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ORIGINAL ARTICLE

Didier Cussac · Adrian Newman-Tancredi · Yann Quentric · Nathalie Carpentier · Guillaume Poissonnet Jean-Gilles Parmentier · Solo Goldstein · Mark J. Millan

Characterization of phospholipase C activity at h5-HT$_{2C}$ compared with h5-HT$_{2B}$ receptors: influence of novel ligands upon membrane-bound levels of [^{3}H]phosphatidylinositols

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Abstract Employing a novel, rapid and sensitive method for evaluation of phospholipase C (PLC) activity, the present study characterized the actions of diverse agonists and antagonists at human (h)5-HT$_{2C}$ receptors expressed in Chinese Hamster Ovary (CHO) cells. In addition, affinities and efficacies at these sites were compared with those obtained at h5-HT$_{2B}$ receptors.

5-HT elicited a robust and rapid reduction in levels of the pre-labelled, membrane-bound substrate of PLC, [^{3}H]phosphatidylinositols ([^{3}H]PI). The time-course of [^{3}H]PI depletion paralleled that of [^{3}H]inositol phosphate ([^{3}H]IP) accumulation, as determined by conventional anion exchange chromatography. Inactivation of h5-HT$_{2C}$ receptors with the alkylating agent, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), revealed a large receptor reserve, with half-maximal PLC activation induced by a concentration of 5-HT occupying only 5% of sites. In analogy to 5-HT (E_{max}=100%), DOI, MK212 and mCPP, as well as the novel ligands, Ro600332, Ro600175 and BW723C86, showed "full" efficacy at h5-HT$_{2C}$ sites. Their efficacies were similar at h5-HT$_{2B}$ sites, with the exception of mCPP and MK212, which acted as partial agonists. Further, lisuride and Ro600869 behaved as partial agonists and antagonists at h5-HT$_{2C}$ and h5-HT$_{2B}$ receptors, respectively. As concerns functional selectivity (potency for induction of [^{3}H]PI depletion), only Ro600175 preferentially activated h5-HT$_{2B}$ sites. In contrast, Ro600332 preferentially activated h5-HT$_{2C}$ receptors. Amongst antagonists, RS102221 and SB242084 displayed a marked preference for h5-HT$_{2C}$ sites, whereas LY266097, S33526 and SB204741 behaved as selective antagonists at h5-HT$_{2B}$ receptors. At both h5-HT$_{2C}$ and h5-HT$_{2B}$ receptors, antagonist potency (pK_b) and binding affinity (pK_i) were highly correlated.

In conclusion, this rapid and innovative method for determination of PLC activity permitted characterization of an extensive range of novel ligands at h5-HT$_{2C}$ receptors. Although several antagonists clearly differentiated h5-HT$_{2C}$ from h5-HT$_{2B}$ receptors under these conditions, highly selective agonists remain to be identified.

Keywords 5-HT$_{2C}$ receptors · 5-HT$_{2B}$ receptors · Phospholipase C · Phosphatidylinositols · Receptor reserve

D. Cussac (✉) · A. Newman-Tancredi · Y. Quentric
N. Carpentier · M.J. Millan
Department of Psychopharmacology,
Institut de Recherches Servier, 125 Chemin de Ronde,
78290 Croissy-Sur-Seine (Paris), France
e-mail: didier.cussac@fr.netgrs.com, Fax: +33-155-722470

G. Poissonnet · J.-G. Parmentier · S. Goldstein
Department of Chemistry A, Institut de Recherches Servier,
11 rue des Moulineaux, 92150 Suresnes (Paris), France

Introduction

5-HT$_{2C}$ receptors are currently attracting considerable interest in view of their broad implication in the control of mood, motor behaviour and cognition, and their potential importance as targets for the improved treatment of anxiety, depression and other psychiatric and neurologic disorders (Stefanski and Goldberg 1997; Jenck et al. 1998a; Roth et al. 1998; Fox and Brotchie 1999; Meltzer 1999; Millan et al. 2000a). Of particular interest, the potent antagonist properties of the atypical antipsychotic, clozapine, at 5-HT$_{2C}$ receptors (Canton et al. 1994; Millan et al. 1998; Cussac et al. 2000a) may contribute to its low extrapyramidal potential, superior control of negative symptoms and improvement of mood (Roth and Meltzer 1995; Meltzer 1999; Reavill et al. 1999). Further, blockade of 5-HT$_{2C}$ receptors may participate in the therapeutic actions of several antidepressant agents, including nefazodone and mirtazapine (Millan et al. 2000a, 2000b). The role of closely related 5-HT$_{2B}$ receptors remains unclear inasmuch as their density is low in the CNS (Foguet et al. 1992; Kursar et al. 1992, 1994; Bonhaus et al. 1995; Duxon et al. 1997a; Hoyer and Martin 1997). However, they may be involved, together with 5-HT$_{2C}$ receptors, in the modulation of appetite (Kennett et al. 1997a). Further, activation of 5-HT$_{2B}$ receptors may, in contrast to 5-HT$_{2C}$ receptor, attenuate anxious states (Kennett et al. 1996a, 1998a;

Duxon et al. 1997b), and, more recently, agonist properties at 5-HT_{2B} receptors have been associated with cardiopathy (Fitzgerald et al. 2000).

In light of the above, much effort has been devoted to identification of ligands differentiating 5-HT_{2C} from 5-HT_{2B} receptors. In the characterization of such agents, behavioural paradigms of actions at 5-HT_{2C} sites have been extensively exploited. However, in vivo models of actions at central 5-HT_{2B} receptors remain to be established. Thus, studies of 5-HT_{2C} and 5-HT_{2B} receptor-coupled signal transduction are of particular importance.

In this regard, the 5-HT_2 receptor family is coupled via a heterotrimeric GTP binding protein, G_q, to phospholipase C (PLC; Conn et al. 1986; Sanders-Bush and Canton 1995; Gerhardt and Heerikhuizen 1997). Activation of PLC elevates cytosolic levels of inositol phosphates, and subsequently increases levels of intracellular calcium, two major parameters exploited for characterization of drug efficacy at 5-HT_2 receptor subtypes (Conn et al. 1986; Porter et al. 1999; Cussac et al. 2000b; Jerman et al. 2001). Although calcium levels show rapid kinetics and yield important information, they are subject to modulation by a plethora of intracellular signals. Further, alterations of calcium levels may reflect actions at 5-HT_{2C} receptors mediated via G-proteins other than G_q (notably G_i; Lucaites et al. 1996; Alberts et al. 1999). This can complicate interpretation of drug actions since, in line with the concept of agonist-directed trafficking, 5-HT_{2C} receptor ligands may differentially modify coupling to distinct effectors (Berg et al. 1998, 1999; Alberts et al. 1999; Miller et al. 2000). Correspondingly, determination of [^{3}H]IP levels offers a more direct and straightforward approach to evaluation of drug actions at 5-HT_{2C} receptors. In support of this contention, employing membrane-permeable peptides, 5-HT_{2C} receptors in choroid plexus were shown to couple to PLC via G_q independently of G_i (Chang et al. 2000).

Standard methodology for determination of [^{3}H]IP levels is, however, cumbersome. We recently developed, therefore, a complementary approach quantifying membrane-bound [^{3}H]phosphatidylinositols ([^{3}H]PI), the substrate of PLC (Cussac et al. 2000a, 2000b). In the present study, we exploited the rapidity of this novel technique for characterization of the actions of an extensive series of "classical" and novel ligands at h5-HT_{2C} receptors in comparison with h5-HT_{2B} receptors.

In these studies, we exploited the VSV (edited) isoform of 5-HT_{2C} receptors which is highly expressed both in rat and in human brain (Burns et al. 1997; Herrick-Davis et al. 1999; Niswender et al. 1999; Wang et al. 2000). This isoform does not, in contrast to the non-edited (INI) isoform, exhibit constitutive activity (Burns et al. 1997; Herrick-Davis et al. 1999). In addition to mRNA editing, a further important factor influencing drug actions and, in particular, coupling efficacy at 5-HT_{2C} receptors, is receptor density (Lucaites et al. 1996; Burns et al. 1997; Berg et al. 1998, 1999; Herrick-Davis et al. 1999; Wang et al. 2000). Thus, we investigated the influence of the high expression level of h5-HT_{2C} receptors in this cell line by quantifying receptor reserve employing irreversible inactivation with the alkylating agent, EEDQ (Atkinson and Minneman 1992; Adham et al. 1993; Cussac et al. 1999).

Materials and methods

Phospholipase C activity: [^{3}H]PI depletion assay. CHO cells stably expressing ~3 pmol/mg of h5-HT_{2B} receptors and ~20 pmol/mg of edited h5-HT_{2C} receptors (VSV isoform), were obtained from Euroscreen (Brussels, Belgium) and grown in adherent culture in 225-cm^2 flasks with UltraCHO medium (BioWhittaker, Verviers, Belgium) containing sodium pyruvate (1 mM), dialysed fœtal calf serum (0.1%) and geneticin (400 μg/ml). At confluence, cells were labelled with 2 μCi/ml of [^{3}H]myo-inositol (10–20 Ci/mmol; Amersham, Les Ulis, France) for 24 h in serum-free UltraCHO medium. Adherent cells were rinsed twice in Krebs-LiCl buffer (NaH_2PO_4 15.6 mM pH 7, NaCl 120 mM, KCl 4.8 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.2 mM, glucose 0.6% w/v, bovine serum albumin 0.04% w/v, LiCl 10 mM), scraped from the flask and washed once again by slow centrifugation. The cells were then resuspended in Krebs-LiCl and left to stand for 15 min at 37°C before use, or kept at –80°C in Krebs-LiCl/DMSO 10% until assay. The resulting suspension of cells loaded with [^{3}H]myo-inositol is referred to as 'loaded cells'.

For determination of [^{3}H]PI depletion, incubation of loaded cells was performed in 96-well plates (typically, one 225-cm^2 culture flask yielded sufficient cells for one 96-well plate containing 2.5×10^5 cells per well) with agonist ligands at 37°C for 20 min (h5-HT_{2C} receptors) and for 30 min (h5-HT_{2B} receptors) in 0.4 ml final volume. Antagonists were preincubated with the cells for 15 min before addition of the agonist. Assays were stopped by addition of 0.4 ml of methanol/HCl (88 ml of 100% methanol + 12 ml of HCl 1 N) and cells were placed at –20°C for at least 2 h to facilitate cell lysis. The 96-well plates were then sonicated for 2 min and membranes recovered using a 'Filtermate' harvester (Packard, Meriden, Conn., USA) by filtration through GF/B filters impregnated with 0.1% v/v polyethyleneimine followed by three washes with distilled, deionized water. Radioactivity was determined using a 'Top-Count' microplate scintillation counter (Packard, Meriden, Conn., USA). Conventional determination of PLC activity was performed with the same loaded cell preparations in the same buffer and incubation conditions as above, and soluble total [^{3}H]IP generation was determined using Dowex anion exchange chromatography as described by Dickenson and Hill (1996). Isotherms were analysed by non-linear regression, using the program 'PRISM' (Graphpad Software, San Diego, Calif., USA) to yield EC_{50} and IC_{50} (concentration of agonist and antagonist, respectively, that produced a half-maximal response) values. K_B values for inhibition of 5-HT (30 nM and 100 nM for h5-HT_{2B} and h5-HT_{2C}, respectively)-stimulated [^{3}H]PI depletion, were calculated according to Lazareno and Birdsall (1993).

$K_B=IC_{50}/(1+(Agonist/EC_{50}))$ where IC_{50}=Inhibitory $Concentration_{50}$ of the antagonist; Agonist=5-HT concentration; EC_{50}=Effective $Concentration_{50}$ of 5-HT alone.

Competition binding assays. Binding affinity at h5-HT_{2B} and h5-HT_{2C} receptors was determined essentially as described (Bonhaus et al. 1995) by competition binding with [^{3}H]mesulergine (1 nM; Amersham, Les Ulis, France) in a buffer containing HEPES (20 mM) pH 7.7, EDTA (2 mM) and ascorbic acid (0.1% w/v). Incubations lasted 2 h at 22°C and non-specific binding was defined by 5-HT (10 μM). Isotherms were analysed by non-linear regression to yield IC_{50} values. Inhibition constants (K_i values) were derived from IC_{50} values according to Lazareno and Birdsall (1993).

5-HT_{2C} receptor alkylation with EEDQ. Intact CHO-h5-HT_{2C} cells were treated in Krebs-LiCl with EEDQ at a final concentration of 100 μM for different incubation times at 37°C followed by application of 5-HT and quantification of [^{3}H]PI depletion as described above. For saturation experiments following EEDQ treatment, CHO-h5-HT_{2C} cells were washed twice by slow centrifugation in

an excess volume of Krebs-LiCl and [^{3}H]mesulergine binding performed on membrane preparation as described above. K_A values were determined by Furchgott analysis, as described by Atkinson and Minneman (1992) and Adham et al. (1993). Plots were derived of 1/[A] vs. 1/[A']; where [A] and [A'] are equiactive concentrations for stimulation of [^{3}H]PI depletion before and after receptor alkylation, respectively. K_A was calculated from K_A=(slope–1)/*y*-intercept. Percentage receptor occupancy (*O*) was calculated by $O=100\times L/(L+K_A)$; where *L* is the concentration of agonist. The curve is fitted by a rectangular hyperbola. All data are expressed as means ± SEM of ≥3 independent determinations performed in triplicate. Protein concentrations were determined colourimetrically using a bicinchonic acid assay kit (Sigma, Saint Quentin Fallavier, France).

Drugs. 5-HT was purchased from Sigma (Saint Quentin Fallavier, France). mCPP (1-(3-chlorophenyl)piperazine), DOI (1-2,5-dimethoxy-4-iodophenyl-2-aminopropane), lisuride, mesulergine and metergoline were purchased from Research Biochemicals International (Natick, Mass., USA), and RS102221 (*N*-[5-[5-(2,5-dioxospiro[imidazolidine-4,4'-piperidin]-1'-yl)pentanoyl]-2,4-dimethoxyphenyl]-4-(trifluoromethyl)benzenesulfonamide) from Tocris Cookson (Southampton, UK). Ro600175 (2(*S*)-1-(6-chloro-5-fluoro-1*H*-indol-1-yl)-2-propanamine fumarate), Ro600332 (1(*S*)-methyl-2-(4,4,7-trimethyl-1,4-dihydroindeno[1,2-b]pyrrol-1-yl)ethylamine fumarate), Ro600869 (*cis*-7-ethyl-6-hydroxy-8-methoxy-2-methyl-1,2,3,3a,4,9b-hexahydro-5*H*-benzo[e]isoindol-5-one), BW723C86 (1-methyl-2-[5-(2-thienylmethoxy)-1*H*-indole-3-yl]ethylamine hydrochloride), SB242084 (6-chloro-5-methyl-*N*-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl]indoline-1-carboxamide), ketanserin, mianserin and ritanserin were synthesized by G. Lavielle, Servier. MK212 (4-(6-chloro-2-pyrazinyl)piperazine), SB215505 (6-chloro-5-methyl-*N*-(5-quinolinyl)-2,3-dihydro-1*H*-indole-1-carboxamide), LY266097 (1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-b]indole hydrochloride) and S33526 ((6-chloro-2,3,4,9-tetrahydro-1*H*-β-carbolin-1-yl)-phenyl-acetic acid ethyl ester) were synthesized by S. Goldstein, J.G. Parmentier and G. Poissonnet, Servier. SB206553 (5-methyl-*N*-(3-pyridyl)-1,2,3,5-tetrahydrobenzo[1,2-b:4,5-b']dipyrrole-1-carboxamide) and SB204741 (*N*-(1-methyl-5-indolyl)-*N*'-(3-methylisothiazol-5-yl)urea) were synthesized by J.-L. Peglion, Servier.

Results

Kinetics of [^{3}H]PI depletion as compared to [^{3}H]IP generation

At CHO-h5-HT_{2B} cells, 5-HT elicited [^{3}H]PI depletion that was expressed linearly over 40 min before attaining a plateau (Fig. 1A). The depletion of membrane-bound [^{3}H]PI at CHO-h5-HT_{2C} cells in response to 5-HT reached a plateau at about 30 min (Fig. 1B). The soluble phase of these samples revealed a similar time-course for h5-HT_{2C} receptor-mediated [^{3}H]IP generation (Fig. 1C). In the absence of 5-HT, no [^{3}H]PI depletion was observed in either cell line. On the basis of these observations, we selected incubation times of 30 min and 20 min for determination of [^{3}H]PI depletion at CHO-h5-HT_{2B} and CHO-h5-HT_{2C} cells, respectively. Compared with a total level of approximately 40,000–45,000 dpm (Fig. 1), 5-HT reduced [^{3}H]PI levels by about 15,000–20,000 dpm in both cell lines, a decrease of ~40% in each case.

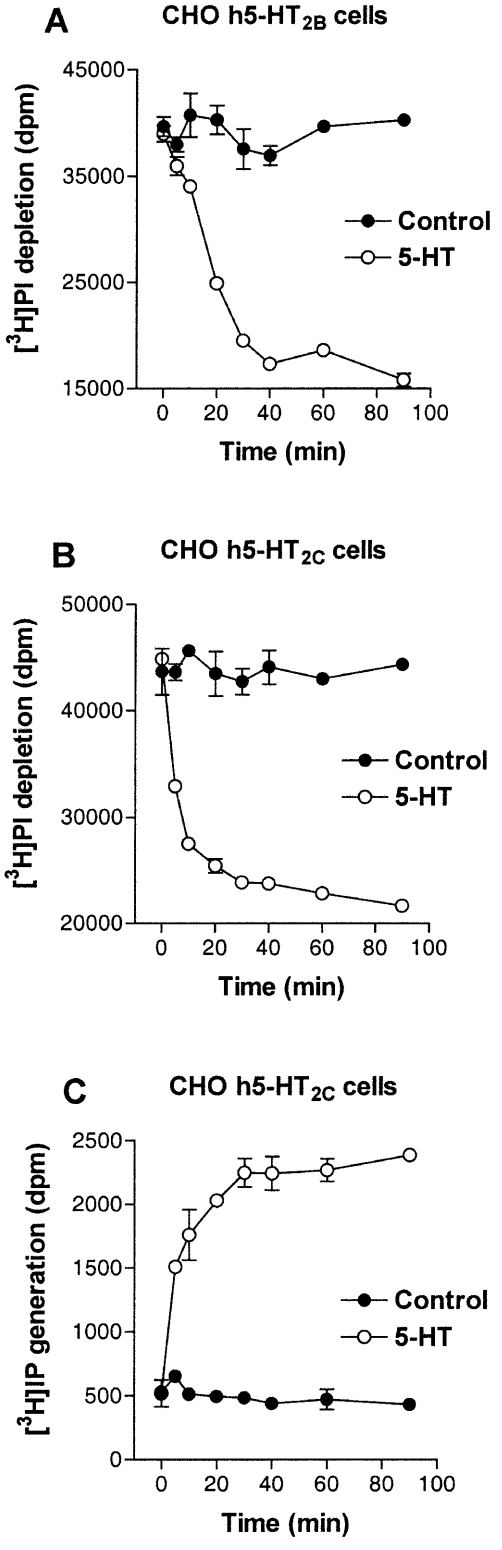

Fig. 1A–C Kinetics of phospholipase C activation at h5-HT_{2B} and h5-HT_{2C} receptors stably expressed in CHO cells. **A,B** Time-course of [^{3}H]phosphatidylinositol ([^{3}H]PI) depletion induced by 5-HT (10 μM) at h5-HT_{2B} and at h5-HT_{2C} cells, respectively. **C** Parallel time-course increase of 5-HT (10 μM)-stimulated soluble [^{3}H]inositol phosphate ([^{3}H]IP) generation at h5-HT_{2C} cells determined by anion exchange chromatography. Similar data were obtained on three occasions. A representative experiment performed in triplicate is shown

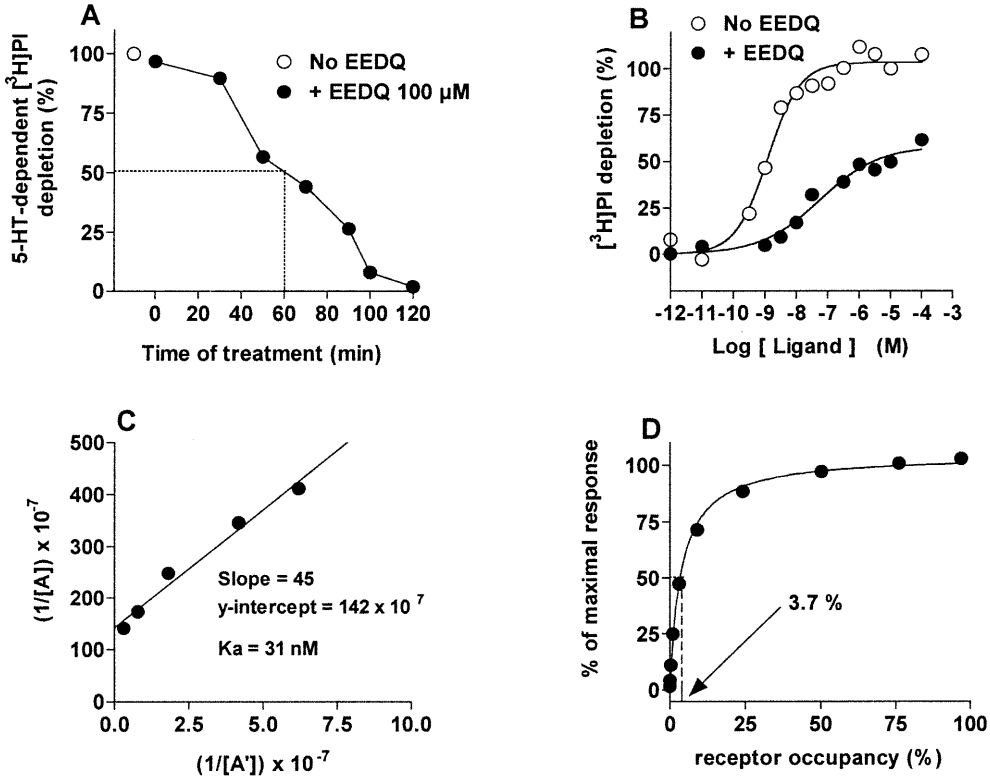

Fig. 2A–D Influence of h5-HT_{2C} receptor inactivation by EEDQ upon 5-HT-stimulated phospholipase C. **A** Time-dependent reduction of 5-HT (10 µM, 20 min)-stimulated [^{3}H]PI depletion at h5-HT_{2C} cells by pre-treatment with 100 µM EEDQ (0–120 min). Similar data were obtained on three occasions. A representative experiment performed in triplicate is shown. **B** Concentration-response of 5-HT-induced [^{3}H]PI depletion at control or EEDQ (100 µM, 60 min)-pre-treated CHO-h5-HT_{2C} cells. [^{3}H]PI depletion is expressed as a percentage of maximal stimulation with 10 µM 5-HT in absence of EEDQ. **C** Double-reciprocal plot of 1/[A] vs. 1/[A'] derived from B, where [A'] and [A] are equiactive concentrations of 5-HT for stimulation of [^{3}H]PI depletion with and without EEDQ treatment, respectively. **D** 5-HT occupancy/response relationship, derived using the value of K_A from C. The hyperbolic isotherm indicates the presence of receptor reserve. For B, C and D, *points* shown are the means of triplicate determinations from a representative experiment repeated on five occasions. The mean K_A value was 53.4±14.6 nM. Mean half-maximal response to 5-HT was observed at 4.6±0.9% occupation of h5-HT_{2C} binding sites

Influence of EEDQ treatment upon h5-HT_{2C} receptors

Addition of EEDQ (100 µM) to CHO-h5-HT_{2C} cells time-dependently reduced depletion of [^{3}H]PI by 5-HT (Fig. 2A) without altering basal [^{3}H]PI levels (not shown). Subsequent experiments were carried out with a treatment time of 60 min, which reduced the influence of 5-HT upon [^{3}H]PI levels by about 50% (Fig. 2A,B). Under these conditions, the pEC_{50} of 5-HT was reduced to 7.50±0.30 compared with 8.87±0.05 for control cells (Fig. 2B; Table 1). The K_A value determined by Furchgott analysis was 53.4± 14.6 nM (Fig. 2C), which corresponded approximately to the affinity of 5-HT at h5-HT_{2C} receptors determined in competition binding experiments (K_i=18 nM; Table 1). The presence of a high degree of receptor reserve was demonstrated in occupancy/response plots which yielded hyperbolic curves, with the mean half-maximal response to 5-HT being observed at 4.6±0.9% occupation of h5-HT_{2C} binding sites (Fig. 2D). EEDQ treatment (60 min at 100 µM) decreased the density of h5-HT_{2C} receptors by over ~97% as determined by [^{3}H]mesulergine saturation binding (Fig. 3; 21.6±3.4 pmol/mg without EEDQ compared with 0.6± 0.2 pmol/mg in the presence of EEDQ). Thus, although only about ~3% of h5-HT_{2C} receptors remained following EEDQ treatment, ~50% of the influence of 5-HT upon [^{3}H]PI levels was retained compared with non-EEDQ-treated cells.

Binding affinities of agonists and induction of [^{3}H]PI depletion at h5-HT_{2B} and h5-HT_{2C} receptors

Based on competition binding with [^{3}H]mesulergine, Ro600332 showed similar affinity at h5-HT_{2B} and h5-HT_{2C} receptors, whereas Ro600869 showed weak selectivity for h5-HT_{2C} receptors (Table 1; Fig. 4). All other agonists showed higher affinity at h5-HT_{2B} receptors. As derived from measures of [^{3}H]PI depletion, the majority of agonists presented functional selectivity for h5-HT_{2C} vs. h5-HT_{2B} receptors. Thus, mCPP, MK212 and Ro600332 induced [^{3}H]PI depletion with potencies ~27-, ~13- and ~12-fold greater than at h5-HT_{2B} receptors, respectively. However, mCPP and MK212 displayed full efficacy at h5-HT_{2C} receptors, whereas they were partial agonists at h5-HT_{2B} receptors. In contrast, 5-HT and DOI acted as full agonists at both sites and displayed mild functional selectivity for

Table 1 Actions of agonists at h5-HT_{2C} compared with h5-HT_{2B} receptors: [^{3}H]PI depletion (pEC_{50}; E_{max}) and binding affinities (pK_is). Agonist efficacies were determined by [^{3}H]PI depletion and are expressed relative to the effects of 5-HT at 10 μM (defined as 100%), which were evaluated in all experiments. Lisuride did not alter [^{3}H]PI depletion at h5-HT_{2B} cells (see Table 2). Affinities (pK_i values) were determined by competition binding experiments with [^{3}H]mesulergine. pEC_{50} and pK_i values are expressed as means ± SEM of 3–10 independent experiments performed in triplicate (*n.c.* not computable)

Ligand	h5-HT_{2C}			h5-HT_{2B}			Selectivity h5-HT_{2B}/h5-HT_{2C}	
	pEC_{50}	E_{max} (%)	pK_i	pEC_{50}	E_{max} (%)	pK_i	EC_{50} ratio	K_i ratio
5-HT	8.87±0.05	104.7±3.6	7.74±0.05	8.52±0.03	102.0± 6.0	8.55±0.04	2.3	0.15
mCPP	8.41±0.18	100.6±0.3	7.07±0.17	6.98±0.18	37.9± 9.1	7.92±0.05	26.9	0.14
MK212	7.69±0.16	103.0±3.6	6.05±0.11	6.58±0.21	75.3±10.3	6.82±0.07	12.9	0.17
Ro600332	8.34±0.14	90.9±3.6	7.86±0.03	7.26±0.10	91.0± 5.0	7.80±0.02	12.0	1.14
DOI	8.55±0.03	101.6±3.1	7.73±0.08	7.98±0.07	103.6± 6.4	8.15±0.12	3.7	0.38
BW723C86	8.14±0.08	103.9±4.3	6.94±0.11	8.10±0.10	107.5± 3.6	8.50±0.04	1.1	0.02
Ro600175	8.72±0.07	97.5±3.8	7.57±0.03	9.23±0.09	90.2± 3.4	9.26±0.03	0.3	0.02
Lisuride	8.11±0.10	74.5±5.6	8.19±0.03	n.c.[a]	0	8.86±0.06	n.c.[a]	0.21
Ro600869	7.67±0.16	37.9±5.4	8.56±0.06	n.c.[a]	0	8.10±0.05	n.c.[a]	2.9

[a]Antagonist; see Table 2

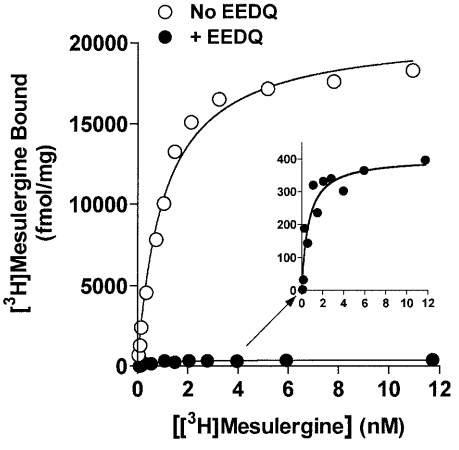

Fig. 3 Reduction of h5-HT_{2C} receptor density by EEDQ. Representative saturation binding isotherms of [^{3}H]mesulergine to CHO-h5-HT_{2C} membranes pre-treated with and without EEDQ (100 μM for 60 min). *Points* shown are means of triplicate determinations from a representative experiment repeated on three occasions. The mean B_{max} values were 21.6±3.4 pmol/mg without EEDQ and 0.6±0.2 pmol/mg in presence of EEDQ, respectively. K_D values were unchanged, ~0.8 nM (the *insert* represents the EEDQ treatment at a higher scale)

h5-HT_{2C} receptors of ~2.3- and ~3.7-fold, respectively. BW723C86 was equipotent at these sites. Ro600175 was the only ligand which exhibited weak functional selectivity for h5-HT_{2B} receptors (~3.3-fold). Lisuride and Ro600869 did not induce [^{3}H]PI depletion at h5-HT_{2B} receptors. Nevertheless, lisuride was a potent and relatively efficacious (75%) agonist at h5-HT_{2C} receptors, at which Ro600869 displayed low efficacy (38%). Interestingly, for h5-HT_{2C} receptors, pEC_{50} values (except for partial agonists) were higher than pK_i values.

Binding affinities of antagonists and their influence upon 5-HT-induced [^{3}H]PI depletion at h5-HT_{2B} and h5-HT_{2C} receptors

There was a marked correlation between antagonist potency (pK_b) and binding affinity (pK_i) for both h5-HT_{2C} (r=0.95, P<0.001) and h5-HT_{2B} sites (r=0.92, P<0.001; Table 2; Fig. 5). All antagonists tested, at both 5-HT_{2B} and 5-HT_{2C} receptors, completely abolished 5-HT-induced PLC activation, demonstrating that endogenous 5-HT_{1B} receptors expressed in CHO cells (Giles et al. 1996) are not involved in PLC regulation. RS102221 displayed pronounced affinity for h5-HT_{2C} receptors and the highest degree of selectivity vs. h5-HT_{2B} receptors (>100-fold). A pronounced preference for h5-HT_{2C} receptors was similarly displayed by SB242084. Although it was more potent than RS102221 at h5-HT_{2C} receptors, it was somewhat less selective (Table 2). Ketanserin showed a modest preference for h5-HT_{2C} vs. h5-HT_{2B} sites as concerns both its binding affinity and its antagonist potency. On the other hand, mesulergine, metergoline, SB206553, mianserin and ritanserin failed to clearly discriminate h5-HT_{2C} and h5-HT_{2B} receptors, based on both their pK_i and pK_b values. While SB215505 behaved as a modestly preferential antagonist at h5-HT_{2B} vs. h5-HT_{2C} sites, this difference was considerably more pronounced for several other agents. Thus, both SB204741 and S33526 manifested a high degree of selectivity for h5-HT_{2B} sites (>30-fold), although the former was less potent at this site. LY266097 was the most potent and selective antagonist at h5-HT_{2B} vs. h5-HT_{2C} sites of all ligands evaluated. Interestingly, lisuride and Ro600869 behaved as h5-HT_{2B} antagonists although, as described above, they possess agonist properties at h5-HT_{2C} receptors. None of the antagonists provoked [^{3}H]PI depletion at h5-HT_{2B} or h5-HT_{2C} sites upon application alone (not shown). Several antagonists, including the h5-HT_{2C}-selective ligands, SB242084 and RS102221, and the h5-HT_{2B}-selective ligands, SB204741, S33526 and LY266097, showed low binding affinities at h5-HT_{2A} receptors la-

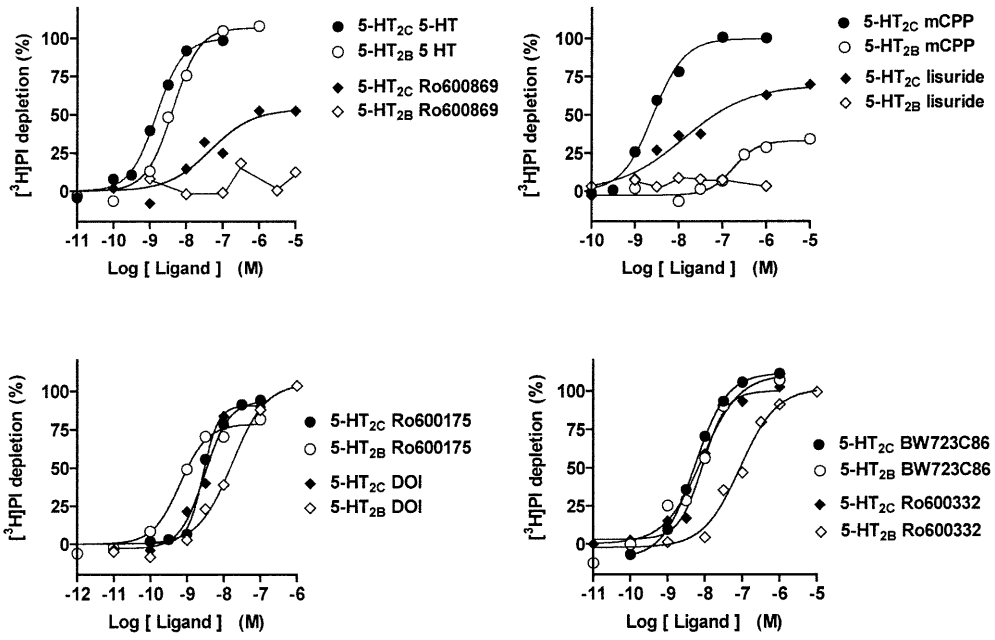

Fig. 4 Concentration-response curves of agonist actions at h5-HT_{2C} and h5-HT_{2B} receptor-mediated phospholipase C activation. [^{3}H]PI depletion is expressed as a percentage of maximal stimulation evoked by 10 μM 5-HT. *Points* shown are means of triplicate determinations from representative experiments repeated on at least 3–10 occasions. E_{max} and pEC_{50} data from these experiments are shown in Table 1

Table 2 Actions of antagonists at h5-HT_{2C} compared with h5-HT_{2B} receptors: inhibition of 5-HT-induced [^{3}H]PI depletion (pK_B) and binding affinities. Antagonist potencies (pK_B values) were calculated from IC_{50} values for inhibition of 5-HT-stimulated [^{3}H]PI depletion (30 nM for h5-HT_{2B} and 100 nM or 30 nM for h5-HT_{2C}). Affinities (pK_i values) were determined by competition binding experiments with [^{3}H]mesulergine. pK_B and pK_i values are expressed as means ± SEM of 3–6 independent experiments performed in triplicate (*n.c.* not computable)

Ligand	h5-HT_{2C}		h5-HT_{2B}		Selectivity h5-HT_{2B}/h5-HT_{2C}	
	pK_B	pK_i	pK_B	pK_i	K_B ratio	K_i ratio
RS102221	8.47±0.10	8.83±0.04	5.99±0.07	6.63±0.05	302	158
SB242084	9.53±0.14	9.32±0.06	7.70±0.03	7.34±0.07	67	95
Ketanserin	7.17±0.13	7.77±0.03	6.36±0.05	6.63±0.06	6.4	14
Mesulergine	9.14±0.08	8.95±0.06	8.58±0.13	8.71±0.02	3.6	1.73
Metergoline	9.30±0.06	9.33±0.07	9.03±0.20	9.15±0.01	1.9	1.51
SB206553	8.35±0.09	8.50±0.13	8.44±0.06	8.26±0.17	0.81	1.73
Mianserin	8.14±0.12	8.82±0.04	8.68±0.09	8.26±0.05	0.28	3.63
Ritanserin	8.84±0.05	8.79±0.10	9.49±0.13	8.90±0.13	0.22	0.77
SB215505	8.47±0.06	7.95±0.06	9.16±0.15	8.83±0.09	0.20	0.13
SB204741	5.95±0.05	5.67±0.11	7.53±0.11	7.29±0.04	0.026	0.023
S33526	6.34±0.05	6.34±0.04	8.11±0.17	8.39±0.07	0.016	0.009
LY266097	7.03±0.09	7.17±0.19	9.68±0.05	9.70±0.07	0.002	0.002
Lisuride	n.c.[a]	8.19±0.03	8.94±0.06	8.86±0.06	n.c.[a]	0.21
Ro600869	n.c.[a]	8.56±0.06	7.02±0.10	8.10±0.05	n.c.[a]	2.88

[a]Agonist; see Table 1

belled by [^{3}H]ketanserin; selectivity of at least 100-fold vs. h5-HT_{2C} and h5-HT_{2B}, respectively (not shown).

Discussion

Technical aspects

Determination of membrane-bound [^{3}H]phosphatidylinositol ([^{3}H]PI) depletion, as compared to generation of cytosolic [^{3}H]inositol phosphates ([^{3}H]IP), offers a rapid technique for evaluation of drug activity at h5-HT_{2C}, h5-HT_{2B} receptors and, in principle, other sites coupled to PLC. As discussed elsewhere (Cussac et al. 2000b), addition of LiCl prevents the recycling of inositol phosphates (Berridge 1993), permitting measurement of the influence of ligands upon membrane-bound [^{3}H]PI. [^{3}H]PI consists, thus, of the substrate of PLC, phosphatidylinositol-4,5 bisphosphate, as well as its precursors, phosphatidylinositol-4-phosphate and phosphatidylinositol (Berridge 1993). Emphasizing the pharmacological specificity of this novel approach, measures of [^{3}H]PI generated results identical to a conventional [^{3}H]IP measurement as concerns actions of 5-HT and the antagonist, SB242084, at h5-HT_{2C} receptors (Cussac

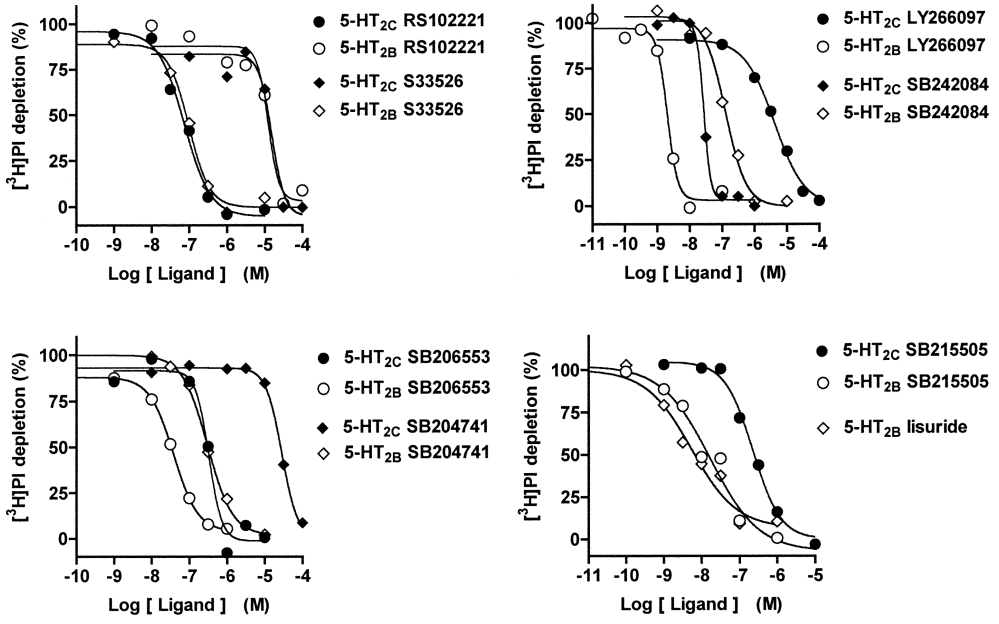

Fig. 5 Concentration-response curves of antagonism actions at h5-HT_{2C} and h5-HT_{2B} receptor-mediated phospholipase C activation. [^{3}H]PI depletion is expressed as a percentage of maximal stimulation elicited by 10 μM 5-HT. *Points* shown are means of triplicate determinations from representative experiments repeated on at least 3–5 occasions. pK_B data from these experiments are shown in Table 2

et al. 2000b). Moreover, for diverse antagonists examined herein, pK_b values for blockade of 5-HT-induced [^{3}H]PI depletion correlated tightly with pK_i values at h5-HT_{2C} and h5-HT_{2B} receptors. The present observation that, at h5-HT_{2C} receptors, the time-course of [^{3}H]PI depletion by 5-HT parallels that for generation of [^{3}H]IP as monitored by anion exchange chromatography under the same assay conditions (Dickenson and Hill 1996) further underpins the validity of the present approach.

Pre-loaded CHO-h5-HT_{2B} and CHO-h5-HT_{2C} cells can be stored frozen (see Materials and methods) without affecting drug actions (not shown). Together with the advantage of a simple filtration step for determination of [^{3}H]PI, this allows for use of the present protocol in the rapid characterization of large numbers of ligands, which would be prohibitively time-consuming employing a standard [^{3}H]IP procedure.

h5-HT_{2C} receptors alkylation with EEDQ reveals receptor reserve

With the exception of lisuride and Ro600869, all agonists at h5-HT_{2C} receptors exhibited high K_i/EC_{50} ratios (7–40) and "full" efficacy relative to 5-HT, suggesting that there exists a substantial receptor reserve (Adham et al. 1993; Brink et al. 2000; Table 1). h5-HT_{2C} receptors have been shown to be sensitive to EEDQ treatment (Ni et al. 1997). Indeed, the presence of spare h5-HT_{2C} receptors was revealed upon their inactivation with EEDQ, which reduced their levels to 3% of control values, yet diminished the 5-HT-induced PLC response by 50%. The presence of pronounced receptor reserve was confirmed by Furchgott analysis which yielded a K_A/EC_{50} ratio of 40 for 5-HT, and a hyperbolic occupancy/response plot with a half-maximal response at 4.6%, similar to the 3% value mentioned above. These data indicate that the present cellular expression system (CHO-h5-HT_{2C}) exhibits very high sensitivity to agonist stimulation. In comparison, in a receptor inactivation study employing a different cell line (NIH3T3) expressing 5 pmol/mg of edited VSV or non-edited INI h5-HT_{2C} isoforms, no receptor reserve was detected for PLC activation (Burns et al. 1997). These observations indicate that careful characterisation of signal transduction parameters in each expression system is important for appropriate interpretation of functional responses (Burstein et al. 1997). It is not known what receptor expression levels are physiological. Saturation binding studies using brain tissue homogenates yield measures of receptor number for a heterogeneous mix of cell types. Hence 5-HT_{2C}-receptor expression levels per 5-HT_{2C}-expressing neuron, for instance, can only be roughly estimated. In the case of rat choroid plexus neurons, 5-HT_{2C} receptor expression is reported to be in the pmol/mg range (Yagaloff and Hartig 1985), thus potentially implicating receptor reserve, as described herein.

Inasmuch as the primary focus of the present study was h5-HT_{2C} receptors, the possible presence of spare receptors at h5-HT_{2B} receptors was not evaluated by receptor inactivation herein. In fact the close correspondence of K_i and EC_{50} values for all agonists except mCPP (see below) suggests that this is unlikely to be pronounced. Nevertheless, this aspect, and the high expression level of h5-HT_{2C} vs. h5-HT_{2B} receptors should be borne in mind in the following comparison of drug actions at h5-HT_{2C} vs. h5-HT_{2B} sites.

Agonist actions at h5-HT_{2B} and h5-HT_{2C} receptors

The high level of receptor reserve in the present CHO-h5-HT_{2C} cell line has important implications for interpretation of agonist properties. Indeed, the potencies and effi-

cacies of several agonists herein were more pronounced compared with previous studies of CHO-expressed h5-HT_{2C} (VSV) receptors (Porter et al. 1999) or SH-SY5Y-expressed h5-HT_{2C} (INI) receptors (Jerman et al. 2001). Thus, while these authors did not directly determine receptor reserve, the expression levels of h5-HT_{2C} receptors in their systems was low (0.2 pmol/mg for VSV and 0.9–6.3 pmol/mg for INI, compared with 20 pmol/mg herein). Notably, these authors did not detect agonist properties of lisuride at h5-HT_{2C} receptors, whereas it displayed substantial efficacy in the present study, in analogy to reports of a highly expressed VNV isoform of h5-HT_{2C} receptors (Egan et al. 1998; Fitzgerald et al. 1999). The novel, selective 5-HT_2 ligand, Ro600869, mimicked the actions of lisuride at h5-HT_{2C} receptors, exhibiting modest partial agonist activity, although it was previously reported to act as an antagonist at h5-HT_{2C} receptors (Boes et al. 1997, 1998). In view of the potential utility of Ro600869 as a selective 5-HT_{2C} ligand and its structural novelty, it would be of interest to extend these observations to other cellular paradigms. Taken together, the present data demonstrate that: (1) a high degree of receptor reserve in the present CHO-h5-HT_{2C} cell line can reveal partial agonist properties of low efficacy ligands and (2) lisuride and Ro600869 display antagonist properties at h5-HT_{2B} receptors.

Inasmuch as mCPP is extensively exploited as an experimental and clinical probe for evaluation of 5-HT_{2C} receptor function, its actions are of particular importance. Indeed, agonist actions of mCPP have been characterised (Kennett and Curzon 1988; Newton et al. 1996; Porter et al. 1999; Miller et al. 2000; Jerman et al. 2001). As concerns actions at recombinant h5-HT_{2B} and h5-HT_{2C} receptors, Thomas et al. (1996) documented antagonist properties at h5-HT_{2B} receptors expressed in HEK293 cells. However, these findings may be characteristic of that expression system inasmuch as, in the present CHO cell line (which expresses a similar level of 5-HT_{2B} receptors), partial agonist properties of mCPP were observed. Moreover, despite a sixfold lower expression of h5-HT_{2B} receptors in another CHO cell line (0.6 pmol/mg compared with 3 pmol/mg herein), partial agonist actions of mCPP were also characterised by an increase in cytosolic Ca^{2+} concentrations (Porter et al. 1999). Further, agonist properties of mCPP at native, rat 5-HT_{2B} receptors have been documented (Baxter et al. 1995).

In contrast to mCPP, BW723C86 was proposed as a tool for exploration of the role of 5-HT_{2B} vs. 5-HT_{2C} receptors based on its selectivity in binding studies (Baxter 1996; Kennett et al. 1996a, 1997a). Indeed, employing a measure of cytosolic Ca^{2+} concentrations, BW723C86 displayed ~100-fold functional selectivity for h5-HT_{2B} sites (Porter et al. 1999; Jerman et al. 2001). In contrast, the present binding studies revealed higher binding affinity of BW723C86 at h5-HT_{2B} sites, but showed comparable potency and efficacy at h5-HT_{2B} and h5-HT_{2C} sites in eliciting [^{3}H]PI depletion. Although the present functional selectivities should be interpreted in the context of the marked h5-HT_{2C} receptor reserve, these data are consistent with the action of BW723C86 in activating PLC at h5-HT_{2C} expressed in HEK293 cells (Thomas et al. 1996; Wood et al. 1997).

Ro600175 was originally suggested to be a selective agonist for 5-HT_{2C} receptors, principally based upon binding studies (Boes et al. 1997). Subsequently, in vivo studies demonstrated that 5-HT_{2C} receptors are indeed involved in its functional actions (Jenck et al. 1998b; Dekeyne et al. 1999; Gobert et al. 2000). However, Ro600175 also behaves as a full agonist at native, rat 5-HT_{2B} receptors (Martin et al. 1998) and it was more potent in elevating cytosolic Ca^{2+} concentrations at CHO-expressed h5-HT_{2B} than h5-HT_{2C} receptors (Porter et al. 1999). The present study corroborates these observations in demonstrating that Ro600175 more potently enhances [^{3}H]PI depletion at h5-HT_{2B} vs. h5-HT_{2C} receptors. In fact, Ro600175 may currently be the most selective h5-HT_{2B} agonist available.

In contrast, Ro600332, while exhibiting equilibrated binding affinity and full agonist properties at both h5-HT_{2B} and h5-HT_{2C} receptors, manifested 12-fold greater potency in enhancing [^{3}H]PI depletion at the latter. In the light of the present results and in vivo studies demonstrating robust agonist actions at 5-HT_{2C} receptors in rodents (Jenck et al. 1998a, 1998b; Martin et al. 1998), Ro600032 may, thus, be a more appropriate ligand than Ro600175 for exploration of its functional significance. Nevertheless, as for all the agonists evaluated herein, caution needs to be exercised in the interpretation of their functional selectivity due to the presence of spare receptors in this CHO-h5-HT_{2C} cell line.

Antagonist actions at h5-HT_{2B} and h5-HT_{2C} receptors

The latter point emphasises the importance of studies with antagonists for differentiating actions at 5-HT_{2C} vs. 5-HT_{2B} receptors. In fact, the rapidity of the present technique permitted the – to our knowledge – most extensive comparative study to date of the functional properties of diverse antagonists at h5-HT_{2C} vs. h5-HT_{2B} receptors. In view of the high degree of correlation between pK_B and pK_i values, comparison of ratios (5-HT_{2B}/5-HT_{2C}) in functional tests as well as binding affinities permitted the identification of three groups of antagonists in accordance with their selectivity (Table 2). The most h5-HT_{2C}-selective ligand found herein, RS102221, was also described as a selective 5-HT_{2C} receptor antagonist based on a microphysiometry assay which yielded a pA_2 of 8.1 (Bonhaus et al. 1997). This value is close to the pK_B of 8.4 (present study), confirming its potent and preferential blockade of h5-HT_{2C} compared with h5-HT_{2B} receptors. Previous studies demonstrated antagonist properties of RS102221 at central 5-HT_{2C} sites under certain conditions (Bonhaus et al. 1997; Sevoz-Couche et al. 2000), and the present data support its utility as a tool for differentiation of actions mediated at 5-HT_{2C} vs. 5-HT_{2B} receptors. This is also the case for SB242084 (Kennett et al. 1997b), which exhibited pronounced selectivity for h5-HT_{2C} receptors, underpinning previous observations of its potent – and competitive – antagonist properties at h5-HT_{2C} receptors in vitro (Cussac et al. 2000b) and in vivo (Dekeyne et al. 1999).

In contrast, the structurally related analogues, SB206553 and SB215505, were potent antagonists at both sites (Kennett et al. 1996b, 1998b; Dekeyne et al. 1999). Although SB215505 was described as a preferential h5-HT_{2B} ligand, the poor selectivity for this site observed herein (<tenfold) encourages caution in the interpretation of its actions. Amongst other weakly discriminative h5-HT_{2C} vs. h5-HT_{2B} receptor ligands, mesulergine, metergoline, mianserin and ritanserin were potent antagonists at both sites, while ketanserin displayed lower potency and a mild preference for h5-HT_{2C} sites (Baxter et al. 1995).

As concerns selective h5-HT_{2B} antagonists, SB204741 was previously described as a selective ligand based on its actions in functional tests in vitro (Baxter et al. 1995; Thomas et al. 1996) and in vivo (Dekeyne et al. 1999; Glusa and Pertz 2000; Gobert et al. 2000). This preference was substantiated herein, although SB204741 showed only modest potency at h5-HT_{2B} sites. On the other hand, LY266097 and the novel ligand, S33526, displayed both high potency and high selectivity for h5-HT_{2B} vs. h5-HT_{2C} sites. These ligands appear, therefore, of particular use for separating actions mediated via h5-HT_{2B} rather than h5-HT_{2C} sites.

Conclusions

Employing an innovative and rapid technique for quantification of the influence of drugs upon PLC activity, the present study comprises the, to date, most comprehensive characterization of novel (and putatively selective) agonists and antagonists at h5-HT_{2C} receptors. The high receptor reserve at h5-HT_{2C} receptors encourages caution as concerns comparisons with h5-HT_{2B} receptors, but the present data indicate that, in contrast to antagonists, selective agonists at these sites remain to be identified. In view of the pleiotropic coupling of 5-HT_{2C} receptors to diverse G-proteins and intracellular cascades (Gerhardt and Heerikhuizen 1997), considerable research is still required to understand mechanisms of intracellular coupling at 5-HT_{2C} receptors, as well as their pathophysiological significance. In this light, the present data provide an extensive framework for further exploration of drug actions at 5-HT_{2C} receptors.

References

Adham N, Ellerbrock B, Hartig P, Weinshank RL, Branchek T (1993) Receptor reserve masks partial agonist activity of drugs in a cloned rat 5-hydroxytryptamine1B receptor expression system. Mol Pharmacol 43:427–433

Alberts GL, Pregenzer JF, Im WB, Zaworski PG, Gill GS (1999) Agonist-induced GTPγ^{35}S binding mediated by human 5-HT_{2C} receptors expressed in human embryonic kidney 293 cells. Eur J Pharmacol 383:311–319

Atkinson BN, Minneman KP (1992) Occupancy-response relationships for β- and α_2-adrenergic receptors exerting opposing effects on cAMP production. Receptor 2:195–206

Baxter GS (1996) Novel discriminatory ligands for 5-HT_{2B} receptors. Behav Brain Res 73:149–152

Baxter GS, Ellis ES, Forbes IT, Jones GE, Kennett GA, Murphy OE, Tilford N (1995) SB 204741: a potent and selective antagonist at 5-HT_{2B} receptors. Br J Pharmacol 114:157P

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. Mol Pharmacol 54:94–104

Berg KA, Stout BD, Cropper JD, Maayani S, Clarke WP (1999) Novel actions of inverse agonists on 5-HT_{2C} receptor systems. Mol Pharmacol 55:863–872

Berridge MJ (1993) Inositol triphosphate and calcium signalling. Nature 361:315–325

Boes M, Martin JR, Moreau JL, Sleight AJ, Wichmann J, Widmer U (1997) Novel agonists of 5-HT_{2C} receptors. Synthesis and biological evaluation of substituted 2-(indol-1-yl)-1-methylethylamines and 2-(indeno(12-b)pyrrol-1-yl)-1-methylethylamines. Improved therapeutics for obsessive compulsive disorder. J Med Chem 40:2762–2769

Boes M, Stadler H, Wichmann J, Jenck F, Martin JR, Moreau JL, Sleight AJ (1998) Syntheses of *O*-methylasparvenone-derived serotonin-receptor antagonists. Helv Chim Acta 81:525–538

Bonhaus DW, Bach C, DeSouza A, Salazar FHR, Matsuoka BD, Zuppan P, Chan HW, Eglen R (1995) The pharmacology and distribution of human 5-hydroxytryptamine$_{2B}$ (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and 5-HT_{2C} receptors. Br J Pharmacol 115:622–628

Bonhaus DW, Weinhardt KK, Taylor M, Desouza A, McNeeley PM, Szczepanski K, Fontana DJ, Trinh J, Rocha CL, Dawson MW, Flippin LA, Eglen RM (1997) RS-102221: a novel high affinity and selective 5-HT_{2C} receptor antagonist. Neuropharmacology 36:621–629

Brink CB, Wade SM, Neubig RR (2000) Agonist-directed trafficking of porcine α_{2A}-adrenergic receptor signaling in Chinese hamster ovary cells: l-isoproterenol selectively activates Gs. J Pharmacol Exp Ther 294:539–547

Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emerson RB (1997) Regulation of serotonin$_{2C}$ receptor G-protein coupling by RNA editing. Nature 387:303–308

Burstein ES, Spalding TA, Brann MR (1997) Pharmacology of muscarinic receptor subtypes constitutively activated by G protein. Mol Pharmacol 51:312–319

Canton H, Verrièle L, Millan MJ (1994) Competitive antagonism of serotonin (5-HT)$_{2C}$ and 5-HT_{2A} receptor-mediated phosphoinositide (PI) turnover by clozapine in the rat: a comparison to other antipsychotics. Neurosci Lett 181:65–68

Chang M, Zhang L, Tam JP, Sanders-Bush E (2000) Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides. Endogenous 5-$HT_{(2C)}$ receptors in choroid plexus epithelial cells. J Biol Chem 275:7021–7029

Conn PJ, Sanders-Bush E, Hoffman B, Hartig PR (1986) A unique serotonin receptor in choriod plexus is linked to phosphatidylinositol turnover. Proc Natl Acad Sci USA 83:4086–4088

Cussac D, Newman-Tancredi A, Pasteau V, Millan MJ (1999) Human dopamine D_3 receptors mediate mitogen-activated protein kinase activation via a phosphatidylinositol 3-kinase and an atypical protein kinase C-dependent mechanism. Mol Pharmacol 56:1025–1030

Cussac D, Newman-Tancredi A, Nicolas J-P, Boutin JA, Millan MJ (2000a) Antagonist properties of the novel antipsychotic S16924 at cloned human serotonin 5-HT_{2C} receptors: a parallel phosphatidylinositol and calcium accumulation comparison with clozapine and haloperidol. Naunyn-Schmiedeberg's Arch Pharmacol 361:549–554

Cussac D, Newman-Tancredi A, Quentric Y, Millan MJ (2000b) An innovative method for rapid characterisation of phospholipase C activity: SB242084 competitively antagonises 5-HT_{2C} receptor-mediated [^{3}H]phosphatidylinositol depletion. Naunyn-Schmiedeberg's Arch Pharmacol 361:221–223

Dekeyne A, Girardon S, Millan MJ (1999) Discriminative stimulus properties of the novel serotonin (5-HT)$_{2C}$ receptor agonist RO 60-0175: a pharmacological analysis. Neuropharmacology 38: 415–423

Dickenson JM, Hill SJ (1996) Synergistic interactions between human transfected adenosine A1 receptors and endogenous cholecystokinin receptors in CHO cells. Eur J Pharmacol 302:141–151

Duxon MS, Flanigan TP, Reavley AC, Baxter GS, Blackburn TP, Fone KCF (1997a) Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. Neuroscience 76:323–329

Duxon MS, Kennett GA, Lightowler S, Blackburn TP, Fone KCF (1997b) Activation of 5-HT_{2B} receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. Neuropharmacology 36:601–608

Egan CT, Herrick-Davis K, Miller K, Glennon RA, Teitler M (1998) Agonist activity of LSD and lisuride at cloned 5-HT_{2A} and 5-HT_{2C} receptors. Psychopharmacology (Berl) 136:409–414

Fitzgerald LW, Deborah S, Conklin DS, Krause CM, Marshall AP, Patterson JP, Tran DP, Iyer G, Kostich WA, Largent BL, Hartig PR (1999) High-affinity agonist binding correlates with efficacy (intrinsic activity) at the human serotonin 5-HT_{2A} and 5-HT_{2C} receptors: evidence favoring the ternary complex and two-state models of agonist action. J Neurochem 72:2127–2134

Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, Sun J-H, Link JR, Abbaszade I, Hollis JM, Largent BL, Hartig PR, Hollis GF, Meunier PC, Robichaud AJ, Robertson DW (2000) Possible role of valvular serotonin 5-HT_{2B} receptors in the cardiopathy associated with fenfluramine. Mol Pharmacol 57:75–81

Foguet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman HO, Stühmer W, Lübbert H (1992) Cloning and functional characterization of the rat stomach fundus serotonin receptor. EMBO J 11:3481–3487

Fox SH, Brotchie JM (1999) A role for 5-HT_{2C} receptor antagonists in the treatment of Parkinson's disease? Drug News Perspect 12:477–483

Gerhardt CC, Heerikhuizen H (1997) Functional characteristics of heterologously expressed 5-HT receptors. Eur J Pharmacol 334: 1–23

Giles H, Lansdell SJ, Bolofo ML, Wilson HL, Martin GR (1996) Characterization of a 5-HT_{1B} receptor on CHO cells: functional responses in the absence of radioligand binding. Br J Pharmacol 117:1119–1126

Glusa E, Pertz HH (2000) Further evidence that 5-HT-induced relaxation of pig pulmonary artery is mediated by endothelial 5-HT_{2B} receptors. Br J Pharmacol 130:692–698

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP, Cistarelli L, Melon C, Millan MJ (2000) Serotonin2C receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic but not serotonergic pathways: a combined dialysis and electrophysiological analysis in the rat. Synapse 36:205–221

Herrick-Davis K, Grinde E, Niswender CM (1999) Serotonin 5-HT_{2C} receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. J Neurochem 73:1711–1717

Hoyer D, Martin G (1997) 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology 36:419–428

Jenck F, Bös M, Wichmann J, Stadler H, Martin JR, Moreau JL (1998a) The role of 5-HT_{2C} receptors in affective disorders. Exp Opin Invest Drugs 7:1587–1599

Jenck F, Moreau JL, Berendsen HH, Boes M, Broekkamp CL, Martin JR, Wichmann J, Van Delft AM (1998b) Antiaversive effects of 5-HT_{2C} receptor agonists and fluoxetine in a model of panic-like anxiety in rats. Eur Neuropsychopharmacol 8:161–168

Jerman JC, Brough SJ, Gager T, Wood M, Coldwell MC, Smart D, Middlemiss DN (2001) Pharmacological characterisation of human 5-HT_2 receptor subtypes. Eur J Pharmacol 414:23–30

Kennett GA, Curzon G (1988) Evidence that mCPP may have behavioural effects mediated by central 5-HT_{1C} receptors. Br J Pharmacol 94:137–147

Kennett GA, Bright F, Trail B, Baxter GS, Blackburn TP (1996a) Effects of the 5-HT_{2B} receptor agonist BW 723C86 on three rat models of anxiety. Br J Pharmacol 117:1443–1448

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP (1996b) In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434

Kennett GA, Ainsworth K, Trail B, Blackburn TP (1997a) BW 723C86, a 5-HT_{2B} receptor agonist, causes hyperphagia and reduced grooming in rats. Neuropharmacology 36:233–239

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Midlemiss DN, Blackburn TP (1997b) SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. Neuropharmacology 36:609–620

Kennett GA, Trail B, Bright F (1998a) Anxiolytic-like actions of BW 723C86 in the rat Vogel conflict test are 5-HT_{2B} receptor mediated. Neuropharmacology 37:1603–1610

Kennett GA, Trail B, Riley G, Bickerdike MJ, Ranson J, Forbes IT, Price GW, Baxter GS, Hunter AJ (1998b) SB-215505, a selective 5-HT_{2B} receptor antagonist in rats. Am Sci Neurosci Abstr 24:1371

Kursar JD, Nelson DL, Wainscott DB, Cohen ML, Baez M (1992) Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine$_{2F}$) from rat stomach fundus. Mol Pharmacol 42:549–557

Kursar JD, Nelson DL, Wainscott DB, Baez M (1994) Molecular cloning, functional expression, and mRNA tissue distribution of a human 5-hydroxytryptamine$_{2B}$ receptor. Mol Pharmacol 46: 227–234

Lazareno S, Birdsall NJM (1993) Estimation of antagonist K_B from inhibition curves in functional experiments: alternatives to the Cheng-Prusoff equation. Trends Pharmacol Sci 14:237–239

Lucaites VL, Nelson DL, Wainscott DB, Baez M (1996) Receptor subtype and density determine the coupling repertoire of the 5-HT_2 receptor sub-family. Life Sci 59:1081–1095

Martin JR, Bös M, Jenck F, Moreau J-L, Sleight AJ, Wichmann J, Andrews JS, Berendsen HHG, Broekkamp CLE, Ruigt GSF, Köhler C, Delft AML van (1998) 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. J Pharmacol Exp Ther 286:913–924

Meltzer HY (1999) The role of serotonin in antipsychotic drug action. Neuropsychopharmacology 21:106–115

Millan MJ, Gobert A, Newman-Tancredi A, Audinot V, Lejeune F, Rivet JM, Cussac D, Nicolas JP, Muller O, Lavielle G (1998) S 16924 ((+)-2-{1-[2-(23-dihydro-benzo [14]dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl}-1-(4-fluoro-phenyl) ethanone), a novel potential antipsychotic with marked serotonin (5-$HT)_{1A}$ agonist properties. I. Receptorial and neurochemical profile in comparison with clozapine and haloperidol. J Pharmacol Exp Ther 286:1341–1355

Millan MJ, Lejeune F, Gobert A (2000a) Reciprocal autoreceptor and heteroreceptor control of serotonergic dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. J Psychopharmacol (Oxf) 14:114–138

Millan MJ, Gobert A, Rivet JM, Adhumeau-Auclair A, Cussac D, Newman-Tancredi A, Dekeyne A, Nicolas JP, Lejeune F (2000b) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic but not serotonergic transmission by blockade of α_2-adrenergic receptors and serotonin$_{2C}$ receptors: a comparison with citalopram. Eur J Neurosci 12:1079–1095

Miller KJ, Robichau AJ, Largent BL (2000) 5-HT_{2C} receptor-mediated activation of IP_3 and cGMP in HEK293 cells: evidence for agonist directed trafficking. Am Sci Neurosci Abstr 26:117

Newton RA, Phipps SL, Flanigan TP, Newberry NR, Carey JE, Kumar C, McDonald B, Chen C, Elliott JM (1996) Characterization of human 5-hydroxytryptamine$_{2A}$ and 5-hydroxytryptamine$_{2C}$ receptors expressed in the human neuroblastoma cell line SH-SY5Y: comparative stimulation by hallucinogenic drug. J Neurochem 67:2521–2531

Ni YG, Camacho N, Miledi R (1997) Irreversible antagonism of $5HT_{2C}$ receptors by *N*-ethoxycarbonyl-2-ethoxy-12-dihydroquinoline (EEDQ). Proc Natl Acad Sci USA 94:2715–2718

Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E (1999) RNA editing of the human serotonin 5-hydroxytryptamine$_{2C}$ receptor silences constitutive activity. J Biol Chem 274:9472–9478

Porter RHP, Benwell KR, Lamb H, Malcom CS, Allen NH, Revell DF, Adams DR, Sheardown MJ (1999) Functional characterisation of agonists at recombinant human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in CHO-K1 cells. Br J Pharmacol 128:13–20

Reavill C, Kettle A, Holland V, Riley G, Blackburn TP (1999) Attenuation of haloperidol-induced catalepsy by a 5-HT_{2C} receptor antagonist. Br J Pharmacol 126:572–574

Roth BL, Meltzer HY (1995) The role of serotonin in schizophrenia. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation in progress. Raven, New York, pp 1215–1228

Roth BL, Willins DL, Kristiansen K, Kroeze WK (1998) 5-hydroxytryptamine$_2$-family receptors (5-hydroxytryptamine$_{2A}$, 5-hydroxytryptamine$_{2B}$, 5-hydroxytryptamine$_{2C}$): where structure meets function. Pharmacol Ther 79:231–257

Sanders-Bush E, Canton H (1995) Serotonin receptors: signal transduction pathways. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation in progress. Raven, New York, pp 1215–1228

Sevoz-Couche C, Spyer KM, Jordan D (2000) Inhibition of rat nucleus tractus solitarius neurones by activation of 5-HT_{2C} receptors. Neuroreport 11:1785–1790

Stefanski R, Goldberg SR (1997) Serotonin 5-HT_2 receptor antagonists: potential in the treatment of psychiatric disorders CNS. Drugs 7:388–409

Thomas DR, Gager TL, Holland V, Brown AM, Wood MD (1996) m-chlorophenylpiperazine (mCPP) is an antagonist at the cloned human 5-HT_{2B} receptor. Neuroreport 7:1457–1460

Wang Q, O'Brian PJ, Chen C-X, Cho D-S, Murray JM, Nishikura K (2000) Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin$_{2C}$ receptors. J Neurochem 74:1290–1300

Wood MD, Thomas DR, Gager TL, Browne MJ, Carey JE, Chambers JK, Gale DG, Hastwell C, Muir AI, Pritchard TR, Robinson JH, Wilson S, Brown A (1997) Pharmacological characterisation of the human 5-HT_{2B} and 5-HT_{2C} receptors in functional studies. Pharmacol Rev Commun 9:259–268

Yagaloff KA, Hartig PR (1985) 125I-lysergic acid diethylamide binds to a novel serotonergic site on rat choroid plexus epithelial cells. J Neurosci 5:3178–3183