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Ca^{2+} Responses in Chinese Hamster Ovary-K1 Cells Demonstrate an Atypical Pattern of Ligand-Induced 5-HT_{1A} Receptor Activation

PETRUS J. PAUWELS and FRANCIS C. COLPAERT

Centre de Recherche Pierre Fabre, Department of Cellular and Molecular Biology, Castres, France

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ABSTRACT

Little experimental evidence has been reported for diverse signaling via 5-hydroxytryptamine (5-$HT)_{1A}$ receptors despite the fact that agonists seem to be more efficacious at dorsal raphe somatodendritic 5-HT_{1A} autoreceptors than at postsynaptic 5-HT_{1A} receptors. The present study investigated Ca^{2+} responses in Chinese hamster ovary (CHO)-K1 cells expressing a human 5-HT_{1A} receptor by 5-HT, prototypical 5-HT_{1A} agonists, *N*-(3-chloro-4-fluorobenzoyl)-4-fluoro-4-[(5-methyl-6-; methylaminopyridin-2-yl)-methylaminomethyl]-piperidine (F 14679), and especially *N*-(3-chloro-4-fluorobenzoyl)-4-fluoro-4-[(5-methylpyridin-2-yl)-; methylaminomethyl]piperidine (F 13640) as representative ligands of a new chemical class (methylamino-pyridine) that combines both high efficacy and selectivity for 5-HT_{1A} receptors. 5-HT (pEC_{50} = 6.70 ± 0.02) induced a pertussis toxin-sensitive, transient high-magnitude Ca^{2+} response. High-magnitude Ca^{2+} responses (E_{max}, percentage versus 5-HT) were also found with F 13640 (107 ± 4), 5-carboxamidotryptamine (100 ± 3), and F 14679 (87 ± 3). In contrast, the prototypical 5-HT_{1A} receptor agonists buspirone, ipsapirone, and 8-(hydroxy-2-(di-*n*-propylamino)tetralin, and also flesinoxan and eptapirone, were virtually inactive (≤5). This atypical pattern of 5-HT_{1A} receptor activation contrasts with the broad spectrum of the ligands' partial agonist properties as observed by measuring guanosine 5′-*O* -(3-[^{35} S]thio)triphosphate ([^{35}S]GTPγS) binding responses with membranes of either CHO-K1 or C6-glial cells stably expressing a human 5-HT_{1A} receptor. Remarkably, differences between ligands that seem small in the [^{35}S]GTPγS binding assay translate into huge differences in the magnitude of Ca^{2+} responses. Therefore, some of these 5-HT_{1A} ligands (i.e., F 13640) may in a selective way induce responses that may be not at all be achieved with other ligands (i.e., buspirone). In conclusion, the pharmacology of 5-HT_{1A} receptor ligands seems to be codetermined by the effector pathway.

Traditional receptor theory provides for the activation of multiple and distinct cellular effectors by G protein-coupled receptor agonists but predicts that the relative degree of activation (relative efficacy) of each effector pathway by an agonist must be the same (Ariëns, 1964; Kenakin, 1997). Recent data (Berg et al., 1998) indicate that certain agonists may have the capacity to selectively activate a subset of the multiple signal transduction pathways that are coupled to a single receptor subtype. Partial agonists would preferentially induce or select receptor conformational states that favor activation of one effector pathway over another. Computational simulations of ligand interactions with the 5-HT_{2A} receptor (Zhang and Weinstein, 1993) and experimental evidence with the 5-HT_{2A} receptor (Shapiro et al., 2000) and β_2-adrenoceptor (Gether et al., 1995; Krumins and Barber, 1997) support the hypothesis of agonist-selective receptor states, although there is some debate as to the number of possible receptor conformational states (Leff et al., 1997). The most likely mechanism by which agonists may preferentially direct a receptor stimulus to different effector mechanisms is via differential G protein coupling. Although the influence of G protein subtype on ligand efficacy has been suggested for α_{2A}-adrenoceptors (Yang and Lanier, 1999), there are to date few experimental data to support this hypothesis. It has been suggested that each agonist may induce a different receptor conformation or set of conformations (Watson et al., 2000). The issue regarding G protein-coupled receptors is not so much whether each ligand produces a distinct active state, but rather, whether the differences in

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine, serotonin; 8-OH-DPAT, 8-(hydroxy-2-(di-*n*-propylamino)tetralin; 5-CT, 5-carboxamidotryptamine; F 14679, *N*-(3-chloro-4-fluorobenzoyl)-4-fluoro-4-[(5-methyl-6-; methylaminopyridin-2-yl)-methylaminomethyl]-piperidine; F 13640, *N*-(3-chloro-4-fluorobenzoyl)-4-fluoro-4-[(5-methylpyridin-2-yl)-; methylaminomethyl]piperidine; CHO, Chinese hamster ovary; GTPγS, 5′-*O* -(3-[^{35} S]thio) triphosphate; WAY 100635, *N*-[2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide; SB 224289 1′-methyl-5-(2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-; carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine].

conformation for different agonists are sufficiently large at the far end of the receptor molecule that interacts with the G protein for the G protein to know which agonist is bound (Colquhoun, 1998). Little experimental evidence has been reported for diverse signaling via 5-HT_{1A} receptors despite the fact that agonists seem more efficacious at dorsal raphe somatodendritic 5-HT_{1A} autoreceptors than at postsynaptic receptors (for instance, in hippocampus; Meller et al., 1990, 2000). Gettys et al. (1994) suggested agonist-dependent coupling of the human 5-HT_{1A} receptor to different Gα_i proteins in recombinant CHO cells.

In the present study, we measured 5-HT ligand-mediated Ca^{2+} responses in CHO-K1 cells transfected with a recombinant human 5-HT_{1A} receptor. Although 5-HT_{1A} receptors can activate phospholipase C (Raymond et al., 1999), this effect is considered to be host-specific and not as efficient as coupling to the inhibition of adenylate cyclase. Several 5-HT ligands were investigated here: prototypical 5-HT_{1A} agonists [i.e., 8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT), buspirone, and ipsapirone], flesinoxan and eptapirone, 5-carboxamidotryptamine (5-CT) as a high-efficacy but nonselective 5-HT_{1A} ligand (Pauwels et al., 1997), *N*-(3-chloro-4-fluorobenzoyl)-4-fluoro-4-[(5-methyl-6-methylaminopyridin-2-yl)-methylaminomethyl]-piperidine (F 14679; Koek et al., 2001) and, especially *N*-(3-chloro-4-fluorobenzoyl)-4-fluoro-4-[(5-methylpyridin-2-yl)-methylaminome-thyl]piperidine (F 13640; Colpaert et al., 2002) as representative ligands of a new chemical class (5-methyl-pyridin-2ylmethyl amine derivatives) that combines both high-efficacy and selectivity for 5-HT_{1A} receptors. We found that highly efficacious 5-HT_{1A} receptor agonists could induce a transient, high-magnitude Ca^{2+} response in CHO-K1 cells; the amplitude of the ligands' Ca^{2+} responses was similar to that of 5-HT. Prototypical 5-HT_{1A} ligands (i.e., 8-OH-DPAT), commonly considered as partial agonists (Koek et al., 2001), were no longer capable to induce a significant Ca^{2+} response. The Ca^{2+} response data were compared with 5′-*O*-(3-[^{35}S]thiotriphosphate ([^{35}S]GTPγS) binding responses as a sensitive read-out for a wide spectrum of 5-HT_{1A} receptor ligand activities. The Ca^{2+} data are discussed with regard to agonist-selective 5-HT_{1A} receptor signaling.

Materials and Methods

Transfection of CHO-K1 Cells with Human Recombinant 5-HT_{1A} Receptor. Subconfluent CHO-K1 cells were transiently transfected with 10 μg of a wild-type human 5-HT_{1A} receptor plasmid containing the entire receptor coding sequence (R.C.2.1.5HT.01.A, GenBank accession no. X57829) by electroporation (Wurch et al., 1996). Cells were plated in 96-well plates with 0.2 ml of nutrient mixture Ham's F12 plus 10% heat-inactivated fetal calf serum and 1% dimethyl sulfoxide at about 60,000 cells/well. Cells were assayed for intracellular Ca^{2+} responses between 24 and 48 h after transfection.

Measurement of Intracellular Ca^{2+} Responses. Intracellular Ca^{2+} responses were measured upon 1-h loading with 2 μM Fluo-3 fluorescent calcium indicator dye as described previously (Pauwels et al., 2000). Either 5-HT or other 5-HT ligands were assayed between 1 nM and 10 μM for their Ca^{2+} responses. Data for Ca^{2+} responses were obtained in arbitrary fluorescent units and were not translated into Ca^{2+} concentrations. Fluorescent readings were made every 2 s for the first 3 min by using a fluorometric imaging plate reader (Molecular Devices Corp., Sunnyvale, CA). E_{max} values were defined as the ligand's maximal high-magnitude Ca^{2+} response in percentage versus that obtained with 10 μM 5-HT. pEC_{50} values correspond to a ligand concentration at which 50% of its own maximal high-magnitude Ca^{2+} response was measured. Antagonists were preincubated for 10 min before 5-HT and the Ca^{2+} response recorded for a further 3 min. Antagonist potency (pIC_{50} value) was defined as the concentration required to antagonize 50% of the Ca^{2+} response induced by 1 μM 5-HT. This was calculated as the difference in surface area between the 5-HT and ligand conditions.

[^{35}S]GTPγS Binding Responses. [^{35}S]GTPγS binding responses were determined on membrane preparations of CHO-K1 or C6-glial cells stably transfected with a recombinant human 5-HT_{1A} receptor as described previously (Pauwels et al., 1997). Incubation mixtures were prepared in glass tubes and consisted of 0.4 ml of membrane preparation (20–40 μg of protein) and 0.05 ml of either 5-HT or another 5-HT ligand in the presence of 30 μM GDP. After an incubation of 30 min at 25°C, 0.05 ml [^{35}S]GTPγS (0.5 nM) was added for an additional period of 30 min. The reactions were stopped by adding 3 ml of ice-cold 20 mM HEPES (pH 7.4) containing 3 mM $MgCl_2$ and rapid filtration as described previously (Pauwels et al., 1997). Maximal stimulation of [^{35}S]GTPγS binding was defined in the presence of 10 μM 5-HT. E_{max} values were expressed as a percentage of the response obtained with 10 μM 5-HT.

Protein Content. Protein levels were estimated with the dye-binding assay using the Bio-Rad kit (Bradford, 1976). Bovine serum albumin was used as a standard.

Statistical Analysis. Statistical analysis was performed on E_{max} values using a two-tailed Student's *t* test.

Materials. CHO-K1 and C6-glial cells were obtained from American Type Culture Collection (Rockville, MD). Cell culture media, fetal calf serum, culture plates, and *Bordetella pertussis* toxin were obtained from Gibco Biocult (Paisley, UK). [^{35}S]GTPγS (1100 Ci/mmol) was obtained from Amersham Biosciences Inc. (Les Ulis, France). Fluo-3 was obtained from Molecular Probes (Eugene, OR). 5-HT and probenicid acid were from Sigma-Aldrich (St. Louis, MO). 5-CT, 8-OH-DPAT, and buspirone were obtained from Sigma/RBI (Natick, MA). Flesinoxan, ipsapirone, *N*-[2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635), 1′-methyl-5-(2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB 224289), F 13640, and F 14679 were synthesized at the Centre de Recherche Pierre Fabre (Castres, France). Stock solutions of compounds were prepared at 10^{-3} M. Serial dilutions were made in incubation buffer.

Results

In contrast to nontransfected CHO-K1 cells, 5-HT (10 μM) induced a Ca^{2+} response in CHO-K1 cells transiently transfected with a human 5-HT_{1A} receptor (Fig. 1). The amplitude of this Ca^{2+} response (3432 ± 244 arbitrary fluorescence units) was smaller than that obtained at the endogenously expressed bradykinin receptor upon stimulation with 10 μM bradykinin (6519 ± 113 arbitrary fluorescence units). The kinetic properties of these two Ca^{2+} responses differed. The onset time of maximal activation was faster with bradykinin (16 ± 1 s) than with 5-HT (30 ± 1 s), whereas the attenuation of the Ca^{2+} signal upon maximal activation was slightly greater for bradykinin than for 5-HT. In contrast to the bradykinin-mediated Ca^{2+} response, pertussis toxin-treatment prevented the 5-HT-mediated Ca^{2+} response (Fig. 1C).

Results on antagonism of the 5-HT-induced Ca^{2+} response are reported in Fig. 2. WAY 100635, methiothepin, and buspirone fully antagonized the 5-HT response. The 5-HT_{1B} receptor antagonist SB 224289 (1 μM) exerted little, if any, effect (Fig. 2B). Prototypical 5-HT_{1A} receptor agonists (i.e., buspirone, 8-OH-DPAT, and ipsapirone) did not induce a

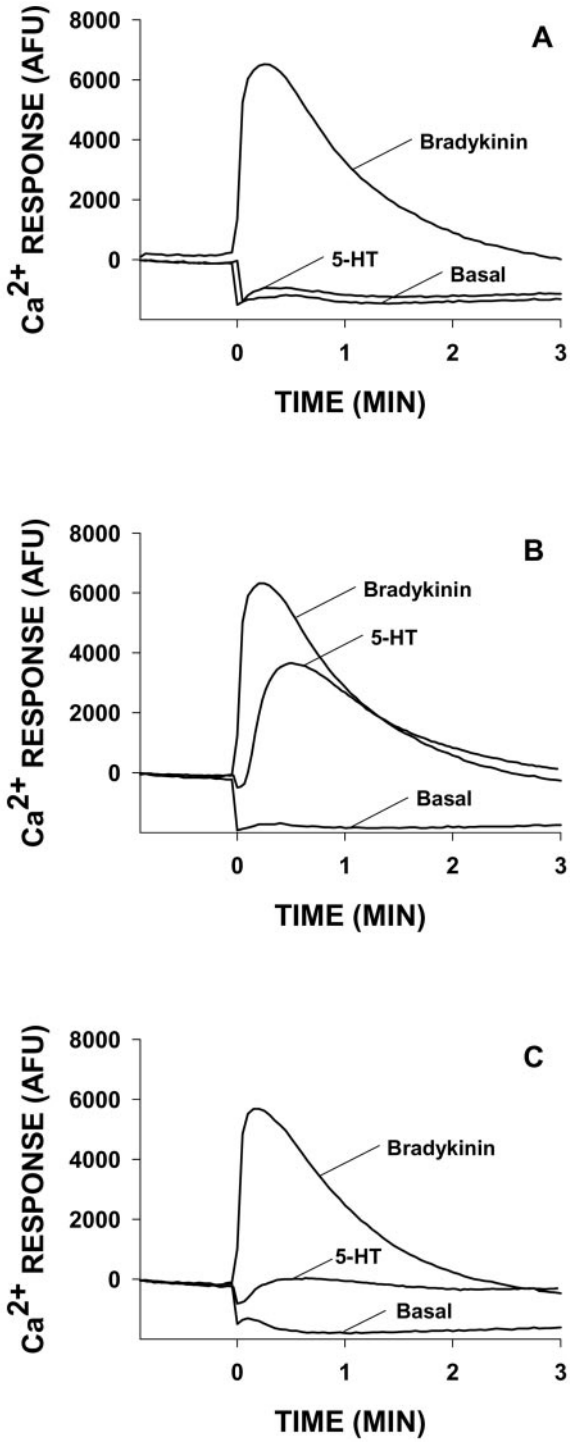

Fig. 1. 5-HT and bradykinin-induced Ca^{2+} responses in nontransfected and 5-HT_{1A} receptor-transfected CHO-K1 cells. CHO-K1 cells were transiently transfected with the human 5-HT_{1A} receptor (10 μg of plasmid). Basal, bradykinin- (10 μM), and 5-HT (10 μM)-induced Ca^{2+} responses were measured every 2 s for 3 min. Curves illustrate a representative experiment. A, nontransfected CHO-K1 cells; B, 5-HT_{1A} receptor-transfected CHO-K1 cells (0.83 ± 0.18 pmol/mg protein binding sites on intact cells as estimated by [^{3}H] WAY 100635, 1 nM); and C, 5-HT_{1A} receptor-transfected CHO-K1 cells upon overnight treatment with pertussis toxin (20 ng/ml).

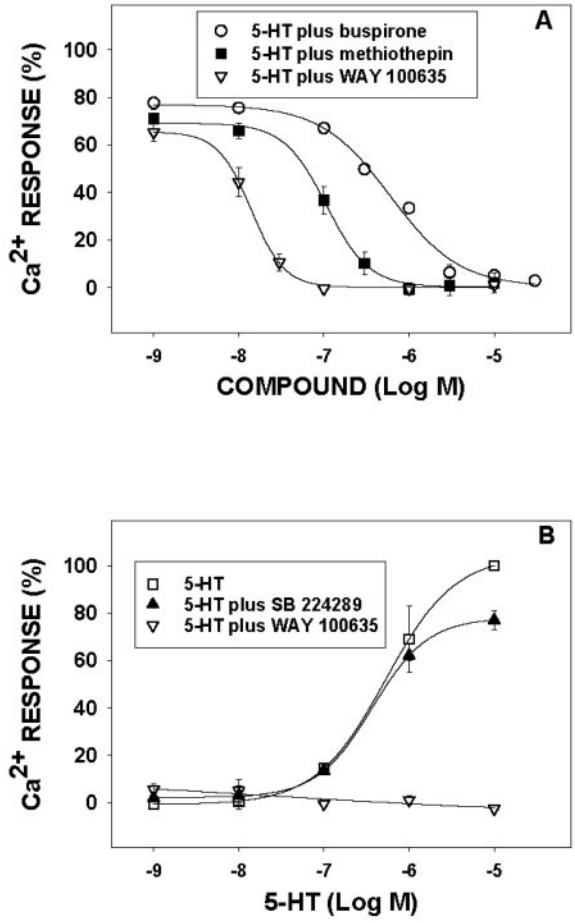

Fig. 2. Antagonism of the 5-HT-induced Ca^{2+} response by buspirone, methiothepin, and WAY 100635, but not SB 224289, in CHO-K1 cells transiently transfected with a 5-HT_{1A} receptor. CHO-K1 cells were transiently transfected with a human 5-HT_{1A} receptor plasmid and assayed for a 5-HT (1 μM)-induced Ca^{2+} response. A, WAY 100635, methiothepin, and buspirone were given at indicated concentrations 10 min before 5-HT (1 μM). Ca^{2+} data are expressed in percentage of the maximal Ca^{2+} response as obtained with 10 μM 5-HT. Curves were constructed using mean values ± S.E.M. obtained in five to seven independent transfection experiments. pIC_{50} values are 7.96 ± 0.13 (WAY 100635), 7.16 ± 0.08 (methiothepin), and 6.34 ± 0.09 (buspirone). B, WAY 100635 (1 μM) and SB 224289 (1 μM) were given at 10 min before 5-HT was assayed at the indicated concentrations. Ca^{2+} data are expressed in percentage of the maximal Ca^{2+} response as obtained with 10 μM 5-HT. Curves were constructed using mean values ± S.E.M. (5-HT alone) and mean values (5-HT + WAY 100635 and 5-HT + SB 224289) obtained in five and two independent transfection experiments.

significant Ca^{2+} response despite the fact that their maximal [^{35}S]GTPγS binding responses as obtained with membranes of 5-HT_{1A} receptor-transfected CHO-K1 cells were between 56 and 79% compared with 5-HT (Table 1); similar findings were obtained with flesinoxan and eptapirone. Even a comparison with [^{35}S]GTPγS binding data as obtained with membranes of C6-glial cells stably transfected with a 5-HT_{1A} receptor (Pauwels et al., 1997) indicated lower but still significant activity for each of these compounds (Table 1). Similarly, these compounds behaved as either partial (i.e., buspirone) or efficacious agonists (i.e., eptapirone) by monitoring their cAMP responses in transfected HeLa cells (Table 1). In contrast, F 13640, 5-CT, and F 14679 induced large Ca^{2+} responses with a magnitude that was similar or apparently identical to that of 5-HT (Table 1). F 13640 and F14679, in contrast to 5-CT, are highly selective for the 5-HT_{1A} receptor. Table 2 compares binding affinities between 5-HT_{1A} and two receptor subtypes (5-HT_{1B} and 5-HT_{2A}) that have been postulated to be endogenously expressed in CHO-K1 cells and

TABLE 1

E_{max} and pEC_{50} values of 5-HT ligands Ca^{2+}, [^{35}S]GTPγS binding, and cAMP responses by recombinant human 5-HT_{1A} receptor

Ca^{2+} responses were determined in 5-HT_{1A} receptor-transfected CHO-K1 cells and expressed as a percentage of the Ca^{2+} response induced by 10 μM 5-HT. [^{35}S]GTPγS binding responses were determined on membranes of stably 5-HT_{1A} receptor transfected CHO-K1 or C6-glial cells in the presence of 30 μM GDP and expressed versus 10 μM 5-HT-mediated response. Mean values ± S.E.M. are given for a minimum of three independent experiments.

	Ca^{2+} Response/CHO-K1		[^{35}S]GTPγS Binding Response/ CHO-K1		[^{35}S]GTPγS Binding Response/C6-glial		cAMP Response/HeLa	
	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}
5-HT	100	6.70 ± 0.02	100	7.74 ± 0.07	100[c]	6.71[c]	100[e]	7.67[e]
F 13640	107 ± 4	7.37 ± 0.02	99 ± 5	7.98 ± 0.12	75 ± 2	7.63 ± 0.13		
5-CT	100 ± 3	7.28 ± 0.19	102 ± 4	8.43 ± 0.15	85[c]	7.98[c]		
F 14679	87[a] ± 3	7.33 ± 0.16	93 ± 6	9.21 ± 0.15	61[d]	8.31[d]	110[d]	8.70[d]
Flesinoxan	5 ± 1		81[b] ± 2	8.02 ± 0.16	45[c]	7.48[c]	100[e]	8.48[e]
Buspirone	4 ± 1		61[b] ± 7	7.08 ± 0.04	22[c]	6.40[c]	47[d]	6.56[d]
8-OH-DPAT	3 ± 1		79[b] ± 3	7.89 ± 0.08	41[c]	7.41[c]	82[d]	7.65[d]
Ipsapirone	2 ± 2		56[b] ± 8	7.69 ± 0.13	26[c]	7.30[c]	58[e]	7.66[e]
Eptapirone	−1 ± 3		79[b] ± 4	7.46 ± 0.05	38[d]	6.68[d]	100[d]	7.11[d]

[a] E_{max} value (relative to 5-HT = 100%) of F 14679 in Ca^{2+} response is significantly different ($P < 0.05$) from that by 5-CT.
[b] E_{max} values (relative to 5-HT = 100%) for [^{35}S]GTPγS binding responses on CHO-K1 membranes are significantly different ($P < 0.001$) from the corresponding Ca^{2+} responses. E_{max} values (relative to 5-HT = 100%) as obtained with the Ca^{2+} response differed significantly from the corresponding E_{max} values as estimated with the [^{35}S]GTPγS binding response in C6-glial membranes for each investigated ligand with the exception of 5-HT.
[c] Pauwels et al. (1997).
[d] Koek et al. (2001).
[e] Pauwels et al. (1993).

TABLE 2

Binding affinities of F 13640 and F 14679 for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptor subtypes

Binding affinities for 5-HT_{1B} and 5-HT_{2A} receptor subtypes were obtained as described previously (Koek et al., 1998).

	Binding Affinities		
	5-HT_{1A} (pK_i)	5-HT_{1B} (pIC_{50})	5-HT_{2A} (pIC_{50})
F 13640	9.49[a]	<5	<5
F 14679	10.23[b]	5.12	<5

[a] Colpaert et al. (2002).
[b] Koek et al. (2001).

C6-glial cells (Giles et al., 1996; Pauwels et al., 1996). F 13640 and F 14679 do not significantly bind to 5-HT_{1B} and 5-HT_{2A} receptors. Moreover, we could not measure a 5-HT-mediated Ca^{2+} response in nontransfected CHO-K1 cells (Fig. 1A). Therefore, the observed Ca^{2+} responses with F 13640 and F 14679 can be considered to be due to activation by 5-HT_{1A} receptors. A comparison with the [^{35}S]GTPγS binding responses in transfected C6-glial membranes (Fig. 3; Table 1) indicated a significantly lower maximal [^{35}S]GTPγS binding response for F 13640 with an unmodified potency ($p > 0.05$), and a significantly lower maximal [^{35}S]GTPγS binding response for 5-CT and F 14679 accompanied with an enhanced potency ($p \leq 0.01$). A comparison with the [^{35}S]GTPγS binding responses in transfected CHO-K1 membranes did not reveal attenuated maximal responses, whereas pEC_{50} values were significantly enhanced ($p \leq 0.005$), although less for F 13640 (4 times) than 5-HT (11 times), 5-CT (14 times), and F 14679 (76 times) (Table 1). Figure 4 illustrates the atypical pattern of 5-HT ligand-mediated maximal Ca^{2+} responses in CHO-K1 cells versus their spectrum of maximal [^{35}S]GTPγS binding responses for

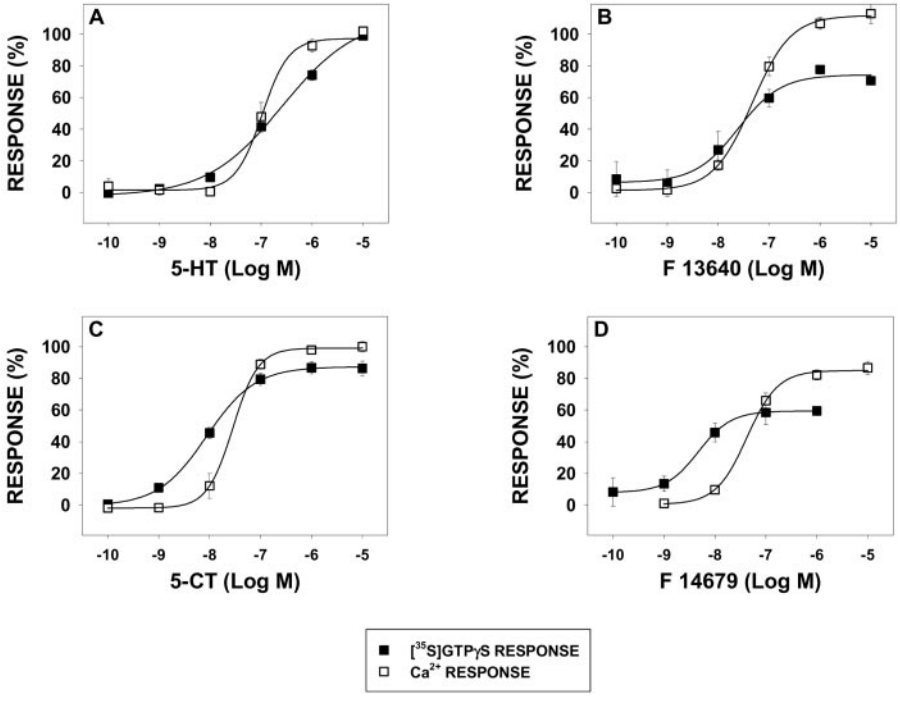

Fig. 3. Concentration-dependent Ca^{2+} and [^{35}S]GTPγS binding responses of 5-HT ligands in either CHO-K1 or C6-glial cells transfected with a 5-HT_{1A} receptor. Ca^{2+} responses in transiently 5-HT_{1A} receptor-transfected CHO-K1 cells were expressed as a percentage of the maximal Ca^{2+} response induced by 10 μM 5-HT. [^{35}S]GTPγS binding responses at membranes of stably transfected C6-glial cells were measured with 30 μM GDP. Data are expressed as a percentage of the 10 μM 5-HT-induced [^{35}S]GTPγS binding response. Curves were constructed using mean values ± S.E.M. for a minimum of three independent transfection experiments. The 5-CT-mediated [^{35}S]GTPγS binding response data are from Pauwels et al. (1997). Mean E_{max} and pEC_{50} values are summarized in Table 1.

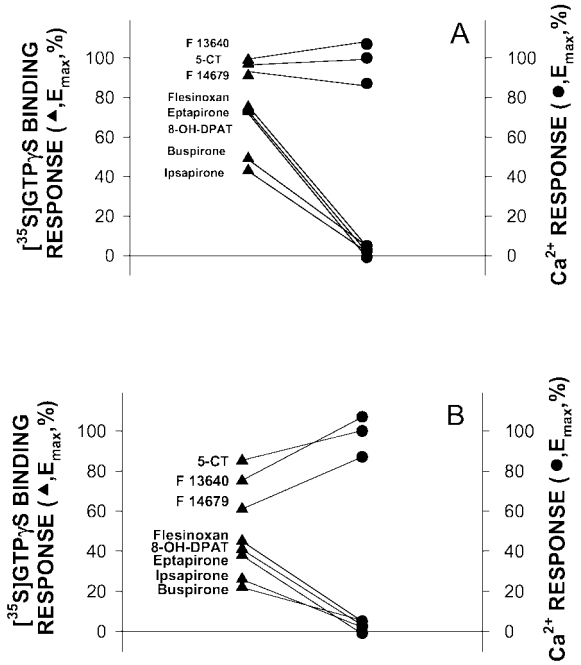

Fig. 4. E_{max} values of 5-HT ligand's Ca^{2+} and [^{35}S]GTPγS binding responses. E_{max} values for Ca^{2+} and [^{35}S]GTPγS binding responses were obtained with 5-HT_{1A} receptor transfected CHO-K1 cells and membranes of stably 5-HT_{1A} receptor-transfected CHO-K1 (A) or C6-glial cells (B), respectively (Table 1).

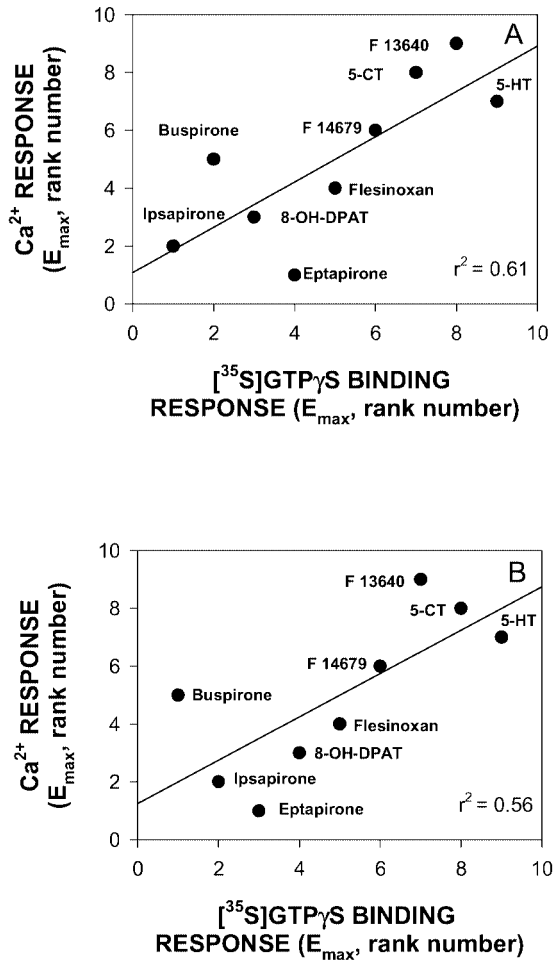

Fig. 5. Relationship between the magnitudes of 5-HT ligand's Ca^{2+} and [^{35}S]GTPγS binding responses (higher rank numbers represent a higher amplitude, and are based on the E_{max} values described in Table 1), r^2 represents Spearman's rank correlation coefficient. A, [^{35}S]GTPγS binding responses in membranes of stably 5-HT_{1A} receptor-transfected CHO-K1 cells. B, [^{35}S]GTPγS binding responses in membranes of stably 5-HT_{1A} receptor transfected C6-glial cells.

both transfected CHO-K1 and C6-glial membranes. The magnitude of Ca^{2+} responses produced by the 5-HT_{1A} receptor ligands examined here in CHO-K1 cells correlated weakly with their [^{35}S]GTPγS binding response at 5-HT_{1A} receptors in CHO-K1 (Spearman's rank correlation, $r^2 = 0.61$, $p = 0.009$, $n = 9$) and C6-glial cells (Spearman's rank correlation, $r^2 = 0.56$, $p = 0.016$, $n = 9$) (Fig. 5).

Discussion

The Ca^{2+} response data as obtained with the 5-HT_{1A} receptor in transfected CHO-K1 cells indicate an atypical pharmacological 5-HT_{1A} receptor profile. Either a highly efficacious Ca^{2+} response or almost no Ca^{2+} response was found with the series of investigated 5-HT ligands. It seemed that the ligands induce either a "yes" or "no" response. The Ca^{2+} response induced by the native ligand 5-HT is likely to occur via activation of the recombinant 5-HT_{1A} receptor; no Ca^{2+} signal could be detected in nontransfected CHO-K1 cells, although the Ca^{2+} pathway was adequately responsive to bradykinin. CHO cells have previously been reported to express endogenous 5-HT_{1B} receptors, which are negatively coupled to adenylyl cyclase and positively coupled to increases in intracellular Ca^{2+} formation (Dickenson and Hill, 1995; Giles et al., 1996). In the present study, the selective 5-HT_{1B} receptor antagonist SB 224289 (Gaster et al., 1998) affected the 5-HT-mediated Ca^{2+} response little, if at all, whereas the selective 5-HT_{1A} receptor antagonist WAY 100635 (Fletcher et al., 1996) fully blocked the response. The 5-HT-mediated Ca^{2+} response was also sensitive to inhibition by pertussis toxin treatment. Thus, like the [^{35}S]GTPγS binding responses, the Ca^{2+} effect in CHO-K1 cells seem to be mediated by endogenous $G_{i/o}$ proteins. The [^{35}S]GTPγS binding response monitors Gα protein activation of endogenous $G_{i/o}$ proteins, whereas the Ca^{2+} response is probably mediated by endogenous Gβγ subunits of activated $G_{i/o}$ proteins in CHO-K1 cells. Although dual coupling of the cloned 5-HT_{1A} receptor to both adenylyl cyclase and phospholipase C in HeLa cells is apparently mediated via the same $G\alpha_{i3}$ protein (Fargin et al., 1991), this may be different for CHO-K1 and C6-glial cells.

The Ca^{2+} response data strongly suggest that these 5-HT_{1A} receptor ligands can be divided in two different classes. A first class of ligands (F 13640, 5-CT, and F 14679) seem to demonstrate a maximal effect that is similar to that induced by 5-HT. These compounds also acted with a significantly higher efficacy in the Ca^{2+} response compared with their [^{35}S]GTPγS binding responses in C6-glial cells. A second class of ligands (buspirone, flesinoxan, 8-OH-DPAT, ipsapirone, and eptapirone), with definite partial agonist properties in the [^{35}S]GTPγS binding responses, were inactive or almost inactive in the Ca^{2+} response. It is possible that both classes of 5-HT receptor ligands recognize a distinct population of 5-HT_{1A} receptor conformations that may affect in a different manner the downstream cascade of effector proteins. Activation of both populations of 5-HT_{1A} receptor con-

formations would result in [^{35}S]GTPγS binding responses with a broad spectrum of partial agonist properties. Activation of only a single population of receptor conformations would result in an efficacious Ca^{2+} response, whereas the other population of receptor conformations would result in very low efficacy. For instance, buspirone was almost free of intrinsic activity in the Ca^{2+} response and fully antagonized the 5-HT-induced Ca^{2+} response. Similar observations for buspirone have previously been obtained using HeLa cells and Ca^{2+} mobilization (Hoyer et al., 1991); this contrasts with buspirone's partial or efficacious agonist activity in [^{35}S]GTPγS binding (e.g., Pauwels et al., 1997) and cAMP responses (Pauwels et al., 1993). Molecular dynamics simulations, considering the 5-HT_{2A} receptor (Shapiro et al., 2000), produced ligand-bound structures using substantially different binding interactions even among structurally similar ligands (differing by as little as one methyl group). Relatively minor changes in either receptor or ligand structure can produce drastic and unpredictable changes in both binding interactions and 5-HT_{2A} receptor activation. Differences in receptor reserve have often been invoked to explain why partial agonists may demonstrate either agonist (i.e., high receptor reserve) or antagonist (i.e., low receptor reserve) behaviors. In spite of prior evidence that dorsal raphe somatodendritic 5-HT_{1A} autoreceptors exhibit high receptor/effector coupling efficiency (receptor reserve) compared with postsynaptic receptors in hippocampus (Meller et al., 1990), there is no clear evidence of a difference at the level of receptor/G protein coupling (Meller et al., 2000). Alternatively, the 5-HT_{1A} receptor is able to couple to different $G_{i/o/z}$ proteins (i.e., Butkerait et al., 1995), one of which may act preferentially on phospholipase C.

Strikingly, F 14679 and flesinoxan displayed a small difference (up to 16%) in their maximal [^{35}S]GTPγS binding response, whereas they showed an 82% difference in their maximal Ca^{2+} response. Therefore, it is unlikely that the herein observed Ca^{2+} results can be explained by the assumption that efficacy in the Ca^{2+} response is observable from a certain threshold of 5-HT_{1A} receptor activation as estimated by the [^{35}S]GTPγS binding response. This would also suggest that the apparent efficacy of each 5-HT ligand in the Ca^{2+} response would be enhanced under conditions of more efficient coupling or attenuated when coupling efficacy would be lower. The present study clearly demonstrates some 5-HT ligands are more efficacious, whereas others are less or not at all efficacious in the Ca^{2+} response. This opposite observation on ligand efficacy suggests the pharmacology of the Ca^{2+} response is different from that observed with the [^{35}S]GTPγS binding response, although both are effected via the 5-HT_{1A} receptor. This would suggest that 5-HT_{1A} ligands inducing a highly efficacious Ca^{2+} response may result in downstream effects that cannot at all be achieved with such prototypical ligands as buspirone. In confirmation of this, F 13640 produces a complete inhibition of formalin-induced pain behaviors in conditions where buspirone exerted no detectable effect (Colpaert et al., 2002).

In conclusion, the pharmacological observations with the Ca^{2+} response indicate two classes of 5-HT_{1A} receptor ligands. The Ca^{2+} response in CHO-K1 cells constitutes a useful tool to identify highly selective 5-HT_{1A} receptor ligands that are distinct from prototypical 5-HT_{1A} ligands. This study further illustrates that the pharmacology of 5-HT_{1A} receptors may be diverse and be codetermined by the effector pathway. The complex area of 5-HT_{1A} receptor-coupled effector systems requires further research to analyze the activation of these effector systems and characterizes this activation by effector-selective ligands.

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References

Ariëns EJ (1964) The mode of action of biologically active compounds, in *Molecular Pharmacology*, vol 1, Academic Press, New York, NY.

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, and 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.

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:**248–254.

Butkerait P, Zheng Y, Hallak H, Graham TE, Miller HA, Burris KD, Molinoff PB, and Manning DR (1995) Expression of the human 5-hydroxytryptamine$_{1A}$ receptor in Sf_9 cells. *J Biol Chem* **270:**18691–18699.

Colpaert FC, Tarayre JP, Koek W, Pauwels PJ, Bardin L, Xu ZJ, Wiesenfeld-Hallin Z, Cosi C, Carilla-Durand E, Assié MB, et al. (2002) Large-amplitude 5-HT_{1A} receptor activation: a new mechanism of profound central analgesia. *Neuropharmacology* **43:**945–958.

Colquhoun D (1998) Binding, gating, affinity and efficacy: the interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. *Br J Pharmacol* **125:**924–947.

Dickenson JM and Hill SJ (1995) Coupling of an endogenous 5-$HT_{1B-like}$ receptor to increases in intracellular calcium through a pertussis toxin-sensitive mechanism in CHO-K1 cells *Br J Pharmacol* **116:**2889–2896.

Fargin A, Yamamoto K, Cotecchia S, Goldsmith PK, Spiegel AM, Lapetina EG, Caron MG, and Lefkowitz RJ (1991) Dual coupling of the cloned 5-HT_{1A} receptor to both adenylyl cyclase and phospholipase C is mediated via the same G_i protein. *Cell Signal* **3:**547–557.

Fletcher A, Forster EA, Bill DJ, Brown G, Ciffe IA, Hartley JE, Jones DE, McLenachan A, Stanhope KJ, Critchely DJP, et al. (1996) Electrophysiological, biochemical, neurohormonal and behavioral studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. *Behav Brain Res* **73:**337–353.

Gaster LM, Blaney FE, Davies S, Duckworth DM, Ham P, Jenkins S, Jennings AJ, Joiner GF, King FD, Mulholland KR, et al. (1998) The selective 5-HT_{1B} receptor inverse agonist 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydro-spiro[furo[2,3-f]indole-3, 4′-piperidine] (SB 224289) potency blocks terminal 5-HT autoreceptor function both in vitro and in vivo. *J Med Chem* **41:**1218–1235.

Gether U, Liu S, and Kobilka BK (1995) Fluorescent labeling of purified β_2 adrenergic receptor: evidence for ligand-specific conformational changes. *J Biol Chem* **270:**28268–28275.

Gettys TW, Fields TA, and Raymond JR (1994) Selective activation of inhibitory G protein α-subunits by partial agonists of the human 5-HT_{1A} receptor. *Biochemistry* **33:**4283–4290.

Giles H, Lansdell SJ, Bolofo ML, Wilson HL, and 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.

Hoyer D, Boddeke H, and Schoeffter P (1991) Second messengers in the definition of 5-HT receptors, in *Serotonin: Molecular Biology, Receptors and Functional Effects*, (Fozard JR and Saxena PR eds) pp 117–132, Basel, Birkhäuser.

Kenakin T (1997) Drug receptor theory, in *Pharmacologic Analysis of Drug-Receptor Interaction*, 3rd ed., pp. 1–42, Lippincott-Raven Press, New York, NY.

Koek W, Patoiseau JF, Assie MB, Cosi C, Kleven MS, Dupont-Passelaigue E, Carilla-Durand E, Palmier C, Valentin JP, John G, et al. (1998) F 11440, a potent, selective, high efficacy 5-HT_{1A} receptor agonist with marked anxiolytic and antidepressant potential. *J Pharmacol Exp Ther* **287:**266–283.

Koek W, Vacher B, Cosi C, Assie MB, Patoiseau JF, Pauwels PJ, and Colpaert FC (2001) 5-HT_{1A} receptor activation and antidepressant-like effects: F 13714 has high efficacy and marked antidepressant potential. *Eur J Pharmacol* **420:**103–112.

Krumins AJ and Barber R (1997) The stability of the agonist β_2-adrenergic receptor-G_s complex: evidence for agonist-specific receptors states. *Mol Pharmacol* **52:**144–154.

Leff P, Scaramellini C, Law C, and McKechnie K (1997) A three-state model of agonist action. *Trends Pharmacol Sci* **18:**355–362.

Meller E, Goldstein M, and Bohmaker K (1990) Receptor reserve for 5-hydroxytryptamine$_{1A}$-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine$_{1A}$ agonists. *Mol Pharmacol* **37:**231–237.

Meller E, Li H, Carr KD, and Hiller JM (2000) 5-Hydroxytryptamine$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in rat brain: absence of regional differences in coupling efficiency. *J Pharmacol Exp Ther* **292:**684–691.

Pauwels PJ, Tardif S, Finana F, Wurch T, and Colpaert FC (2000) Ligand-receptor interactions as controlled by wild-type and mutant Thr(370)Lys α2B-adrenoceptor-Gα15 fusion proteins. *J Neurochem* **74:**375–384.

Pauwels PJ, Tardif S, Wurch T, and Colpaert FC (1997) Stimulated [^{35}S]GTP γ S binding by 5-HT_{1A} receptor agonists in recombinant cell lines. Modulation of

apparent efficacy by G-protein activation state. *Naunyn-Schmiedeberg's Arch Pharmacol* **356:**551–561.
Pauwels PJ, Van Gompel P, and Leysen JE (1993) Activity of 5-HT receptor agonists, partial agonists and antagonists at cloned human 5-HT_{1A} receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. *Biochem Pharmacol* **45:**375–383.
Pauwels PJ, Wurch T, Amoureux MC, Palmier C, and Colpaert FC (1996) Stimulation of cloned human serotonin 5-$HT_{1D\beta}$ receptor sites in stably transfected C6 glial cells promotes cell growth. *J Neurochem* **66:**65–73.
Raymond JR, Mukhin YV, Gettys TW, and Garnovskaya MN (1999) The recombinant 5-HT_{1A} receptor: G protein coupling and signaling pathways. *Br J Pharmacol* **127:**1751–1764.
Shapiro DA, Kristiansen K, Kroeze WK, and Roth BL (2000) Differential model of agonist binding to 5-hydroxytryptamine$_{2A}$ serotonin receptors revealed by mutation and molecular modeling of conserved residues in transmembrane region 5. *Mol Pharmacol* **58:**877–886.
Watson C, Chen G, Irving P, Way J, Chen WJ, and Kenakin T (2000) The use of stimulus-biased assay systems to detect agonist-specific receptor active states: implications for the trafficking of receptor stimulus by agonists. *Mol Pharmacol* **58:**1230–1238.
Wurch T, Chastagnier C, Palmier C, Colpaert FC, and Pauwels PJ (1996) A 413 bp region upstream the human 5-HT_{1A} receptor gene is sufficient for its in vitro expression. *Neurosci Res Commun* **19:**75–82.
Yang Q and Lanier SM (1999) Influence of G protein type on agonist efficacy. *Mol Pharmacol* **56:**651–656.
Zhang D and Weinstein H (1993) Signal transduction by a 5-HT_2 receptor: a mechanistic hypothesis from molecular dynamics stimulations of the three-dimensional model of the receptor complexed to ligands. *J Med Chem* **36:**934–938.

Address correspondence to: Dr. Petrus J. Pauwels, Centre d'Immunologie Pierre Fabre, 5, avenue Napoléon III-BP 497, F 74164 Saint-Julien-en-Genevois Cedex, France. E-mail: peter.pauwels@pierre-fabre.com