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Human 5-HT_{1F} receptor-stimulated [^{35}S]GTPγS binding: correlation with inhibition of guinea pig dural plasma protein extravasation

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Abstract

To determine the potency and efficacy of 5-HT_{1F} receptor ligands, a [^{35}S]GTPγS binding assay was developed and optimized for the human 5-HT_{1F} receptor. Compounds which are known to be effective in the abortive treatment of migraine were tested for efficacy and potency in this assay. Naratriptan, sumatriptan, zolmitriptan, and rizatriptan all had agonist activity. The 5-HT_{1F} receptor ligand LY334370 (4-fluoro-*N*-[3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl]-benzamide) was the most potent compound tested with an EC_{50} of 2.13 ± 0.15 nM. LY302148 (5-fluoro-3-[1-[2-(1-methyl-1*H*-pyrazol-4-yl)ethyl]-4-piperidinyl]-1*H*-indole), methysergide, LY306258 (3-dimethylamino-2,3,4,9-tetrahydro-1*H*-carbazol-6-ol), dihydroergotamine (DHE), L-694,247 and CP-122,288 were also investigated for potency and efficacy. There was a statistically significant correlation between the pEC_{50} for the stimulation of [^{35}S]GTPγS binding and the pID_{50} for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig. In the course of these studies, it was found that the purportedly selective 5-HT_{1D} receptor antagonist GR127935 inhibited 5-HT_{1F} receptor-stimulated [^{35}S]GTPγS binding with a K_i of 39.6 ± 9.5 nM. These studies demonstrate that 5-HT_{1F} receptor-mediated stimulation of [^{35}S]GTPγS binding in a clonal cell system is a reproducible, high throughput assay that is predictive of an in vivo model of 5-HT_{1F} receptor activation.

Keywords: 5-HT_{1F} receptor; [^{35}S]GTPγS binding

1. Introduction

The 5-HT_{1F} receptor is a member of the 5-HT_1 receptor family of G protein-coupled receptors. The human (Adham et al., 1993; Lovenberg et al., 1993), rat (Lovenberg et al., 1993), mouse (Amlaiky et al., 1992) and guinea pig forms (Adham et al., 1997) of the receptor have been cloned and expressed. Recently, it has been demonstrated that there is a statistically significant correlation between the affinity and potency of compounds for the 5-HT_{1F} receptor and their potency for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation, which is used as an animal model of migraine headache (Johnson et al., 1997). One consequence of activation of the 5-HT_{1F} receptor is the inhibition of forskolin-stimulated cAMP formation which can be used as a functional measure of activity at this receptor (Adham et al., 1993). However, an assay capable of rapidly determining the potency and efficacy of compounds at the 5-HT_{1F} receptor would be very useful for testing large numbers of compounds. Agonist activation of G protein-coupled receptors results in the release of GDP from the α-subunit of the G protein and the subsequent binding of GTP. The binding of the stable analogue [^{35}S]GTPγS can be used as an indicator of this receptor activation (Wieland and Jakobs, 1994). [^{35}S]GTPγS binding has been shown to be a useful tool for the quantification of the potency and efficacy of compounds at a variety of G protein-coupled receptors, including the 5-HT_{1A} (Newman-Tancredi et al., 1996), 5-HT_{1B} and 5-HT_{1D} (Pauwels et al., 1997; Thomas et al., 1995), muscarinic (Lazareno et al., 1993), opioid (Traynor and Nahorski, 1995), cannabinoid (Selley et al., 1996), mGluR1α (Akam et al., 1997) and A_1 adenosine (Lorenzen et al., 1993) receptors. The purpose of this study was to develop a reproducible, high throughput assay to assess the functional activation of the 5-HT_{1F} receptor by exploratory compounds. The results show that 5-HT_{1F} receptor-activated [^{35}S]GTPγS binding is an assay that provides these characteristics and is predictive of compound po-

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tency for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig.

2. Materials and methods

2.1. Chemicals

Dihydroergotamine (DHE), ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA), Tris base and sodium pyrophosphate ($Na_4P_2O_7$) were purchased from Sigma (St. Louis, MO). Magnesium chloride and sodium chloride were purchased from Mallinkrodt Specialty Chemicals (Paris, KY). Serotonin (5-HT) was purchased from Research Biochemicals (Natick, MA) or Sigma. Methysergide was provided by Sandoz Pharmaceuticals (East Hanover, NJ). Guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) and guanosine-5′-diphosphate (GDP) were purchased from Boehringer Mannheim (Indianapolis, IN). [^{35}S]GTPγS (1000–1250 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA). L-694,247 was purchased from Tocris-Cookson (Ballwin, MO). CP-122,288 was prepared by the Kansas Advanced Synthesis Laboratory (Lawrence, KS). Sumatriptan was isolated from 100 mg Imigran® tablets. Briefly, Imigran tablets (33×100 mg) were added to water (300 ml) and the mixture was stirred vigorously for 2 h. The resulting cloudy solution was filtered, made basic (pH > 8) with saturated Na_2CO_3 solution, and extracted with a 3:1 mixture of $CHCl_3$:*i*-PrOH (8×250 ml). The extract was dried (Na_2SO_4) and concentrated to give a white solid (3.37 g). Purification by flash chromatography (CH_2Cl_2:MeOH:NH_4OH; 100:20:1) made sumatriptan appear as white crystals (2.90 g). Recrystallization from EtOH (50 ml) made sumatriptan appear also as white crystals (2.39 g, m.p. 174–176°C). All other chemicals were synthesized at the Lilly Research Laboratories. Mouse LM(tk$^-$) cells stably expressing the human 5-HT$_{1F}$ receptor at a density of approximately 4.4 pmol/mg protein (Adham et al., 1993) were provided by Synaptic Pharmaceutical (Paramus, NJ).

2.2. [^{35}S]GTPγS binding studies

2.2.1. Membrane preparation

Mouse LM(tk$^-$) cells expressing the human 5-HT$_{1F}$ receptor and grown in suspension were harvested by centrifugation, resuspended in 50 mM Tris–HCl, pH 7.4, as aliquots of 2×10^8 cells and frozen at −70°C until the day of the assay. On the assay day, an aliquot of cells was thawed, resuspended in 35 ml of 50 mM Tris–HCl, pH 7.4, and centrifuged at $39\,800 \times g$ for 10 min at 4°C. The resulting pellet was resuspended in 50 mM Tris–HCl, pH 7.4, incubated for 10 min at 37°C and centrifuged at $39\,800 \times g$ for 10 min at 4°C. The pellet was resuspended and centrifuged once more, with the final pellet being suspended in 4 mM $MgCl_2$, 160 mM NaCl, 0.267 mM EGTA, 67 mM Tris–HCl, pH 7.4.

2.2.2. [^{35}S]GTPγS binding

The assay was modified from published conditions (Sim et al., 1995; Thomas et al., 1995). All incubations were

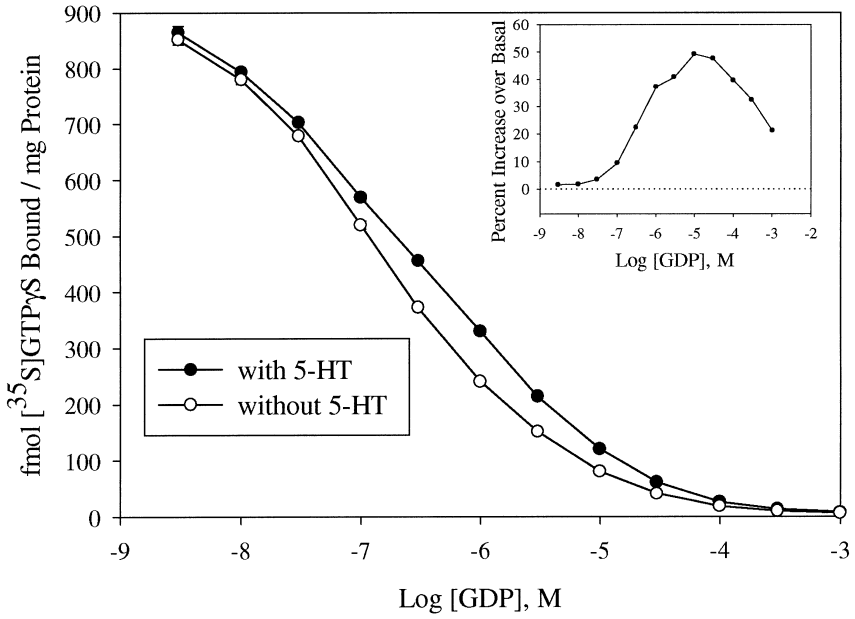


Fig. 1. Representative experiment of the effect of increasing the concentration of GDP on the binding of [^{35}S]GTPγS in the presence and absence of 10 μM 5-HT. The concentrations of $MgCl_2$, NaCl, EGTA and Tris were 5 mM, 100 mM, 0.2 mM and 50 mM, respectively. Each point is the mean ± standard error of triplicate determinations within the same experiment. Inset: the data are expressed as the percent increase over basal, with basal being the amount bound in the absence of 5-HT.

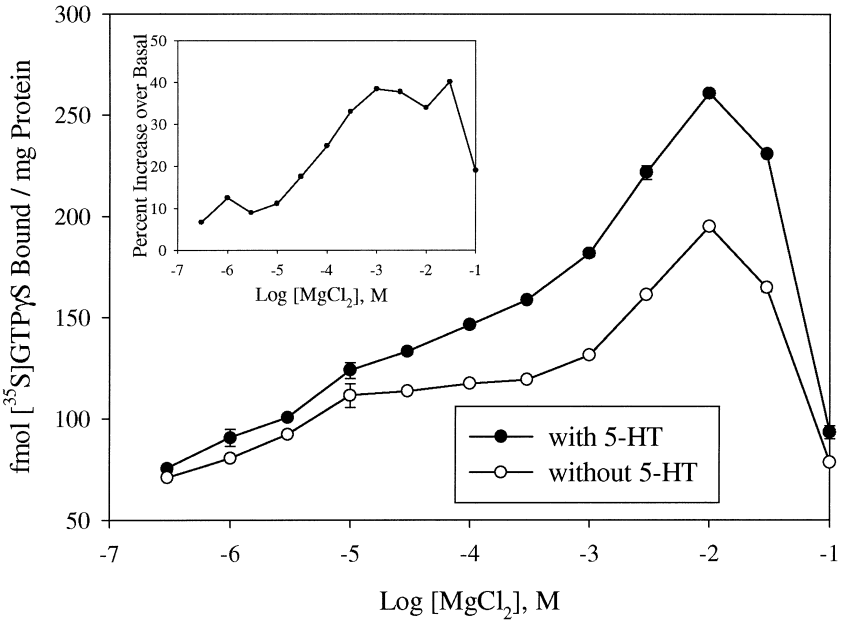


Fig. 2. Representative experiment of the effect of increasing the concentration of $MgCl_2$ on the binding of [^{35}S]GTPγS in the presence and absence of 10 μM 5-HT. The concentrations of GDP, NaCl, EGTA and Tris were 10 μM, 100 mM, 0.2 mM and 50 mM, respectively. Each point is the mean ± standard error of triplicate determinations within the same experiment. Inset: the data are expressed as the percent increase over basal, with basal being the amount bound in the absence of 5-HT.

performed in triplicate in a total volume of 800 μl. Test compounds in water, 200 μl, were added to 400 μl of Tris–HCl, pH 7.4, containing $MgCl_2$, NaCl, EGTA, GDP and [^{35}S]GTPγS. Membrane homogenate (200 μl) was added and the tubes were incubated for 30 min at 37°C. The final optimized concentrations of $MgCl_2$, NaCl, EGTA, GDP, [^{35}S]GTPγS and Tris were 3 mM, 120 mM, 0.2 mM, 10 μM, 0.1 nM and 50 mM, respectively. Using a Brandel cell harvester (model MB-48R, Brandel, Gaithersburg, MD), the incubations were terminated by vacuum filtration through Whatman GF/B filters which had been wetted with water or 20 mM $Na_4P_2O_7$ and pre-cooled with 4 ml of ice-cold 50 mM Tris–HCl, pH 7.4. The filters were then rapidly washed with 4 ml of

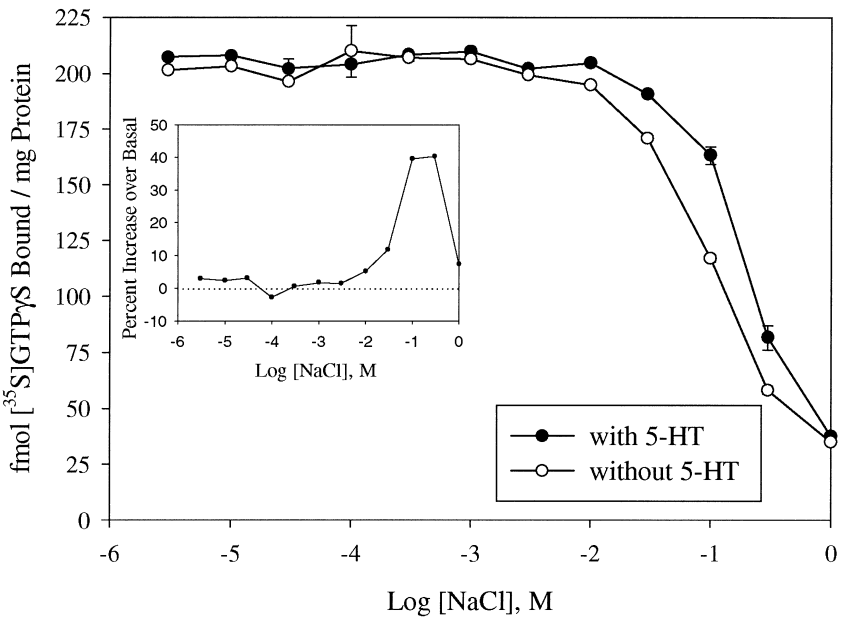


Fig. 3. Representative experiment of the effect of increasing the concentration of NaCl on the binding of [^{35}S]GTPγS in the presence and absence of 10 μM 5-HT. The concentrations of GDP, $MgCl_2$, EGTA and Tris were 10 μM, 5 mM, 0.2 mM and 50 mM, respectively. Each point is the mean ± standard error of triplicate determinations within the same experiment. Inset: the data are expressed as the percent increase over basal, with basal being the amount bound in the absence of 5-HT.

Table 1
Comparison of [^{35}S]GTPγS binding to homogenates of cells expressing the human 5-HT$_{1F}$ receptor with inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig

Compound	[^{35}S]GTPγS binding		Extravasation potency
	EC_{50} (nM) (n)	E_{max} (% 5-HT)	ID_{50} (pmol/kg)
LY334370	2.13 ± 0.15 (9)	94.6 ± 1.9[c]	0.06457[a]
LY302148	5.23 ± 0.11[b] (3)	17.0 ± 0.5[c]	0.7943[a]
Naratriptan	42.8 ± 2.4[b] (5)	94.5 ± 1.5[c]	0.3802[a]
CP-122,288	65.1 ± 10.6[b] (3)	95.8 ± 2.3	1.660
Methysergide	83.1 ± 15.6[b] (3)	82.7 ± 3.2[c]	
Serotonin	172 ± 6[b] (11)	102 ± 1.0	
LY306258	172 ± 9[b] (3)	89.2 ± 1.7[c]	8.710[a]
DHE	257 ± 38[b] (3)	67.8 ± 0.8[c]	23.44[a]
Sumatriptan	506 ± 33[b] (3)	93.0 ± 3.0[c]	43.65[a]
Zolmitriptan	611 ± 22[b] (3)	96.1 ± 2.1	8.710[a]
Rizatriptan	5380 ± 410[b] (3)	103 ± 1	219[a]
L-694,247	> 100 000 (3)		21 900[a]

[a] Denotes values taken from Johnson et al. (1997).
[b] Denotes EC_{50} values statistically different from the EC_{50} value for LY334370 ($P < 0.05$).
[c] Denotes E_{max} values statistically different from the E_{max} value for 5-HT ($P < 0.05$).
For [^{35}S]GTPγS binding, values are the mean ± standard error of the number of experiments in parentheses.

ice-cold 50 mM Tris–HCl, pH 7.4. The amount of [^{35}S]GTPγS captured on the filters was determined by liquid scintillation spectrometry. GTPγS, 10 μM, defined nonspecific binding. Protein was determined by the method of Bradford (1976).

2.3. Inhibition of trigeminal nerve-stimulated dural plasma protein extravasation

Experiments using animals were conducted according to the institutional guidelines of Eli Lilly, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). These studies were carried out as previously described (Johnson et al., 1997; Phebus et al., 1997). Briefly, the trigeminal ganglion of anesthetized guinea pigs was electrically stimulated to induce ipsilateral neurogenic dural plasma protein extravasation which was measured using fluorescence methods. The test compounds were administered intravenously 10 min prior to the trigeminal ganglion being stimulated for 3 min (1 mA, 5 Hz, 4 ms). Animals were sacrificed 15 min following stimulation, the amount of plasma extravasation was determined, and dose–response curves were generated.

2.4. Statistical analysis

Efficacy values for selected compounds were expressed as the percent of [^{35}S]GTPγS binding relative to 10 μM 5-HT which was run as a standard with each concentration–response curve. Nonlinear regression analysis was performed on the concentration response curves using a four-parameter logistic equation described by De Lean et al. (1982). Analysis of variance, followed by the Tukey–Kramer Honestly Significant Difference test (JMP; SAS

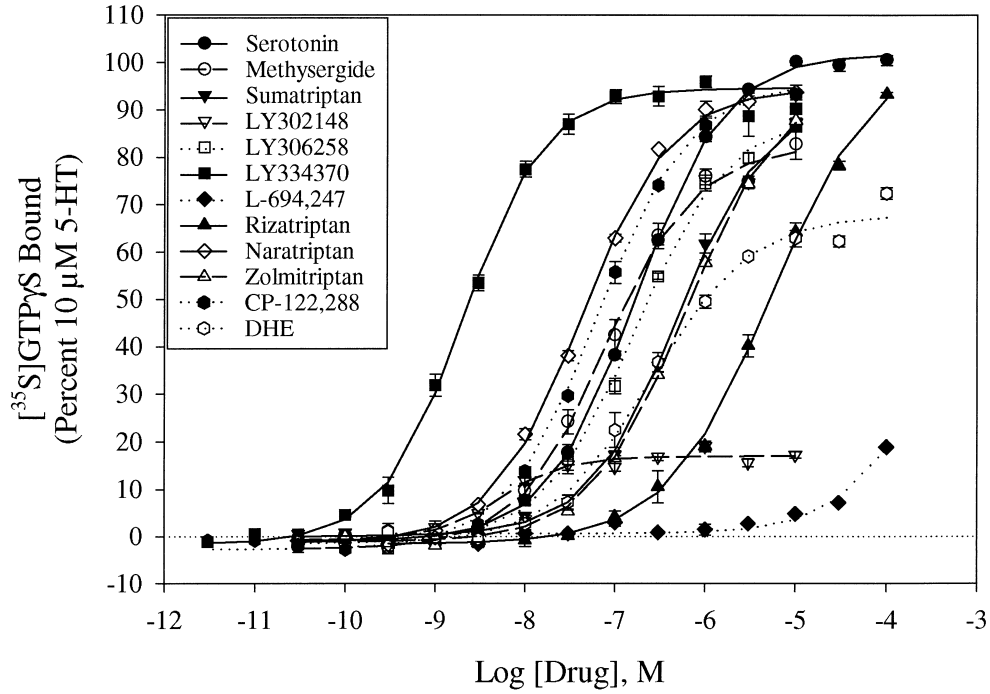
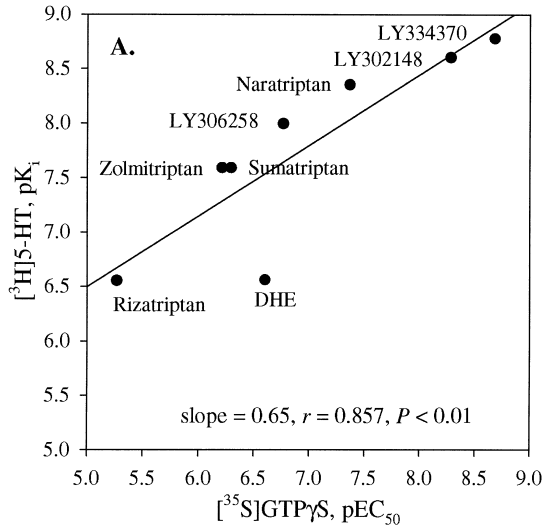


Fig. 4. Stimulation of [^{35}S]GTPγS binding to homogenates of cells expressing the human 5-HT$_{1F}$ receptor. Points are the mean ± standard error of at least three separate experiments except for the CP-122,288 curve, where 1 experiment was a 12-point curve and 6-point curves were run for two experiments.



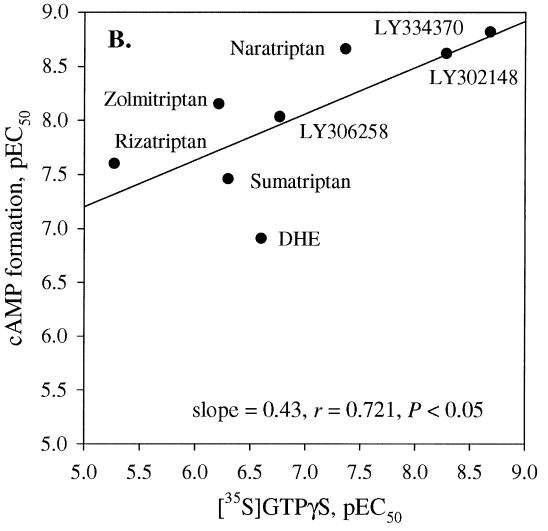


Fig. 5. (A) Correlation between the pK_i (−log, M) for the inhibition of [^{3}H]5-HT binding (Johnson et al., 1997) and the pEC_{50} (−log, M) for the stimulation of [^{35}S]GTPγS binding to homogenates of cells expressing the human 5-HT_{1F} receptor. (B) Correlation between the pEC_{50} (−log, M) for the inhibition of forskolin-stimulated cAMP formation (Johnson et al., 1997) in cells expressing the human 5-HT_{1F} receptor and the pEC_{50} (−log, M) for the stimulation of [^{35}S]GTPγS binding to homogenates of cells expressing the human 5-HT_{1F} receptor.

Institute, Cary, NC) was performed on the pEC_{50} values and the E_{max} values. For GR127935, the IC_{50} values were converted to K_i values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

3. Results

3.1. Characterization of the [^{35}S]GTPγS binding assay

Initially, conditions for the binding assay were determined by first optimizing the concentration of GDP in the assay. The binding assay was performed in the presence and absence of 10 μM 5-HT while increasing the concentration of GDP from 3 nM to 1 mM. A representative experiment is shown in Fig. 1. The optimal concentration of GDP was determined to be 10 μM. Using 10 μM GDP, the effect of changing $MgCl_2$ and NaCl concentrations was investigated. While holding the concentration of NaCl constant at 100 mM, the concentration of $MgCl_2$ added to the assay was increased from 300 nM to 100 mM in the presence and absence of 10 μM 5-HT (Fig. 2). Similarly, while holding $MgCl_2$ constant at 5 mM, the concentration of NaCl added to the assay was increased from 3 μM to 1 M in the presence and absence of 10 μM 5-HT (Fig. 3). Note that there is no appreciable agonist-induced stimulation of binding until the NaCl concentration reaches at least 30 to 100 mM. The optimal concentrations of $MgCl_2$ and NaCl were determined to be 3 mM and 120 mM, respectively. Additionally, an optimal time for incubation at 37°C was determined to be 30 min. The concentration of [^{35}S]GTPγS was chosen to be 0.1 nM, since this yielded a reproducible signal while conserving radioligand. These conditions resulted in a very reproducible assay with a typical signal of approximately 100% to 150% above basal values.

3.2. Concentration–response curves

A number of different 5-$HT_{1B/1D}$ and 5-HT_{1F} ligands were tested for their ability to stimulate the binding of [^{35}S]GTPγS to membrane homogenates of cells expressing the human 5-HT_{1F} receptor. Among these compounds, LY302148 (5-fluoro-3-[1-[2-(1-methyl-1*H*-pyrazol-4-

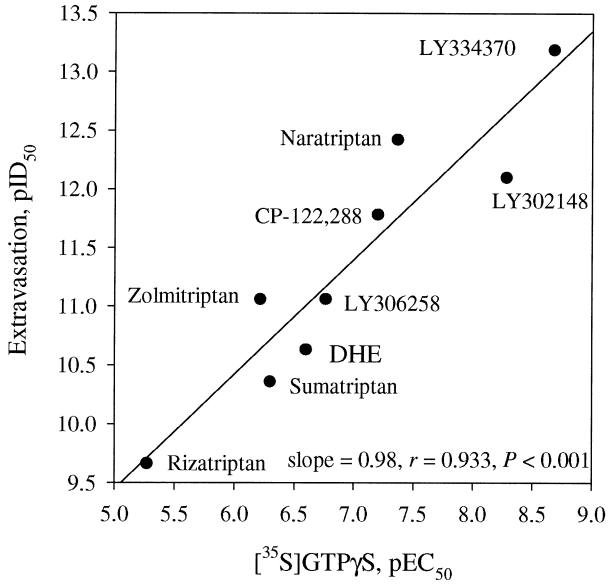


Fig. 6. Correlation between the potency of compounds for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig, pID_{50} (−log, mol/kg), (Table 1; Johnson et al., 1997) and the pEC_{50} (−log, M) for the stimulation of [^{35}S]GTPγS binding to homogenates of cells expressing the human 5-HT_{1F} receptor.

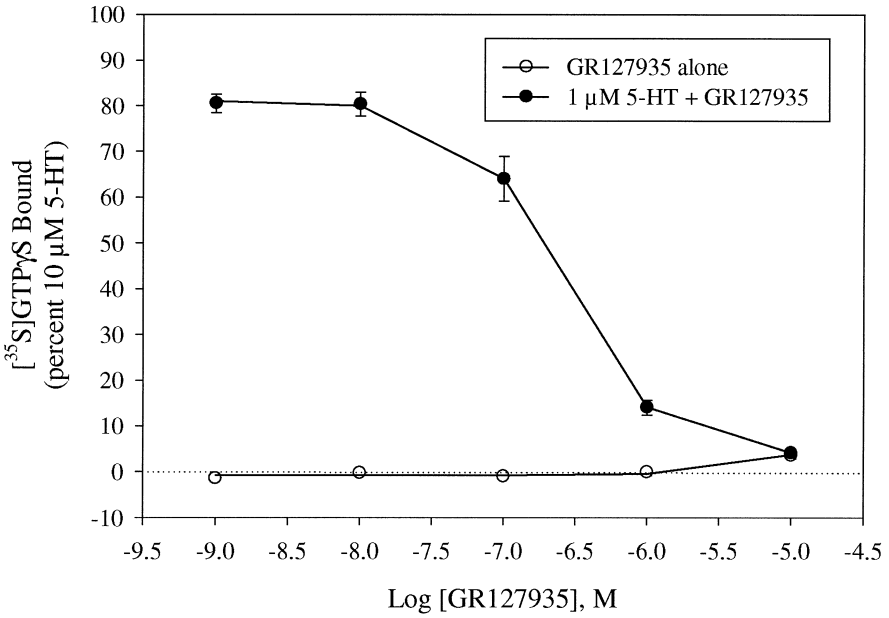


Fig. 7. GR127935 does not stimulate the binding of [^{35}S]GTPγS at concentrations up to 10 μM. GR-127,935 inhibits 1 μM 5-HT-stimulated [^{35}S]GTPγS binding to homogenates of cells expressing the human cloned 5-HT$_{1F}$ receptor in a concentration-dependent manner with an IC$_{50}$ of 270 ± 65 nM, which results in a calculated K_i of 39.6 ± 9.5 nM ($n = 3$).

yl)ethyl]-4-piperidinyl]-1*H*-indole), LY306258 (3-dimethylamino-2,3,4,9-tetrahydro-1*H*-carbazol-6-ol) and LY334370 (4-fluoro-*N*-[3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl]-benzamide) show relative selectivity for the 5-HT$_{1F}$ receptor compared to the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Johnson et al., 1997). The EC$_{50}$ and efficacy values for each compound are shown in Table 1. The efficacy of all agonists was calculated relative to 10 μM 5-HT, which was run as a standard with each concentration–response curve. Serotonin displayed an EC$_{50}$ of 172 ± 6 nM (n = 11) (Table 1, Fig. 4). The rank order of potency was LY334370 > LY302148 > naratriptan = CP-122,288 = methysergide > LY306258 = serotonin > DHE > sumatriptan = zolmitriptan > rizatriptan > L-694,247. Relative to the E_{max} for 5-HT, the compounds LY302148 and DHE were clearly partial agonists. The E_{max} values for methysergide, LY306258, LY334370, naratriptan and sumatriptan were also statistically different from the E_{max} for 5-HT; however, the magnitude of the difference is small. Though the efficacy values for these seven compounds were statistically different from serotonin in the [^{35}S]GTPγS binding studies, they were full agonists (methysergide was not tested) when tested for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig (Johnson et al., unpublished observations).

There was a statistically significant correlation between the pEC$_{50}$ (−log, M) for the stimulation of [^{35}S]GTPγS binding (current study) and the pK_i (−log, M) for the inhibition of [^{3}H]5-HT binding (Johnson et al., 1997) to homogenates of cells expressing the human 5-HT$_{1F}$ receptor ($r = 0.857$, $P < 0.01$, Fig. 5A). In addition, there was a statistically significant correlation between the pEC$_{50}$ for the stimulation of [^{35}S]GTPγS binding (current study) and the pEC$_{50}$ (−log, M) for the inhibition of forskolin-stimulated cAMP formation (Johnson et al., 1997) in cells expressing the human 5-HT$_{1F}$ receptor ($r = 0.721$, $P < 0.05$, Fig. 5B). Even more interesting was the statistically significant correlation ($r = 0.933$, $P < 0.001$, Fig. 6) between the pEC$_{50}$ for the stimulation of [^{35}S]GTPγS binding (current study) to homogenates of cells expressing the human 5-HT$_{1F}$ receptor and the pID$_{50}$ (−log mol/kg) for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig (Table 1; Johnson et al., 1997). Note that the slope value for this correlation is 0.98.

The 5-HT$_{1B/1D}$ antagonist GR127935 was also tested for its ability to stimulate [^{35}S]GTPγS binding. Up to 10 μM, GR127935 displayed no appreciable agonist activity relative to 10 μM 5-HT (Fig. 7). However, GR127935 was able to inhibit 1 μM 5-HT-stimulated [^{35}S]GTPγS binding with a K_i of 39.6 ± 9.5 nM ($n = 3$).

4. Discussion

A [^{35}S]GTPγS binding assay has been developed for the human cloned 5-HT$_{1F}$ receptor. As has been described for many other G protein-coupled receptors, conditions for the 5-HT$_{1F}$ receptor expressed in the LM(tk$^-$) cell were optimized. These included the concentrations of $MgCl_2$, NaCl and GDP. It was determined that the best concentrations of $MgCl_2$, NaCl and GDP were 3 mM, 120 mM and 10 μM, respectively. Once these conditions were established, the

incubation time and [^{35}S]GTPγS concentrations were optimized to 30 min at 37°C and 0.1 nM.

Serotonergic inhibition of trigeminal nerve-stimulated dural plasma protein extravasation has been attributed to a 5-HT$_{1B}$ mechanism in the rat or a 5-HT$_{1D}$ mechanism in the guinea pig (Matsubara et al., 1991). Some confusion exists as to the nature of these receptor interactions, due in part to the changing nomenclature of the 5-HT$_{1B/1D}$ receptors (Hoyer et al., 1994). Thus, receptors previously referred to as 5-HT$_{1D}$ in the literature often likely refer to what are now called non-rodent 5-HT$_{1B}$ receptors according to this new nomenclature. Rizatriptan (MK-462) in the rat (Shepheard et al., 1995), naratriptan in the rat (Connor et al., 1997) and zolmitriptan (BW311C90) in the guinea pig (Martin et al., 1997) have also been described as being effective in the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation via a 5-HT$_{1B}$ and/or 5-HT$_{1D}$ receptor mechanism.

Several authors have concluded that an additional 5-HT$_1$-like receptor other than 5-HT$_{1B/1D}$ is responsible for the effects of certain compounds for the inhibition of trigeminal nerve-stimulated extravasation. For example, CP-122,288 was approximately 1000-fold more potent than sumatriptan for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig (Lee and Moskowitz, 1993) and in the rat (Gupta et al., 1995), while binding affinities for the compounds at human 5-HT$_{1D}$ (formerly 5-HT$_{1D\alpha}$) and human 5-HT$_{1B}$ (formerly 5-HT$_{1D\beta}$) receptors did not correlate with this difference in potency (Gupta et al., 1995). In another study in the rat, CP-122,288 was also greater than 1000-fold more potent than sumatriptan for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation, while there was a little difference in potency between CP-122,288 and sumatriptan in a [^{35}S]GTPγS binding assay using the cloned rat 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Shepherd et al., 1997). Note that in our laboratories, the difference between the potency of CP-122,288 and sumatriptan for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation is only about 26-fold, since we find sumatriptan to have greater potency. This is most likely due to a difference in experimental methodology.

CP-122,288, rizatriptan, naratriptan, sumatriptan and zolmitriptan all had agonist activity in the 5-HT$_{1F}$ GTPγS binding assay relative to 5-HT (Table 1). There is a statistically significant correlation between the potency of compounds tested in the [^{35}S]GTPγS binding assay (current study) and their [^{3}H]5-HT binding affinities (Johnson et al., 1997) for the human 5-HT$_{1F}$ receptor (Fig. 5A). In addition, there is a statistically significant correlation between the potency for the stimulation of [^{35}S]GTPγS binding in homogenates of cells expressing the human 5-HT$_{1F}$ receptor (current study) and the potency for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig (Table 1; Johnson et al., 1997) (Fig. 6). These results provide further evidence that the 5-HT receptor which is responsible for inhibiting dural plasma protein extravasation in the guinea pig is the 5-HT$_{1F}$ receptor.

To date, no selective 5-HT$_{1F}$ receptor antagonists have been described in the literature. GR127935 has been described as a selective antagonist for the 5-HT$_{1B/1D}$ receptor (Skingle et al., 1996). In some studies, GR127935 has been demonstrated to have agonist activity at the cloned human 5-HT$_{1D}$ (formerly 5-HT$_{1D\alpha}$) receptor using the inhibition of forskolin-stimulated cAMP formation (Pauwels and Colpaert, 1995), and partial agonist activity at cloned human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors expressed in cell lines using a [^{35}S]GTPγS binding assay (Watson et al., 1996). GR127935 has been reported to antagonize sumatriptan but not CP-122,288 inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig (Yu et al., 1997). The authors concluded that sumatriptan was probably acting via a 5-HT$_{1B/1D}$ receptor and not the 5-HT$_{1F}$ receptor since GR127935 was reported to have low affinity for the 5-HT$_{1F}$ receptor in guinea pig forebrain homogenates. In the current study, GR127935 had no efficacy as an agonist at the 5-HT$_{1F}$ receptor in the [^{35}S]GTPγS binding assay. However, GR-127,935 inhibited 1 μM 5-HT-stimulated binding with a K_i of 39.6 nM (Fig. 7). The measure of affinity of GR127935 for the 5-HT$_{1F}$ receptor in the present study agrees with that of Pauwels et al. (1997), who found an affinity of 45.7 nM when measured against [^{3}H]5-HT binding. It appears from the current studies that GR127935 can antagonize 5-HT$_{1F}$ receptors with reasonable potency. Thus, these data demonstrate that caution must be observed in the interpretation of results obtained using GR127935 as an antagonist in vitro and especially in vivo.

In summary, 5-HT$_{1F}$ receptor-stimulated [^{35}S]GTPγS binding in cells expressing the cloned human receptor is a very reproducible, high throughput assay that can be run using previously frozen cell pellets and is very predictive of the potency for the inhibition of trigeminal nerve-stimulated dural plasma protein extravasation in the guinea pig, an in vivo model of 5-HT$_{1F}$ receptor activation.

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