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

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Received June 19, 2003; accepted July 30, 2003

**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, neurotransmitter-like ligands, and especially some of these 5-HT$_{1A}$ ligands (i.e., F 13640) may in a selective way induce responses that may not be 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 (Arienès, 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 their activation contrasts with the broad spectrum of the ligands’ partial agonist properties as observed by measuring guanosine 5’-O-(3-[35 S]triphosphate ([35 S]GTP$\gamma$S) 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]GTP$\gamma$S 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).

**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 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 (methylaminopyridine) 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$_{\text{max}}$, 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]triphosphate ([35 S]GTP$\gamma$S) 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]GTP$\gamma$S 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.
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-HT1A receptors despite the fact that agonists seem more efficacious at dorsal raphe somatodendritic 5-HT1A 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-HT1A receptor to different Goi 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-HT1A receptor. Although 5-HT1A 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-HT1A agonists [i.e., 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), buspirone, and ipsapirone], fleinoxan and eptapirone, 5-carboxamidotryptamine (5-CT) as a high-efficacy but nonselective 5-HT1A 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-HT1A receptors. We found that highly efficacious 5-HT1A 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-HT1A 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′-thiotriphosphate ([35S]GTPγS) binding responses as a sensitive read-out for a wide spectrum of 5-HT1A receptor ligand activities. The Ca2+ data are discussed with regard to agonist-selective 5-HT1A receptor signaling.

**Materials and Methods**

**Transfection of CHO-K1 Cells with Human Recombinant 5-HT1A Receptor.** Subconfluent CHO-K1 cells were transiently transfected with 10 μg of a wild-type 5-HT1A 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). Emax values were defined as the ligand’s maximal high-magnitude Ca2+ response in percent age versus that obtained with 10 μM 5-HT. pEC50 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 (pIC50) 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-glial cells stably transfected with a recombinant human 5-HT1A 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]GTP(S) (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. Emax 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 Emax 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, 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 probenecid acid were from Sigma-Aldrich (St. Louis, MO). 5-CT, 8-OH-DPAT, and buspirone were obtained from Sigma/RBI (Natick, MA). Fleinoxan, ipsapirone, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyrindinyl)cyclohexanecarboxamide (WAY 100635), 1′-methyl-5′-(2′-methyl-4′-(5-methyl-1,2-oxadiazol-3-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydropiprofuro[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-HT1A 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-HT1B receptor antagonist SB 224289 (1 μM) exerted little, if any, effect (Fig. 2B). Prototypical 5-HT1A receptor agonists (i.e., buspirone, 8-OH-DPAT, and ipsapirone) did not induce a
significant Ca\(^{2+}\)/H\(_{11001}\) response despite the fact that their maximal [\(^{35}\)S]GTP/\(_{9253}\)S 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]GTP/\(_{9253}\)S 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\(^{2+}\)/H\(_{11001}\) responses with a magnitude that was similar or apparently identical to that of 5-HT (Table 1). F 13640 and F 14679, 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**

<table>
<thead>
<tr>
<th>Binding Affinities</th>
<th>CHO-K1</th>
<th>CHO-K1</th>
<th>CHO-K1</th>
<th>CHO-K1</th>
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<tr>
<td>5-HT (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>100 (±0.02)</td>
<td>100 (±0.07)</td>
<td>100 (±0.1)</td>
<td>100 (±0.2)</td>
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<tr>
<td>F 13640 (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>107 (±0.02)</td>
<td>99 (±0.15)</td>
<td>61 (±0.15)</td>
<td>110 (±0.15)</td>
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<tr>
<td>5-CT (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>7.32 (±0.19)</td>
<td>8.43 (±0.15)</td>
<td>8.31 (±0.15)</td>
<td>7.98 (±0.15)</td>
</tr>
<tr>
<td>F 14679 (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>87 (±0.16)</td>
<td>9.21 (±0.16)</td>
<td>100 (±0.16)</td>
<td>7.67 (±0.16)</td>
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<tr>
<td>Flesinoxan (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>5 (±1)</td>
<td>8.02 (±0.16)</td>
<td>7.48 (±0.16)</td>
<td>8.48 (±0.16)</td>
</tr>
<tr>
<td>Buspirone (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>4 (±1)</td>
<td>7.08 (±0.04)</td>
<td>6.40 (±0.04)</td>
<td>6.56 (±0.04)</td>
</tr>
<tr>
<td>8-OH-DPAT (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>3 (±1)</td>
<td>7.89 (±0.08)</td>
<td>7.41 (±0.08)</td>
<td>7.65 (±0.08)</td>
</tr>
<tr>
<td>Igesapirone (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>2 (±2)</td>
<td>7.69 (±0.13)</td>
<td>7.30 (±0.13)</td>
<td>7.66 (±0.13)</td>
</tr>
<tr>
<td>Eptapirone (pEC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>1 (±3)</td>
<td>7.46 (±0.05)</td>
<td>6.65 (±0.05)</td>
<td>7.10 (±0.05)</td>
</tr>
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</table>

* E<sub>max</sub> value (relative to 5-HT = 100%) of F 14679 in Ca<sup>2+</sup> response is significantly different (P < 0.05) from that by 5-CT.

* E<sub>max</sub> values (relative to 5-HT = 100%) for [35S]GTPγS binding responses on CHO-K1 membranes are significantly different (P < 0.001) from the corresponding Ca<sup>2+</sup> responses.

* E<sub>max</sub> values (relative to 5-HT = 100%) as obtained with the Ca<sup>2+</sup> response differed significantly from the corresponding E<sub>max</sub> values as estimated with the [35S]GTPγS binding response in C6-gial membranes for each investigated ligand with the exception of 5-HT.

* Colpaert et al. (2002).

* Koek et al. (2001).

* Pauwels et al. (1997).

* Pauwels et al. (1993).

C6-gial cells (Giles et al., 1996; Pauwels et al., 1996). F 13640 and F 14679 do not significantly bind to 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors. Moreover, we could not measure a 5-HT-mediated Ca<sup>2+</sup> response in nontransfected CHO-K1 cells (Fig. 1A). Therefore, the observed Ca<sup>2+</sup> responses with F 13640 and F 14679 can be considered to be due to activation by 5-HT<sub>1A</sub> receptors. A comparison with the [35S]GTPγS binding responses in transfected C6-gial membranes (Fig. 3; Table 1) indicated a significantly lower maximal [35S]GTPγS binding response for F 13640 with an unmodified potency (p > 0.05), and a significantly lower maximal [35S]GTPγS binding response for 5-CT and F 14679 accompanied with an enhanced potency (p ≤ 0.01). A comparison with the [35S]GTPγS binding responses in transfected CHO-K1 membranes did not reveal attenuated maximal responses, whereas pEC<sub>50</sub> values were significantly enhanced (p ≤ 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<sup>2+</sup> responses in CHO-K1 cells versus their spectrum of maximal [35S]GTPγS binding responses for the other ligands.
both transfected CHO-K1 and C6-glial membranes. The magnitude of Ca\(^{2+}\)/H11001 responses produced by the 5-HT1A receptor ligands examined here in CHO-K1 cells correlated weakly with their \[^{35}\text{S}]\text{GTP}^\gamma\text{S} binding response at 5-HT1A 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-HT1A receptor in transfected CHO-K1 cells indicate an atypical pharmacological 5-HT1A 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-HT1A 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-HT1B 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-HT1B receptor antagonist SB 224289 (Gaster et al., 1998) affected the 5-HT-mediated Ca\(^{2+}\) response little, if at all, whereas the selective 5-HT1A 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}]\text{GTP}^\gamma\text{S} binding responses, the Ca\(^{2+}\) effect in CHO-K1 cells seem to be mediated by endogenous G\(_{\text{i/o}}\) proteins. The \[^{35}\text{S}]\text{GTP}^\gamma\text{S} binding response monitors G\(_{\text{G}1}\) protein activation of endogenous G\(_{\text{i/o}}\) proteins, whereas the Ca\(^{2+}\) response is probably mediated by endogenous G\(_{\beta\gamma}\) subunits of activated G\(_{\text{i/o}}\) proteins in CHO-K1 cells. Although dual coupling of the cloned 5-HT1A receptor to both adenylyl cyclase and phospholipase C in HeLa cells is apparently mediated via the same G\(_{\text{G}1}\) 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-HT1A 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}]\text{GTP}^\gamma\text{S} 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}\text{S}]\text{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-HT1A receptor conformations that may affect in a different manner the downstream cascade of effector proteins. Activation of both populations of 5-HT1A 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-HT2A 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-HT2A 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-HT1A 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-HT1A receptor is able to couple to different $\text{G}_{i/o}$ proteins (i.e., Butkerait et al., 1995), one of which may act preferentially on phospholipase C.

Strikingly, F 14679 and fleroxaxin 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-HT1A 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-HT1A receptor. This would suggest that 5-HT1A 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-HT1A receptor ligands. The Ca2+ response in CHO-K1 cells constitutes a useful tool to identify highly selective 5-HT1A receptor ligands that are distinct from prototypical 5-HT1A ligands. This study further illustrates that the pharmacology of 5-HT1A receptors may be diverse and be codetermined by the effector pathway. The complex area of 5-HT1A receptor-coupled effector systems requires further research to analyze the activation of these effector systems and characterize this activation by effector-selective ligands.

Acknowledgments
We thank Dr. T. Wurch for critical reading of the manuscript. We also thank S. Bernoís and F. Finana for excellent technical assistance and S. Brignatzi for expert secretarial assistance.

References
Pauwels PJ, Tarifif S, Wurch T, and Colpaert FC (1997) Stimulated [35S]GTPγS binding by 5-HT1A receptor agonists in recombinant cell lines. Modulation of...


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