

Ca²⁺ Responses in Chinese Hamster Ovary-K1 Cells Demonstrate an Atypical Pattern of Ligand-Induced 5-HT_{1A} Receptor Activation

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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 Ca²⁺ 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 (pEC₅₀ = 6.70 ± 0.02) induced a pertussis toxin-sensitive, transient high-magnitude Ca²⁺ response. High-magnitude Ca²⁺ responses (E_{max}, percentage versus 5-HT) were also found with F 13640 (107 ± 4), 5-car-

boxamidotryptamine (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-[³⁵S]thio)triphosphate ([³⁵S]GTPγS) binding responses with membranes of either CHO-K1 or C6-glia cells stably expressing a human 5-HT_{1A} receptor. Remarkably, differences between ligands that seem small in the [³⁵S]GTPγS binding assay translate into huge differences in the magnitude of Ca²⁺ 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 ev-

idence with the 5-HT_{2A} receptor (Shapiro et al., 2000) and β₂-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-[³⁵S]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 G_α_i proteins in recombinant CHO cells.

In the present study, we measured 5-HT ligand-mediated Ca²⁺ 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)-methylaminomethyl]piperidine (F 13640; Colpaert et al., 2002) as representative ligands of a new chemical class (5-methyl-pyridin-2-ylmethyl 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 Ca²⁺ response in CHO-K1 cells; the amplitude of the ligands' Ca²⁺ 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 Ca²⁺ response. The Ca²⁺ response data were compared with 5'-*O*-(3-[³⁵S]thiotriphosphate ([³⁵S]GTPγS) binding responses as a sensitive read-out for a wide spectrum of 5-HT_{1A} receptor ligand activities. The Ca²⁺ 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 Ca²⁺ responses between 24 and 48 h after transfection.

Measurement of Intracellular Ca²⁺ Responses. Intracellular Ca²⁺ 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 Ca²⁺ responses. Data for Ca²⁺ responses were obtained in arbitrary fluorescent units and were not translated into Ca²⁺ 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 Ca²⁺ response in percent-

age versus that obtained with 10 μM 5-HT. p*E*C₅₀ values correspond to a ligand concentration at which 50% of its own maximal high-magnitude Ca²⁺ response was measured. Antagonists were preincubated for 10 min before 5-HT and the Ca²⁺ response recorded for a further 3 min. Antagonist potency (p*E*C₅₀ value) was defined as the concentration required to antagonize 50% of the Ca²⁺ response induced by 1 μM 5-HT. This was calculated as the difference in surface area between the 5-HT and ligand conditions.

[³⁵S]GTPγS Binding Responses. [³⁵S]GTPγS binding responses were determined on membrane preparations of CHO-K1 or C6-gial 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 [³⁵S]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 MgCl₂ and rapid filtration as described previously (Pauwels et al., 1997). Maximal stimulation of [³⁵S]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-gial 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). [³⁵S]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 Ca²⁺ response in CHO-K1 cells transiently transfected with a human 5-HT_{1A} receptor (Fig. 1). The amplitude of this Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ signal upon maximal activation was slightly greater for bradykinin than for 5-HT. In contrast to the bradykinin-mediated Ca²⁺ response, pertussis toxin-treatment prevented the 5-HT-mediated Ca²⁺ response (Fig. 1C).

Results on antagonism of the 5-HT-induced Ca²⁺ 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

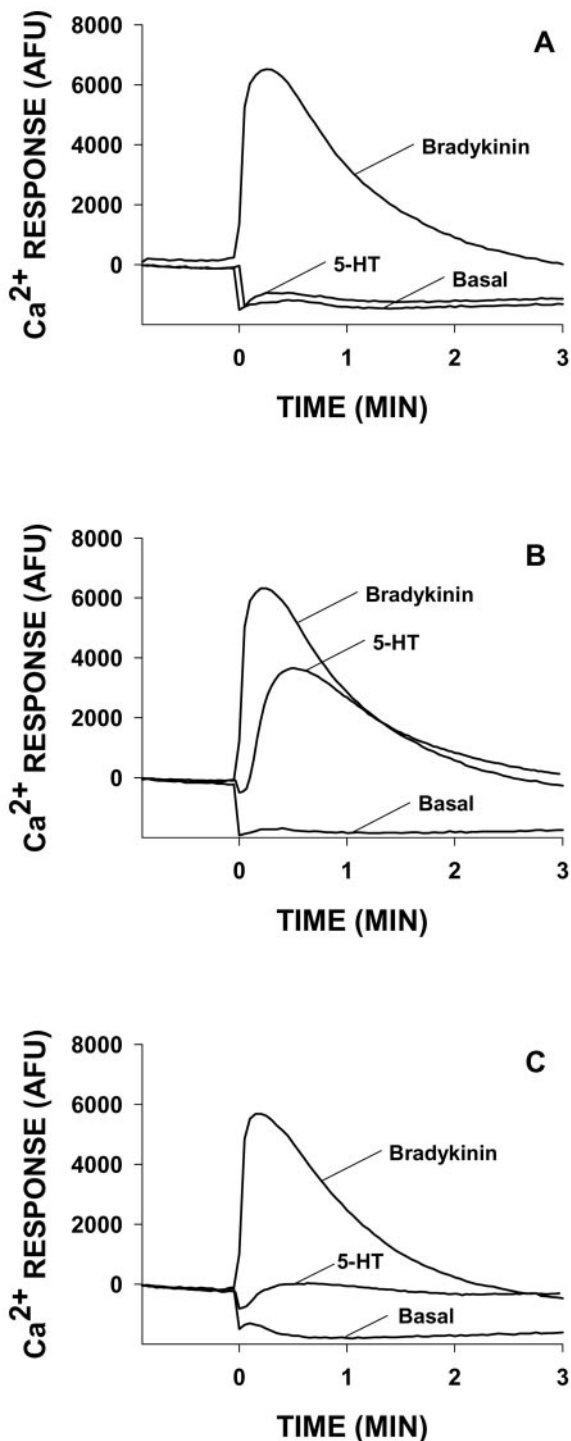


Fig. 1. 5-HT and bradykinin-induced Ca^{2+} responses in nontransfected and 5-HT_{1A} receptor-transfected CHO-K1 cells. CHO-K1 cells were transiently transfected with the human 5-HT_{1A} receptor (10 μg of plasmid). Basal, bradykinin- (10 μM), and 5-HT (10 μM)-induced Ca^{2+} responses were measured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

significant Ca^{2+} response despite the fact that their maximal [³⁵S]GTP γ S binding responses as obtained with membranes of 5-HT_{1A} receptor-transfected CHO-K1 cells were between

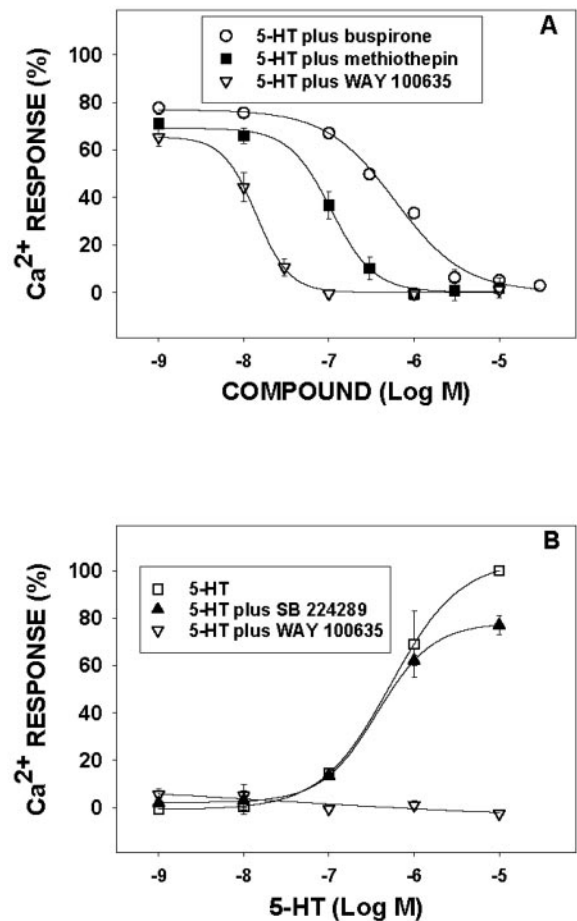


Fig. 2. Antagonism of the 5-HT-induced Ca^{2+} response by buspirone, methiothepin, and WAY 100635, but not SB 224289, in CHO-K1 cells transiently transfected with a 5-HT_{1A} receptor. CHO-K1 cells were transiently transfected with a human 5-HT_{1A} receptor plasmid and assayed for a 5-HT (1 μM)-induced 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 \pm S.E.M. obtained in five to seven independent transfection experiments. pIC_{50} values are 7.96 \pm 0.13 (WAY 100635), 7.16 \pm 0.08 (methiothepin), and 6.34 \pm 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 \pm 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.

56 and 79% compared with 5-HT (Table 1); similar findings were obtained with flesinoxan and eptapirone. Even a comparison with [³⁵S]GTP γ S binding data as obtained with membranes of C6-glia 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 Ca²⁺, [³⁵S]GTP γ S binding, and cAMP responses by recombinant human 5-HT_{1A} receptor. Ca²⁺ responses were determined in 5-HT_{1A} receptor-transfected CHO-K1 cells and expressed as a percentage of the Ca²⁺ response induced by 10 μ M 5-HT. [³⁵S]GTP γ S binding responses were determined on membranes of stably 5-HT_{1A} receptor transfected CHO-K1 or C6-glia cells in the presence of 30 μ M GDP and expressed versus 10 μ M 5-HT-mediated response. Mean values \pm S.E.M. are given for a minimum of three independent experiments.

	Ca ²⁺ Response/CHO-K1		[³⁵ S]GTP γ S Binding Response/CHO-K1		[³⁵ S]GTP γ S Binding Response/C6-glia		cAMP Response/HeLa	
	E_{\max} (%)	pEC_{50}	E_{\max} (%)	pEC_{50}	E_{\max} (%)	pEC_{50}	E_{\max} (%)	pEC_{50}
5-HT	100	6.70 \pm 0.02	100	7.74 \pm 0.07	100 ^c	6.71 ^c	100 ^e	7.67 ^e
F 13640	107 \pm 4	7.37 \pm 0.02	99 \pm 5	7.98 \pm 0.12	75 \pm 2	7.63 \pm 0.13		
5-CT	100 \pm 3	7.28 \pm 0.19	102 \pm 4	8.43 \pm 0.15	85 ^c	7.98 ^c		
F 14679	87 ^a \pm 3	7.33 \pm 0.16	93 \pm 6	9.21 \pm 0.15	61 ^d	8.31 ^d	110 ^d	8.70 ^d
Flesinoxan	5 \pm 1		81 ^b \pm 2	8.02 \pm 0.16	45 ^c	7.48 ^c	100 ^e	8.48 ^e
Buspirone	4 \pm 1		61 ^b \pm 7	7.08 \pm 0.04	22 ^c	6.40 ^c	47 ^d	6.56 ^d
8-OH-DPAT	3 \pm 1		79 ^b \pm 3	7.89 \pm 0.08	41 ^c	7.41 ^c	82 ^d	7.65 ^d
Ipsapirone	2 \pm 2		56 ^b \pm 8	7.69 \pm 0.13	26 ^c	7.30 ^c	58 ^e	7.66 ^e
Eptapirone	-1 \pm 3		79 ^b \pm 4	7.46 \pm 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 Ca²⁺ response is significantly different ($P < 0.05$) from that by 5-CT.

^b E_{\max} values (relative to 5-HT = 100%) for [³⁵S]GTP γ S binding responses on CHO-K1 membranes are significantly different ($P < 0.001$) from the corresponding Ca²⁺ responses. E_{\max} values (relative to 5-HT = 100%) as obtained with the Ca²⁺ response differed significantly from the corresponding E_{\max} values as estimated with the [³⁵S]GTP γ S binding response in C6-glia 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-glia 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 Ca²⁺ response in nontransfected CHO-K1 cells (Fig. 1A). Therefore, the observed Ca²⁺ responses with F

13640 and F 14679 can be considered to be due to activation by 5-HT_{1A} receptors. A comparison with the [³⁵S]GTP γ S binding responses in transfected C6-glia membranes (Fig. 3; Table 1) indicated a significantly lower maximal [³⁵S]GTP γ S binding response for F 13640 with an unmodified potency ($p > 0.05$), and a significantly lower maximal [³⁵S]GTP γ S binding response for 5-CT and F 14679 accompanied with an enhanced potency ($p \leq 0.01$). A comparison with the [³⁵S]GTP γ S 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 Ca²⁺ responses in CHO-K1 cells versus their spectrum of maximal [³⁵S]GTP γ S binding responses for

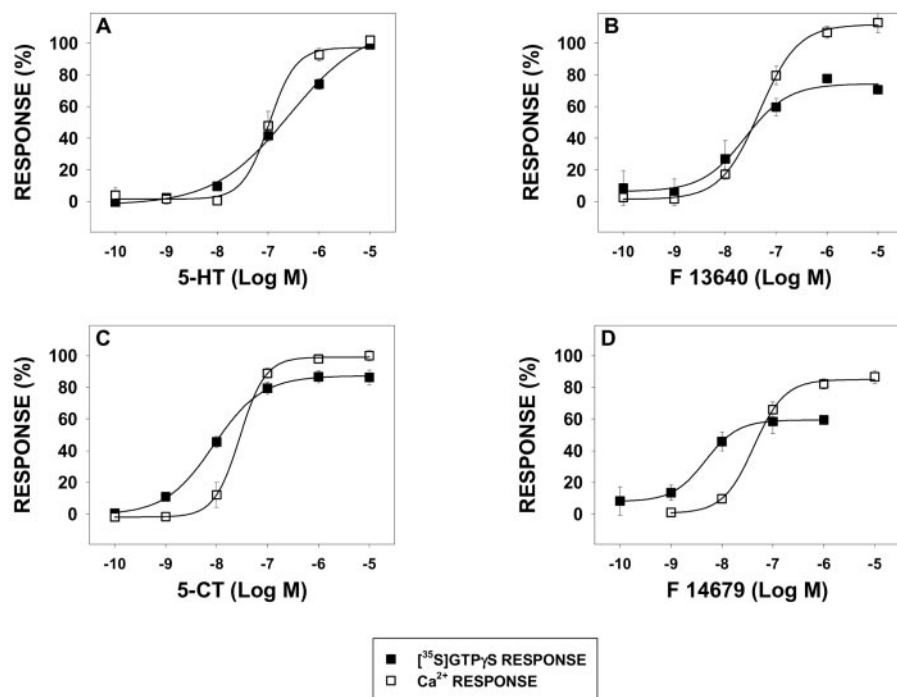


Fig. 3. Concentration-dependent Ca²⁺ and [³⁵S]GTP γ S binding responses of 5-HT ligands in either CHO-K1 or C6-glia cells transfected with a 5-HT_{1A} receptor. Ca²⁺ responses in transiently 5-HT_{1A} receptor-transfected CHO-K1 cells were expressed as a percentage of the maximal Ca²⁺ response induced by 10 μ M 5-HT. [³⁵S]GTP γ S binding responses at membranes of stably transfected C6-glia cells were measured with 30 μ M GDP. Data are expressed as a percentage of the 10 μ M 5-HT-induced [³⁵S]GTP γ S binding response. Curves were constructed using mean values \pm S.E.M. for a minimum of three independent transfection experiments. The 5-CT-mediated [³⁵S]GTP γ S binding response data are from Pauwels et al. (1997). Mean E_{\max} and pEC_{50} values are summarized in Table 1.

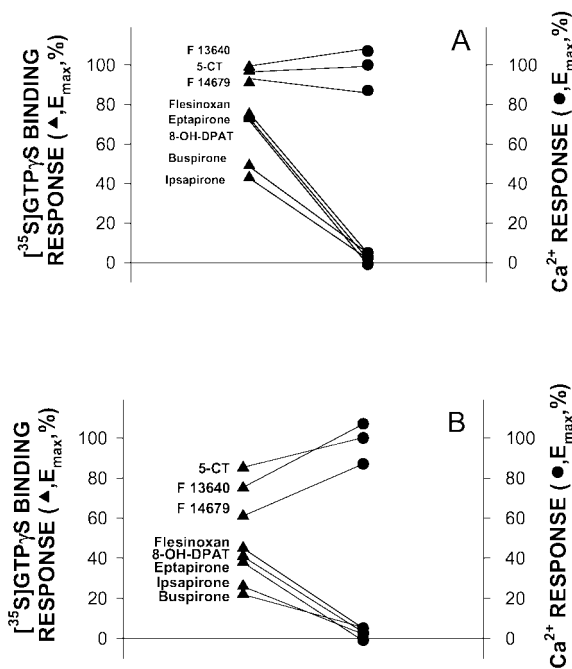


Fig. 4. E_{\max} values of 5-HT ligand's Ca^{2+} and $^{35}\text{S]GTP}\gamma\text{S}$ binding responses. E_{\max} values for Ca^{2+} and $^{35}\text{S]GTP}\gamma\text{S}$ binding responses were obtained with 5-HT_{1A} receptor transfected CHO-K1 cells and membranes of stably 5-HT_{1A} receptor-transfected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

both transfected CHO-K1 and C6-gial 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 $^{35}\text{S]GTP}\gamma\text{S}$ 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-gial 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 $^{35}\text{S]GTP}\gamma\text{S}$ binding responses, the Ca^{2+} effect in CHO-K1 cells seem to be mediated by endogenous $G_{i/o}$ proteins. The $^{35}\text{S]GTP}\gamma\text{S}$ binding response monitors $G\alpha$ protein activation of endoge-

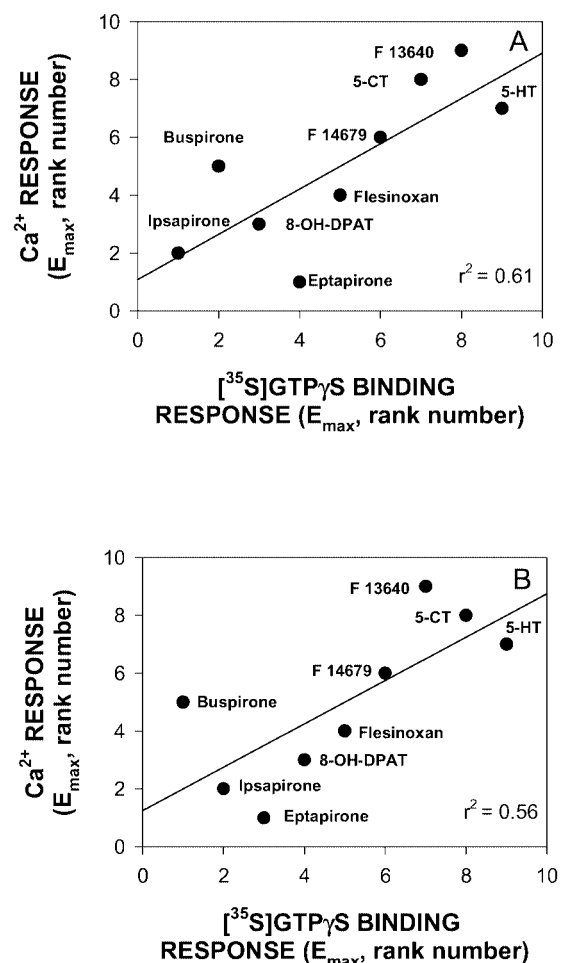


Fig. 5. Relationship between the magnitudes of 5-HT ligand's Ca^{2+} and $^{35}\text{S]GTP}\gamma\text{S}$ binding responses (higher rank numbers represent a higher amplitude, and are based on the E_{\max} values described in Table 1), r^2 represents Spearman's rank correlation coefficient. A, $^{35}\text{S]GTP}\gamma\text{S}$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

nous $G_{i/o}$ proteins, whereas the Ca^{2+} response is probably mediated by endogenous $G\beta\gamma$ 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-gial 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 $^{35}\text{S]GTP}\gamma\text{S}$ binding responses in C6-gial cells. A second class of ligands (buspirone, flesinoxan, 8-OH-DPAT, ipsapirone, and eptapirone), with definite partial agonist properties in the $^{35}\text{S]GTP}\gamma\text{S}$ 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 [³⁵S]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 Ca²⁺ 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 Ca²⁺ response and fully antagonized the 5-HT-induced Ca²⁺ response. Similar observations for buspirone have previously been obtained using HeLa cells and Ca²⁺ mobilization (Hoyer et al., 1991); this contrasts with buspirone's partial or efficacious agonist activity in [³⁵S]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 [³⁵S]GTPγS binding response, whereas they showed an 82% difference in their maximal Ca²⁺ response. Therefore, it is unlikely that the herein observed Ca²⁺ results can be explained by the assumption that efficacy in the Ca²⁺ response is observable from a certain threshold of 5-HT_{1A} receptor activation as estimated by the [³⁵S]GTPγS binding response. This would also suggest that the apparent efficacy of each 5-HT ligand in the Ca²⁺ 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 Ca²⁺ response. This opposite observation on ligand efficacy suggests the pharmacology of the Ca²⁺ response is different from that observed with the [³⁵S]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 Ca²⁺ 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 Ca²⁺ response indicate two classes of 5-HT_{1A} receptor ligands. The Ca²⁺ 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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