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DIFFERENTIATION BETWEEN PARTIAL AND SILENT 5-HT$_{1D\beta}$ RECEPTOR ANTAGONISTS USING RAT C6-GLIAL AND CHINESE HAMSTER OVARY CELL LINES PERMANENTLY TRANSFECTED WITH A CLONED HUMAN 5-HT$_{1D\beta}$ RECEPTOR GENE

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Abstract—Intrinsic activities of serotonin (5-HT) receptor ligands at cloned human 5-HT$_{1D\beta}$ receptor sites were determined by measuring cAMP responses in two permanently transfected cell types: rat C6-glial and Chinese hamster ovary (CHO)-K1 cells. Both transfected cell lines expressed a similar 5-HT$_{1D\beta}$ receptor density (361 to 448 fmol/mg protein) and displayed a number of similar cAMP responses: marked inhibition of forskolin-stimulated cAMP formation by 5-HT; a similar agonist potency and efficacy with 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine, bufotenine, sumatriptan, 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrolo-(1,2-a)quinoxaline (CGS 12066B), 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)1*H*-indole (RU 24,969), and tryptamine, their maximal effect being comparable to that of 5-HT; less agonist efficacy with m-trifluoro-phenyl-piperazine (TFMPP) (it inhibited at most 63% of stimulated cAMP formation); and antagonist activity against the 5-CT-mediated agonist response with methiothepin, 2′-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127,935), and ritanserin. Metergoline and 1-naphtylpiperazine showed different intrinsic activities. In contrast to their pronounced antagonist activity in the transfected CHO-K1 cell line, the antagonist effect was only partial and absent for metergoline and 1-naphtylpiperazine in the transfected C6-glial cell line, respectively. In conclusion, these cell lines are useful as a tool to measure with high sensitivity differences in intrinsic activities of 5-HT receptor ligands and, therefore, discriminate between silent antagonists (no intrinsic activity) and antagonists with intrinsic activity (*i.e.* partial agonists), even though this intrinsic activity may be relatively weak.

Key words: cloned human 5-HT$_{1D\beta}$ receptor; permanent cell transfection; intrinsic activity; agonist-partial agonist-antagonist

Seven major populations of 5-HT† receptors have been identified: 5-HT$_1$ to 5-HT$_7$ [1]. 5-HT$_1$ receptors have been further characterized as 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, 5-ht$_{1E}$ and 5-ht$_{1F}$ [2, 3, 4, 5]. 5-HT$_{1D}$ receptors may be involved in cardiovascular function and perhaps in depression and anxiety [5, 7] 5-HT$_{1D}$ receptors may also be involved in vasospasm and migraine [8]. A functional involvement of these receptors with dopaminergic systems suggests that selective 5-HT$_{1D}$ agents may be of value for treatment of movement disorders [9]. The human 5-HT$_{1D}$ receptor consists of 2 subtypes: 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ [10, 11]. 5-HT$_{1D\beta}$ receptors mediate contractile responses in bovine and human cerebral arteries [12, 13], whereas the 5-HT$_{1D\alpha}$ receptor gene seems to be selectively expressed in human trigeminal ganglia [14]. Nevertheless, the precise function of these receptors remains to be defined. Through cloning, it has been demonstrated that there is a highly significant transmembrane amino acid homology (96%) between 5-HT$_{1D\beta}$ and 5-HT$_{1B}$ receptor populations [15]. Selective 5-HT$_{1D\beta}$ receptor ligands (agonists and antagonists) are almost unknown, and the development of new pharmacological tools is necessary to further characterize these receptors and the ligands that interact with them.

A full characterization of available ligands requires that both their affinity for and their activity at a number of receptors be established. For example, the arylpiperazines not only have limited selectivity for 5-HT$_{1B}$ *vs.* certain populations of 5-HT receptors but, also, behave as agonists at 5-HT$_{1B}$ receptors and as antagonists or partial agonists at other 5-HT receptors [16, 17]. Mammalian cell lines permanently transfected with cloned human receptor genes have often been used for the determination of intrinsic activity of compounds. However, it has been repeatedly documented in recent years that a substantial number of antagonists tend to show intrinsic agonist activity in transfected cell lines. Many of these compounds display antagonism *in situ*, except in situations where receptor reserve is very high and/or receptor effector coupling is very good [18].

In this paper, we report on the intrinsic activity of 5-HT receptor ligands at 5-HT$_{1D\beta}$ receptor sites in rat C6-glial and CHO cells permanently transfected with a cloned human 5-HT$_{1D\beta}$ receptor gene. The following parameters were measured: receptor density on intact cells using the radioligand ^{3}H 6-CT, 5-HT$_{1D\beta}$ receptor-

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† Abbreviations: 5-HT, serotonin; CHO, Chinese hamster ovary; 5-CT, 5-carboxamidotryptamine; CGS 12066B, 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrolo-(1,2-a)quinoxaline; RU 24,969, 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)1*H*-indole; GR 127,935, 2′-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide; TFMPP, m-trifluoro-phenyl-piperazine.

mediated inhibition of stimulated cAMP formation with a series of 5-HT receptor agonists and the antagonism of 5-CT-mediated agonist activity in the presence of various putative 5-HT receptor antagonists. It is shown that, although both transfected cell types express a similar 5-$HT_{1D\beta}$ receptor density, certain compounds display different intrinsic activities.

METHODS

Cell culture

CHO-K1 (ATCC, CCL 61, Chinese hamster) and C6-glial (ATCC, CCL 107, rat) cells were permanently transfected with a cloned human 5-$HT_{1D\beta}$ receptor gene (11) and cultured in 24-well tissue culture plates as previously described [19, 20]. Cultures were maintained at 37°C in an air/CO_2 (95/5) water-saturated atmosphere.

5-$HT_{1D\beta}$ receptor binding on intact cells

Transfected cells were washed twice with 1.0 mL controlled salt solution (CSS: 120 mM NaCl, 5.4 mM KCL, 0.8 mM $MgCl_2$, 5 mM glucose, 25 mM Tris-HCl, pH 7.4) and incubated for 30 min at 37°C with 0.5 mL CSS containing 10 μM chloroquine and 7 to 8 concentrations of ^{3}H 5-CT ranging from 0.1 to 13 nM in the absence and presence of 10 μM 5-HT. The incubation was stopped by washing the cultures three times with 1.0 mL ice-cold CSS. The cells were lysed by collecting them in 0.5 mL 0.1 N NaOH. To quantify ^{3}H 5-CT binding, 0.5 mL of the cell extract was mixed with 5.0 mL Emulsifier-Safe and the mixture counted in a Packard Tricarb liquid scintillation counter. Specific binding of ^{3}H 5-CT was defined as the portion of total binding inhibited by 10 μM 5-HT. Data were analysed in Scatchard plots. Cellular protein was estimated with the dye-binding assay using the Bio-Rad kit [21]. Bovine serum albumin was used as a standard.

5-$HT_{1D\beta}$ receptor-mediated inhibition of forskolin-stimulated cAMP formation

5-$HT_{1D\beta}$ receptor-mediated inhibition of forskolin-stimulated cAMP formation in transfected C6-glial cells was measured as previously described for CHO-K1/5-$HT_{1D\beta}$ cells [19]. Cultures were washed with 1.0 mL CSS and incubated for 5 min at 37°C with 1.0 mL CSS containing 1 mM isobutylmethylxanthine in the presence of 100 μM forskolin and compound. Basal accumulation of cAMP was measured in the absence of forskolin and compound. The reaction was stopped by the addition of 0.1 mL ice-cold $HClO_4$ to a final concentration of 0.04 N and neutralized afterwards. Cellular cAMP content was assayed using a radioimmunoassay kit (Immunotech, Marseille, France). Inhibition of 100 μM forskolin-induced cAMP formation was calculated as the percentage of that obtained with 1 μM 5-HT. EC_{50}-values (concentration of test agent yielding 50% of the inhibition of forskolin-induced cAMP formation produced by 1 μM 5-HT) and E_{max}-values (maximal percentage inhibition of forskolin-induced cAMP formation versus that obtained with 1 μM 5-HT) were derived. The antagonism of 5-CT-mediated inhibition of cAMP formation was assayed after 20 min preincubation with the test agent. Dissociation constants (K_B) of antagonists were calculated according to $K_B = (B)/(A'/A) - 1$, where B is the concentration of the antagonist, and A and A' are the EC_{50}-values of agonist concentration measured in the absence and presence of antagonist, respectively, assuming competitive antagonism.

Materials

Culture media, genecticin, foetal calf serum and 24-well tissue culture plates were obtained from Gibco Biocult. Laboratories (Paisley, U.K.). ^{3}H 5-CT (15–30 Ci/mmol) was obtained from New England Nuclear (Les Ulis, France). GR 127,935 was prepared by Dr. S. Halazy and Dr. C. Jorand according to a patent procedure (European Patent Application 0533268-A1). Other drugs were kindly supplied by the companies of origin. The stock solutions of compounds were prepared in water or ethanol. Dilutions were made in CSS containing 10% ethanol.

RESULTS

Intrinsic activities of 5-HT receptor ligands were measured in transfected C6-glial and CHO-K1 cells expressing a similar 5-$HT_{1D\beta}$ receptor density. The ^{3}H 5-CT saturation binding curves on intact cells and the derived Scatchard analyses suggest the presence of a single high affinity binding site for ^{3}H 5-CT for both cell lines with a mean B_{max}-value between 360 to 450 fmol/mg protein (Fig. 1). Control experiments with the nontransfected cell lines did not reveal specific ^{3}H 5-CT binding nor inhibition or stimulation of cAMP formation by 5-HT. The transfected cell lines displayed no increase in cAMP content by 5-HT but marked inhibition of forskolin-stimulated cAMP formation in the presence of 1 μM 5-HT; it attained >70% and >90% of 100 μM forskolin-stimulated cAMP formation for the transfected CHO-K1 and C6-glial cell line, respectively. Figure 2 compares the dose-response curves for inhibition of forskolin-induced cAMP formation for a series of 5-HT receptor agonists (5-CT, 5-methoxytryptamine, bufotenine, sumatriptan, CGS 12066B, RU 24,969, tryptamine, and TFMPP) in transfected C6-glial and CHO-K1 cell lines. The cAMP-mediated agonist response of each tested compound in both cell lines was almost similar. The corresponding EC_{50}-values are summarized in Table 1. With the exception of TFMPP, which appeared to inhibit at most 63% in both cell lines, all other compounds that elicited this inhibitory response did so by 85% to 100%. The most potent compound in inhibiting forskolin-induced stimulation of cAMP formation was 5-CT with an EC_{50}-value between 2.8 and 3.8 nM. This compound was further used to test the antagonist activity of GR 127,935, methiothepin, ritanserin, metergoline, and 1-naphtylpiperazine. Whereas methiothepin and ritanserin did not affect forskolin-stimulated cAMP formation at concentrations up to 10 μM in either transfected cell line, slight (13% to 16%) to partial inhibition (32% to 53%) of forskolin-stimulated cAMP formation was preferentially apparent in the transfected C6-glial cell line with micromolar concentrations of metergoline, GR 127,935, and 1-naphtylpiperazine (Table 1). The dose-response curves for inhibition of forskolin-stimulated cAMP formation by 5-CT in the presence of these various compounds are illustrated in Fig. 3. One micromolar methiothepin induced an almost similar and parallel rightward shift of the dose-response curve for 5-CT in both transfected cell lines. GR 127,935 also antagonised the 5-CT-mediated responses; the antagonist effect appeared to be more pronounced in the transfected CHO-K1 cell line and slightly more potent than for methiothepin. Ritanserin was a

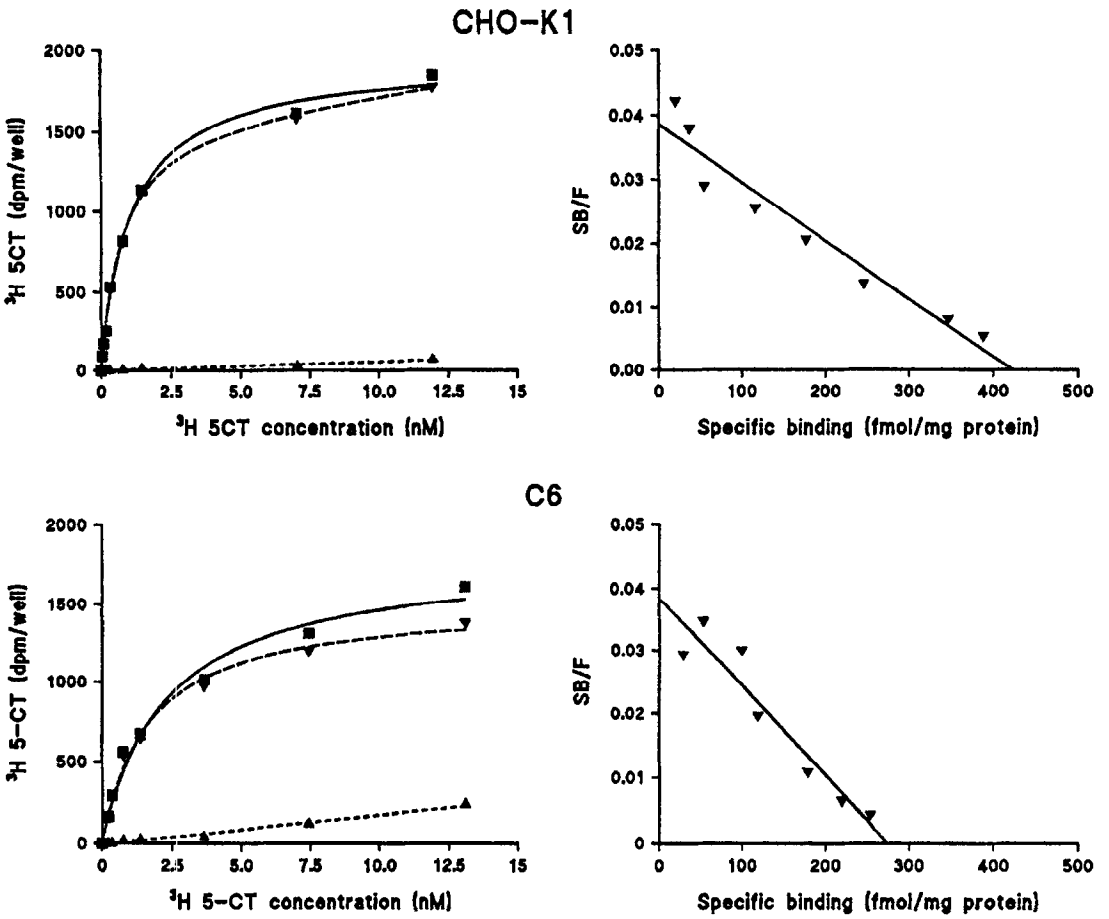

Fig. 1. Saturation binding curves and Scatchard plots of ^{3}H 5-CT binding to 5-HT$_{1D\beta}$ receptor sites in transfected C6-glial and CHO-K1 cells. Binding assays were performed on intact cells as described under "Methods." Nonspecific binding (▲) was performed in the presence of 10 μM 5-HT. Curves were constructed using mean values of binding data of a representative experiment. SB (▼), specific ^{3}H 5-CT binding, total bound (■) ^{3}H 5-CT minus nonspecifically bound. F, free ^{3}H 5-CT, concentration calculated as the added concentration of ^{3}H 5-CT minus the total concentration bound. Analysis of the ligand saturation binding curves by the nonlinear square curve fitting program. Ligand indicated the presence of a single binding site; mean Kd- and B_{max}-values are 1.60 ± 0.67 and 1.67 ± 0.17 nM, and 359 ± 99 and 448 ± 36 fmol/mg protein for transfected C6-glial (*n*: 6) and CHO-K1 cells (*n*: 2), respectively.

much less potent antagonist; at 10 μM it shifted the 5-CT response slightly more in the CHO-K1 cell line. One micromolar of metergoline fully displaced the 5-CT dose-response curve in the transfected CHO-K1 cell line with a K_B-value similar to that of methiothepin (Table 2). A different response was measured with this compound in the transfected C6-glial cell line; the 5-CT response curve was only partially displaced at 1 μM and higher concentrations. In contrast to the potent antagonist activity of 1 μM of 1-naphtylpiperazine in the transfected CHO-K1 cell line, this compound was devoid of antagonist activity against 5-CT in the transfected C6-glial cell line. Finally, no effects were observed on forskolin-induced cAMP formation with GR 127,935, metergoline, and 1-naphtylpiperazine in nontransfected CHO-K1 and C6-glial cells.

DISCUSSION

This paper compares 5-HT$_{1D\beta}$ receptor-mediated cAMP responses of various 5-HT receptor ligands in 2 permanently transfected cell types, C6-glial and CHO-K1 cells. The observed inhibition of forskolin-stimulated cAMP production by 5-HT in these cell lines is in agreement with previous reports on 5-HT$_{1D\beta}$ receptor-mediated coupling mechanisms [10, 22, 23, 24]. The main finding of this paper is that differences in intrinsic activity were found for certain compounds, such as metergoline and 1-naphtylpiperazine, under conditions where the 5-HT$_{1D\beta}$ receptor density was similar.

The cAMP data obtained with the transfected C6-glial and CHO-K1 cell lines show a number of similarities: the agonist potencies and efficacies for 5-CT, 5-methoxytryptamine, bufotenine, sumatriptan, CGS 12066B, RU 24,959, and tryptamine, their maximal agonist effect being comparable to that of 5-HT; the partial inhibition of forskolin-stimulated cAMP formation with TFMPP; and the full antagonism by methiothepin of 5-CT-induced responses. Minor differences between both transfected cell lines were apparent with the antagonist effects of GR 127, 935 and ritanserin. GR 127,935 also showed some inhibition of forskolin-stimulated cAMP formation in the transfected C6-glial cell line in contrast to the apparently silent antagonists methiothepin and ri-

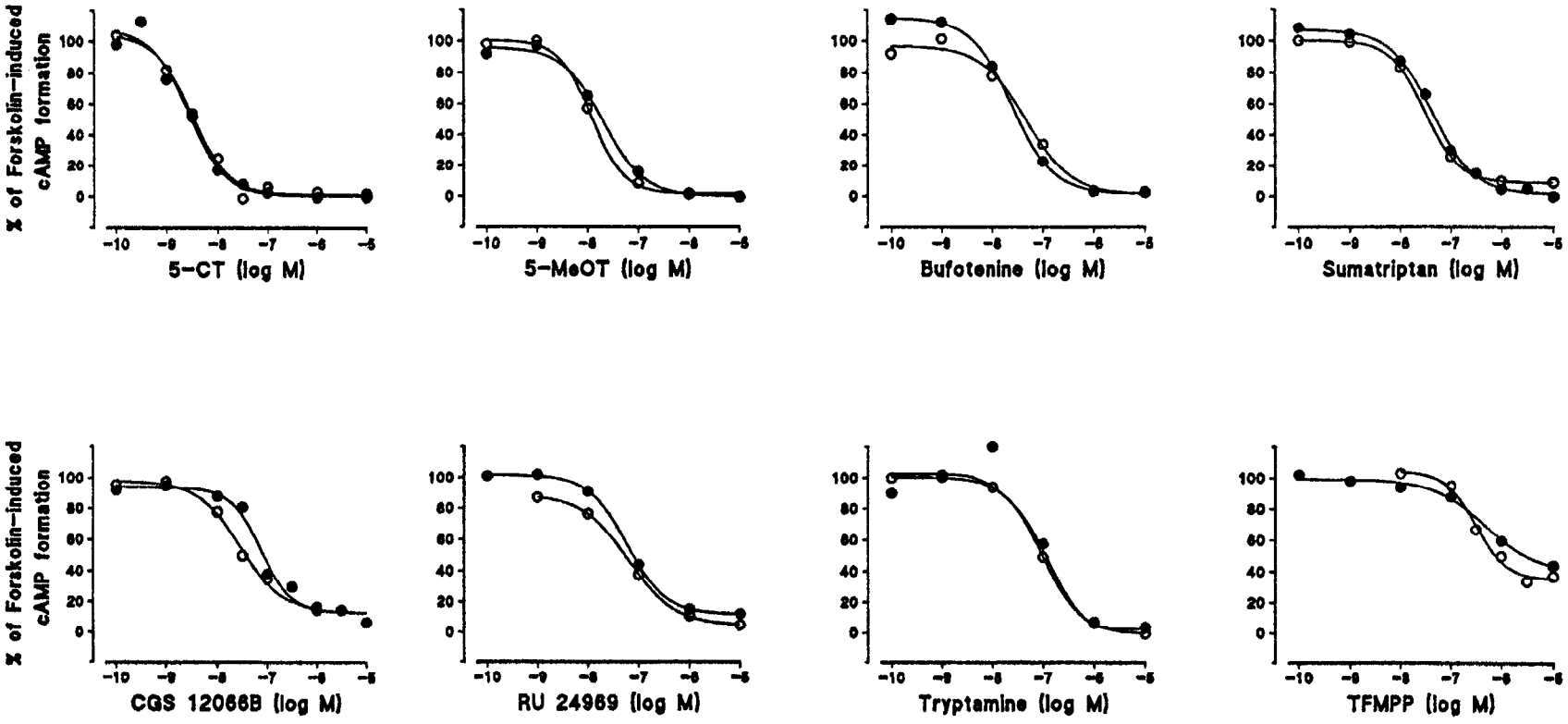

Fig. 2. Agonist effects of 5-HT receptor ligands on forskolin-induced cAMP formation in cultures of C6-glial/5-$HT_{1D\beta}$ and CHO-K1/5-$HT_{1D\beta}$ cells. C6-glial/5-$HT_{1D\beta}$ cells were cultured as described under "Methods" and exposed for 5 min to 100 μM forskolin and the indicated concentrations of compounds. Inhibition of forskolin-induced cAMP formation is expressed as a percentage of that obtained with 1 μM 5-HT. Curves were constructed using mean values of 3 to 4 independent experiments, each performed in triplicate. Curves for inhibition of forskolin-induced cAMP formation in CHO-K1/5-$HT_{1D\beta}$ cells were taken from Ref. 19. EC_{50}- and E_{max} values are presented in Table 1. Filled symbols: C6-glial/5-$HT_{1D\beta}$ cells; open symbols: CHO-K1/5-$HT_{1D\beta}$ cells.

Table 1. EC_{50}- and E_{max}-values of 5-HT receptor ligands for inhibitions of forskolin-induced cAMP formation in C6-glial/5-HT$_{1D\beta}$ and CHO-K1/5$_{1D\beta}$ cells

	Inhibition of 100 μM forskolin-stimulated cAMP formation	
	C6-glial/5-HT$_{1D\beta}$ EC_{50}, nM (E_{max}, %)	CHO-K1-5-HT$_{1D\beta}$* EC_{50}, nM (E_{max}, %)
5-CT	2.8 ± 1.1 (100)	3.8 (9.8)
5-Methoxytryptamine	21.0 ± 10.5 (99)	12.8 (98)
Bufotenine	41.0 ± 11.5 (97)	58.0 (96)
Sumatriptan	51.0 ± 21.3 (100)	40.3 (91)
CGS 12066B	72.0 ± 31.2 (93)	58.0 (85)
RU 24,969	79 ± 23.5 (85)	49.5 (89)
Tryptamine	100 ± 47.7 (96)	110 (94)
TFMPP	490 ± 99[b] (56)	317† (63)
Metergoline	≈10,000 (47)	7,664 (53)
GR 127,935	>10,000 (37)	>10,000 (16)
1-Napthylpiperazine	>10,000 (32)	>10,000 (13)
Methiothepin	>10,000 (0)	>10,000 (6.5)
Ritanserin	>10,000 (0)	>10,000 (0)

Inhibition of forskolin-induced cAMP accumulation in C6-glial/5-HT$_{1D\beta}$ cells was measured as described under "Methods." Basal accumulation of cAMP, 15.7 ± 4.5 pmol/well and accumulation of cAMP induced by 100 μM forskolin, 291 ± 60 pmol/well and accumulation of cAMP induced by 100 μM forskolin in the presence of 1 μM 5-HT, 26.3 ± 4.6 pmol/well. EC_{50} and E_{max} values are the mean ± SD and mean values obtained in 3 to 4 independent experiments, each performed in triplicate.

* EC_{50} values for inhibition of forskolin-induced cAMP formation in CHO-K1/5-HT$_{1D\beta}$ cells were taken from Ref. 19.

† Concentration (nM) to obtain 50% of the maximal inhibition induced by TFMPP.

tanserin. It would, thus, appear that neither of the transfected cell lines differentiates completely between the intrinsic activities of the above-mentioned full agonists, the partial agonist TFMPP, and the apparently silent antagonists methiothepin and ritanserin. The 5-HT$_{1D\beta}$ receptors in the transfected C6-glial cell line seem to be more sensitive to agonist activity because they detect some intrinsic activity for GR 127,735. This latter compound, an orally active 5-HT$_{1D}$ receptor antagonist [25] can, therefore, not be considered as an entirely silent 5-HT$_{1D\beta}$ receptor antagonist. Moreover, this compound also shows intrinsic activity at 5-HT$_{1D\alpha}$ [20], 5-HT$_{1B}$, and 5-HT$_{1A}$ receptor sites [26].

Different intrinsic activities between both cell lines were observed with metergoline and 1-naphtylpiperazine. In contrast to their pronounced antagonist activity in the transfected CHO-K1 cell line, partial antagonist and lack of antagonist activity was found in the transfected C6-glial cell line. These latter two compounds show apparently mixed antagonist/agonist properties and display partial agonist to antagonist activity, dependent on the target cell. The transfected CHO-K1 cell line seems to express the antagonist activity of these compounds; the calculated K_B values are very close to their *Ki* values (metergoline: 4.6 nM; 1-naphtylpiperazine: 8.8 nM; 19). These compounds have previously been reported to act as agonist, partial agonist, and/or antagonist at 5-HT$_{1D}$ and/or 5-HT$_{1B}$ receptor sites. Metergoline was found to act as an agonist at 5-HT$_{1D\beta}$ receptor sites in a homogenate of transfected LMtk-fibroblasts [27] (EC_{50}: 10 nM) and transfected CHO-K1 cells [23] (EC_{50}: 98 nM), and at native 5-HT$_{1B}$ receptor sites in opossum kidney cells [28] (EC_{50}: 347 nM) but as an antagonist at native 5-HT$_{1B}$ receptor sites in Chinese hamster lung fibroblasts [29] (EC_{50}: >10,000 nM, K_B value: 87 nM). 1-naphtylpiperazine shows partial agonist activity at 5-HT autoreceptors in slices of the substantia nigra and hypothalamus of guinea pigs, full antagonist activity *in vivo* in the substantia nigra of freely moving guinea pigs [30] and agonist activity at native 5-HT$_{1B}$ receptor sites in opossum kidney cells [28] (EC_{50}: 77 nM). These results emphasize the importance of the host cell in determining the downstream cascade coupling of a receptor and its functional consequences [24].

Several reasons may account for differences in intrinsic activities of a given drug. Receptor number has generally been considered as an determinant in explaining large variations in EC_{50} values and intrinsic activity for compounds as shown for transfected muscarinic receptors [31] and 5-HT$_{1A}$ receptors [18, 32]. In this study, this factor can be excluded because both transfected cell lines expressed a similar 5-HT$_{1D\beta}$ receptor number. In addition to receptor number, the subtype of guanine nucleotide-binding protein and effector may determine apparent ligand efficacy and consequently, explain differences between different systems. The procedures described here offer one approach to discriminate in a highly sensitive way between agonist and/or antagonist activities of compounds at 5-HT$_{1D\beta}$ receptors. Extrapolation of intrinsic data obtained with one particular cell system should be done with extreme caution. We propose that at least 2 independent cell systems be used, as described above for 5-HT$_{1D\beta}$ receptor sites, to define the intrinsic activity of compounds. This approach may further help the development of highly efficacious agonists and silent antagonists at 5-HT$_{1D\beta}$ receptors.

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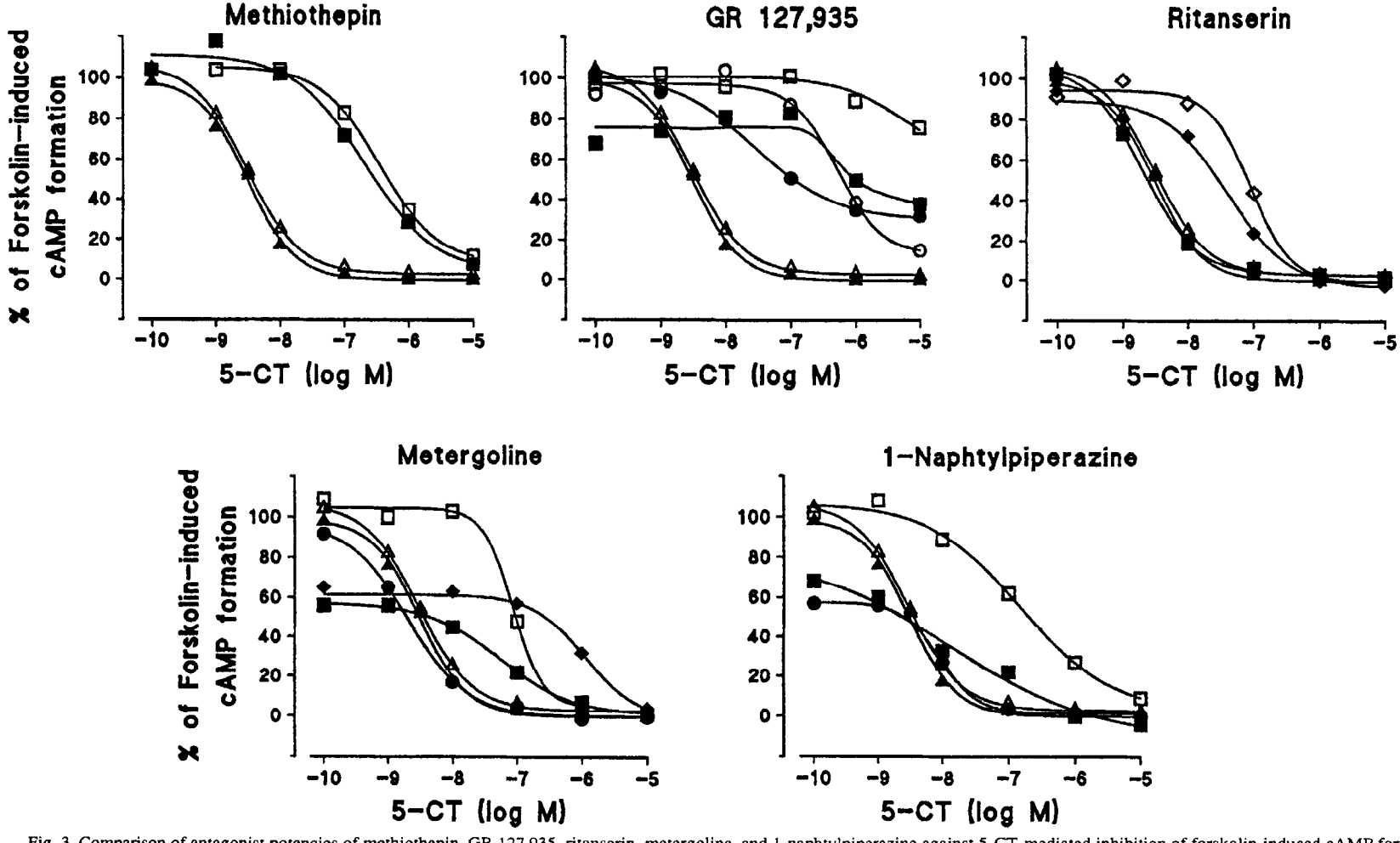

Fig. 3. Comparison of antagonist potencies of methiothepin, GR 127,935, ritanserin, metergoline, and 1-naphtylpiperazine against 5-CT-mediated inhibition of forskolin-induced cAMP formation in C6-glial/5-$HT_{1D\beta}$ and CHO-K1/5-$HT_{1D\beta}$ cells. C6-glial/5-$HT_{1D\beta}$ cells were cultured as described in the legend to Fig. 2 and exposed for 15 min with the indicated compound before cAMP formation was measured for 5 min in the presence of the indicated concentrations of 5-CT with the indicated compound. Inhibition of forskolin-induced cAMP formation is expressed as a percentage of that obtained with 1 μM 5-HT. Curves were constructed using mean values of 1 to 3 independent experiments, each performed in triplicate. Curves for antagonism of 5-CT-mediated inhibition of forskolin-stimulated cAMP formation in CHO-K1/5-$HT_{1D\beta}$ cells were taken from Ref. 19. EC'_{50} values of 5-CT and calculated K_B values are presented in Table 2. Filled symbols: C6-glial/5-$HT_{1D\beta}$ cells; Open symbols: CHO-K1/5-$HT_{1D\beta}$ cells. ▲/△: 5-CT alone; 5-CT in the presence of 0.1 μM, ●/○; 1 μM, ■/□ and 10 μM, ◆/◇ of compound.

Table 2. EC'_{50} values of 5-CT for inhibition of forskolin-induced cAMP accumulation in C6-glial/5-$HT_{1D\beta}$ and CHO-K1/5-$HT_{1D\beta}$ cells in the absence or presence of 0.1 to 10 μM of putative antagonists and calculated K_B values

		5-$HT_{1D\beta}$ receptor-mediated cAMP accumulation					
		C6-glial/5-$HT_{1D\beta}$			CHO-K1/5-$HT_{1D\beta}$		
Compound	Concentration (μM)	EC'_{50} 5-CT (nM)	n	K_B antagonist (nM)	EC'_{50} 5-CT (nM)	n	K_B antagonist (nM)
Methiothepin	1	50–700	3	4–59	340–700	3	8.6–10.7
GR 127,935	0.1	55–400	2	0.7–5.4	240–950	3	0.53–2.1
	1	1,000	1	–	>10,000	6	–
Ritanserin	1	1.4–4.5	3	–			
	10	36	1	843	32–130	3	172–631
Metergoline	0.1	0.9–3.8	2	–			
	1	partial	2	–	70–170	2	9.5–21.9
	10	partial	1				
1-Naphtylpiperazine	0.1	partial	2	–			
	1	partial	1	–	140–500	2	3.8–27.9

Experiments were performed as described in the legend to Fig. 3. The range of observed EC'_{50} values of 5-CT was given and K_B values calculated as described under "Methods." Partial inhibition of forskolin-stimulated cAMP formation (without addition of 5-CT) was obtained in C6-glial/5-$HT_{1D\beta}$ cells with 1 μM (36%) and 10 μM (55%) metergoline, and 0.1 μM (42%) and 1 μM (51%) 1-naphtylpiperazine. *n:* number of independent experiments, each performed in triplicate.

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