	6.65±0.11	0.22	7.20±0.20	0.24	7.09±0.04	0.65
TFMPP	6.79±0.12	0.20	6.54±0.63	0.34	7.13±0.05	0.54
ORG-12962	6.38±0.10	0.54	6.28±0.15	0.41	7.01±0.10	0.62
MK-212	NA	<0.5	6.53±0.07	0.62	6.67±0.08	0.84
Ro 60-0175	6.35±0.09	0.69	9.05±0.29	0.79	7.49±0.08	0.84
5,6-difluoroindol-methylethylamine	6.12±0.08	0.68	9.01±0.17	0.73	7.43±0.05	0.85
Nor-d-fenfluramine	5.98±0.11	0.54	8.06±0.16	0.66	6.77±0.10	0.77
ORG-37684	7.11±0.05	0.45	7.96±0.26	0.34	8.17±0.08	0.55
(±) DOI	9.05±0.11	0.61	8.85±0.13	0.65	8.10±0.11	0.57
(±) DOB	8.82±0.10	0.74	8.54±0.09	0.69	8.11±0.11	0.65
LSD	7.67±0.14	0.44	8.05±0.13	0.51	7.34±0.06	0.29
(S)-lisuride	5.16	0.14	NA	<0.1	NA	<0.1
(R)-lisuride	5.96	0.20	NA	<0.1	NA	<0.1
Quipazine	6.51±0.08	0.62	6.75±0.55	0.17	6.47±0.05	0.57
(R)-8-OH-DPAT	>5	~0.3	6.05±0.10	0.78	NA	<0.10
RU 24969	7.09±0.08	0.53	7.58±0.25	0.36	6.55±0.08	0.48
SCH-23390	7.88±0.13	0.20	7.15±0.41	0.46	7.39±0.16	0.32

pEC$_{50}$ values are the mean±s.e.mean of 3–8 independent experiments. Relative efficacy values are the corresponding fraction of the response elicited by the compounds compared to 10 μM 5-HT. Each data point was calculated from the peak response obtained from each compound at every concentration. NA = not appropriate, due to a relative efficacy value of less than 0.1.

All tryptamine analogues tested, including 5-HT, showed a rank order of potency of 5-HT$_{2B}$, > 5-HT$_{2C}$ > 5-HT$_{2A}$ (Figures 1 and 3A, D and F). The piperazines mCPP, ORG-12962, TFMPP and MK-212 tended to be most selective and efficacious at the 5-HT$_{2C}$ receptor (Table 1, Figure 3A and C). However this was not universal as ORG-12962 also exhibited high efficacy for the 5-HT$_{2A}$ receptor (54% compared to 62% at the 5-HT$_{2C}$ receptor).

In contrast, quipazine was approximately equipotent at all three receptor subtypes, and of equal efficacy at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, but of lower efficacy for the 5-HT$_{2B}$ receptor (Figure 3D). However it should be noted that it is difficult in functional studies to attribute accurate potency values for low efficacy compounds such as mCPP at 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, and for quipazine at 5-HT$_{2B}$ receptors due to poor reproducibility of their effects.

The selective 5-HT$_{2A}$ phenylisopropylamine receptor agonists DOI and DOB were found to be most potent and efficacious for the 5-HT$_{2A}$ receptor, although only by a factor of approximately 2 and 10 fold compared to the 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors respectively (Figure 3E). LSD, on the other hand, was found to be slightly more potent and efficacious at the 5-HT$_{2B}$ receptor than the 5-HT$_{2A}$ receptor. However, it was more potent at the 5-HT$_{2A}$ receptor than the 5-HT$_{2C}$ receptor where it exhibited relatively low efficacy (Table 1, Figure 3F).

ORG-37684 was a highly potent agonist (pEC$_{50}$ = 8.17) at the 5-HT$_{2C}$ receptor with a relative efficacy of 55%. Its selectivity for the 5-HT$_{2C}$ receptor was approximately 2.5 times over the 5-HT$_{2B}$ and ten times for the 5-HT$_{2A}$ receptor, where it exhibited partial agonism of 34 and 45% respectively (Table 1, Figure 3C).

Ro 60-0175 was discovered to be a highly potent (pEC$_{50}$ = 9.05) and high efficacy agonist (79%) at 5-HT$_{2B}$ receptors (Table 1, Figure 3B). Ro 60-0175 was also an agonist at 5-HT$_{2C}$ receptors with a pEC$_{50}$ of 7.49. Its selectivity over the 5-HT$_{2A}$ receptor was approximately 13 fold. This contrasts with a reported selectivity of 25 fold in binding studies (Boes *et al*., 1997), and 2–3 fold functionally (Martin *et al*., 1998). In order to confirm this result the 5,6-difluoro analogue of Ro 60-0175 (5,6-difluoroindolmethylethylamine) was synthesized (see Figure 1) as it had previously been claimed to be over 100 fold more selective for 5-HT$_{2C}$ receptors than 5-HT$_{2A}$ receptors in binding (Boes *et al*., 1997). However, in the present study, while it was a little more selective than Ro 60-0175 for 5-HT$_{2C}$ receptors over 5-HT$_{2A}$ receptors, it was still most potent at 5-HT$_{2B}$ receptors (Table 1).

D-norfenfluramine, a major metabolite of d-fenfluramine, was found to have a rank order of potency of 5-HT$_{2B}$ > 5-HT$_{2C}$ > 5-HT$_{2A}$ (Table 1, Figure 3B), in contrast to D-fenfluramine itself which had no direct effect (data not shown).

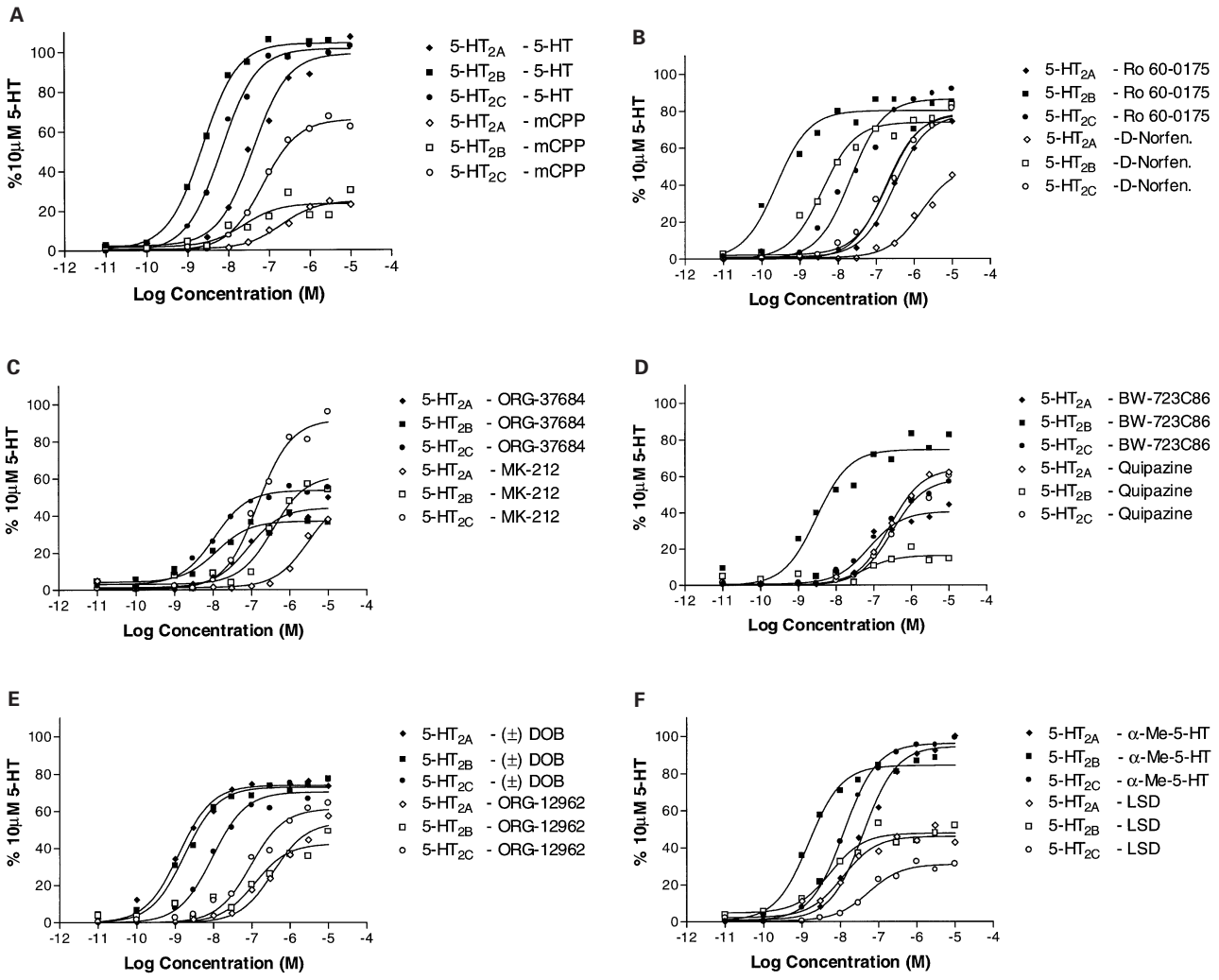

Figure 3 Concentration response curves at 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. Data points represent the maximum fluorescence signal obtained at each concentration and are representative from a single experiment performed in duplicate that was repeated 3–8 times.

Discussion

To our knowledge, this is the most comprehensive functional characterization of agonists at the serotonin 5-HT$_2$ receptors described to date. Unfortunately it is not possible to study the receptors in native CNS tissue due to the lack of complete segregation of all three receptors in discrete brain regions (Bonhaus *et al*., 1995; Duxon *et al*., 1997; Pompiano *et al*., 1994). The use of recombinant expression systems is therefore a useful approach, and also allows the study of human receptors. The three-receptor subtypes were all transfected into CHO-K1 cells thus ensuring they were studied in an identical genetic background. Although CHO cells have previously been reported to express the 5-HT$_{1B}$ receptor (Giles *et al*., 1996), this is unlikely to have compromized the present study for several reasons. The 5-HT$_{1B}$ receptor is endogenously expressed at extremely low levels, and we have been unable to obtain responses to 5-HT from non-transfected CHO-K1 cells using cytosensor microphysiometry (unpublished data), suggesting the receptor protein if present in these cells, is below the threshold level for detection. Additionally, parental non-transfected CHO-K1 cells when examined under an identical experimental paradigm do not exhibit a concentration dependent rise in calcium levels in response to 5-HT.

All of the compounds characterized in the present study also failed to elicit a significant response when tested at 10 μM in parental CHO-K1 cells (data not shown). The endogenous expression of serotonin receptors is a common issue in recombinant receptor expression studies. For example NIH-3T3 cells and SHSY5Y cells, both previously used for the study of recombinant 5-HT$_2$ receptors have been reported to endogenously express 5-HT$_2$ receptors (Saucier *et al*., 1998; Saucier & Albert, 1997, Schmuck *et al*., 1994; Duxon *et al*., 1997). It is generally assumed however that the expression level of endogenous receptors in these cell lines is sufficiently low so as not to interfere with the study of recombinant receptors whose expression levels are generally significantly higher.

The nature of the response obtained in assays that measure the functional consequences of receptor activation is dependent on receptor expression level (Lucaites *et al*, 1996). The above cell lines were therefore chosen as they express the receptors of interest at relatively low levels (183 fmol mg^{-1}, 614 fmol mg^{-1} and 238 fmol mg^{-1} at the 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors respectively) and best fitted the limited data available in the literature.

This study characterized compounds of diverse chemical structures in the hope of discovering trends between the chemical classes for receptor selectivity (Figure 1). Where this study overlaps with previous studies which characterized a range of compounds our results are in good agreement. For example, a recent study by Newton *et al*. (1996) expressed human 5-HT$_{2A}$ and 5-HT$_{2C}$ (isoform unknown) receptors in SHSY5Y cells. Both studies found 5-HT to be more potent at 5-HT$_{2C}$ receptors than 5-HT$_{2A}$ receptors and mCPP to be more efficacious at 5-HT$_{2C}$ receptors. Additionally both studies found quipazine to be almost equi-potent at the two receptors with very similar relative efficacies. However, in the same study Newton and colleagues describe DOI and LSD displaying between one and two orders of magnitude greater selectivity for 5-HT$_{2A}$ receptors, whereas we found a much smaller degree of selectivity, particularly for LSD, although both studies agreed closely on the relative efficacies obtained (Newton *et al*., 1996). In one of the few studies characterizing human 5-HT$_{2B}$ receptors, Wood *et al*. (1997) recently reported limited pharmacology of 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors expressed in HEK-293 cells measuring PI hydrolysis. In this study 5-HT was more potent at 5-HT$_{2C}$ receptors than 5-HT$_{2B}$ receptors and mCPP did not elicit a response, but acted as a potent antagonist at 5-HT$_{2B}$ receptors (Thomas *et al*., 1996, Wood *et al*., 1997). More striking was their observation that BW 723C86 was equi-potent at the two receptors with pEC$_{50}$s of approximately 7.0 (Wood *et al*., 1997). This is almost two orders of magnitude less potent than we observed (pEC$_{50}$ = 8.92) at 5-HT$_{2B}$ receptors. These differences in the pharmacology of human 5-HT$_{2B}$ receptors are likely to be methodological as we used lower expressing recombinant cell lines. Comparison of results obtained from studies using native tissue and recombinant systems is often useful, and contraction of the rat stomach fundus has been used as an index of 5-HT$_{2B}$ receptor activity. The results of Baxter *et al*. (1994) agree well with that obtained for the 5-HT$_{2B}$ cell line used in this study, although the fundic strip appeared to offer slightly greater efficacy. For example we report a relative efficacy of 24% for mCPP, as opposed to 38% reported for the stomach fundus; MK-212 and RU 24969 are described as having relative efficacies of 100 and 62% respectively, whereas in our hands they have a relative efficacy of 62 and 36% respectively (Table 1, Baxter *et al*., 1994). It is possible that the stomach fundus has a higher expression level, and possibly receptor reserve accounting for the higher efficacy. Alternatively, the recombinant expression system used in the present study may offer a cleaner pharmacological profile. However, an additional factor is the observation that although the 5-HT$_{2B}$ receptor is expressed at 614 fmoles mg^{-1} tissue, almost three times higher than either the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors, it consistently elicits a smaller calcium signal (Figure 2). This suggests that the 5-HT$_{2B}$ receptor may couple less efficiently to PLC, and that other signal transduction cascades may also be physiologically relevant (Luciates *et al*., 1996; Berg *et al*., 1998).

The 5-HT$_{2A}$ receptor is considered to be a site of action of hallucinogenic compounds such as DOI, DOB and LSD (Titeler *et al*., 1988; Marek & Aghajanian, 1996; Egan *et al*., 1998). However, there was no clear distinction in the pharmacology of such hallucinogenic compounds from non-hallucinogens also tested such as quipazine and lisuride (Table 1). Nevertheless it was clear that these compounds were generally more potent at the 5-HT$_{2A}$ receptor than at the 5-HT$_{2C}$ receptor. However, the relative efficacy of compounds gave no clear indication of hallucinogenic activity. For example several compounds thought not to elicit hallucinogenic activity such as quipazine and Ro 60-0175, had greater or equal efficacy to the known hallucinogens DOI, DOB and LSD. From this study, a reasonable conclusion that can be drawn is that hallucinogenic-like activity is consistent with high affinity at 5-HT$_{2A}$ receptors, and less so with affinity at 5-HT$_{2C}$ receptors.

The CNS function of 5-HT$_{2B}$ receptors is currently unknown, largely due to their extremely low expression in the brain (Kursar *et al*., 1994; Duxon *et al*., 1997), and the paucity of available pharmacological tools. Hence the relatively potent response of LSD at the 5-HT$_{2B}$ receptor is of unknown significance (Table 1, Figure 3D). BW 723C86 is a selective agonist for rat 5-HT$_{2B}$ receptors (Baxter, 1996), and this study suggests it retains its selectivity for human 5-HT$_{2B}$ receptors (see also Wood *et al*., 1997). Of particular interest is the 80 fold selectivity of BW-723C86 for 5-HT$_{2B}$ receptors, and the finding that it exhibited greater efficacy at 5-HT$_{2B}$ receptors than at either 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors. This suggests that it may be a useful tool for characterizing the CNS function of the 5-HT$_{2B}$ receptor. Indeed, Kennett and colleagues have recently reported that BW 723C86 induces anxiolytic and hyperphagic

effects in rats, and attribute these actions to activation of central 5-HT$_{2B}$ receptors (Kennett *et al*., 1996; 1997). Other striking pharmacological characteristics of the 5-HT$_{2B}$ receptor were its high affinity for the native ligand 5-HT, and especially for α-methyl 5-HT. Interestingly however, despite its potent effect, α-methyl-5-HT was a partial agonist at the 5-HT$_{2B}$ receptor, but a full agonist at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Table 1, Figure 3F). The mainly peripheral expression of 5-HT$_{2B}$ receptors, and the few studies reporting any central effects attributable to the 5-HT$_{2B}$ receptor suggests that its physiological role in the brain require further clarification. However, the high sensitivity of the 5-HT$_{2B}$ receptor to 5-HT, if reflected *in vivo* may imply that its low CNS expression levels should not necessarily be interpreted as a lack of a physiological role.

Ro 60-0175 has previously been claimed to be 25 fold selective for the 5-HT$_{2C}$ receptor over the 5-HT$_{2A}$ from binding studies (Boes *et al*., 1997). However, in agreement with Martin *et al*. (1998), we report relatively little separation for Ro 60-0175 between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in functional studies using human recombinant receptors. However others have reported a degree of selectivity of greater than 500 fold for Ro 60-0175 for the 5-HT$_{2C}$ receptor over the 5-HT$_{2A}$ receptor (Zhang *et al*., 1998). Martin *et al*., (1998) also reported Ro 60-0175 to be most potent at stimulating contraction of the rat stomach fundus, an index of 5-HT$_{2B}$ receptor activation with a relative efficacy of 70%. In agreement with this study, we also report that Ro 60-0175 is a potent agonist at the human 5-HT$_{2B}$ receptor with a relative efficacy of 79%. The functional selectivity of Ro 60-0175 for 5-HT$_{2C}$ receptors is therefore questionable and behavioural effects should be interpreted with care.

We have studied the VSV isoform of the 5-HT$_{2C}$ receptor as it is one of the major isoforms expressed in both rat and human brains (Burns *et al*., 1997; Niswender *et al*., 1998; Iyer *et al*., 1998). The consequence of editing at 5-HT$_{2C}$ receptors is thought to be a reduced efficiency of G protein–receptor coupling. The functional outcome of this has been reported to be a reduced affinity and efficacy of agonists at the edited isoforms relative to the unedited INI isoform, with a concomitant reduced constitutive activity of the receptor when expressed in cell lines (Burns *et al*., 1997; Niswender *et al*., 1998; 1999; Iyer *et al*., 1998). Hence it is possible that the values we are reporting for the 5-HT$_{2C}$ represent the agonists with lower potency and efficacy than might be expected for the unedited receptor. However, as the majority of the receptors are edited in human brain, and the VSV isoform is a major variant (Niswender *et al*., 1999; Iyer *et al*., 1998), our results are likely to be representative of human brain 5-HT$_{2C}$ receptors, and hence the degree of selectivity reported appropriate.

Previous reports have demonstrated limited species differences between rat and human 5-HT$_{2B}$ receptors. For example several antagonists including spiperone, ketanserin, risperidone and clozapine showed increased affinity for the human 5-HT$_{2B}$ receptor over the rat. In contrast, 5-HT has been reported to be approximately 100 times less potent at the mouse than either the rat or human 5-HT$_{2B}$ receptors (Baxter, 1996; Wainscott *et al*., 1996, Kursar *et al*., 1994). Species differences between rat and human 5-HT$_{2A}$ receptors have also been reported for the tryptamines, mCPP and certain ergolines (Johnson *et al*., 1993, Nelson *et al*., 1993; Bonhaus *et al*., 1995). In the case of the 5-HT$_{2A}$ receptor, a single amino acid difference in TM5 between the two species has been implicated (Johnson *et al*., 1994). These findings emphasize the need to carefully consider the species when examining the pharmacology of different 5-HT$_2$ receptors.

In conclusion, this study has used FLIPR to measure the agonist pharmacology of human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ (VSV isoform) receptors recombinantly expressed in CHO-K1 cells. The use of FLIPR to measure a calcium rise in these cells is thought to be advantageous over other methods such as PI hydrolysis due to the ability to simultaneously measure baseline fluorescence while adding compounds. This allows the very rapid measurement of receptor activation, which is particularly useful for receptors such as the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, which rapidly undergo desensitization. (The desensitization rate of the 5-HT$_{2B}$ receptor is currently unknown). The fact that our data does not always reflect the literature in terms of absolute potency and relative efficacy may be a reflection of the different cell lines and methodologies employed in previous studies. It is hoped that the present study will therefore provide a useful reference point for the pharmacology of compounds at 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors.

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