Development of a radioligand binding assay for 5-HT₄ receptors in guinea-pig and rat brain

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- 1 The 5-HT₄ receptor antagonist, GR113808, has been radiolabelled to a high specific activity with tritium.
- 2 Characterization of specific [³H]-GR113808 binding in homogenates of guinea-pig striatum and hippocampus revealed a single site of high affinity (K_d values 0.20 and 0.13 nM respectively).
- 3 [³H]-GR113808 binding was reversible and displayed rapid kinetics such that association and dissociation were complete within 3 min.
- 4 Specific [³H]-GR113808 binding was potently and stereoselectively inhibited by agonists and antagonists acting at the 5-HT₄ receptor but not by compounds selective for other 5-HT receptors or other neurotransmitter receptors.
- 5 Autoradiographic analysis revealed a discrete localization in both guinea-pig and rat brain with high concentrations of binding in brain areas such as the striatum, substantia nigra and olfactory tubercle.
- 6 [³H]-GR113808 binding to homogenates of guinea-pig striatum meets the criteria for labelling of the 5-HT₄ receptor and, as such, represents the first characterization of this receptor in a radioligand binding assay.

Keywords: 5-HT₄ receptors; GR113808; radioligand binding; guinea-pig hippocampus; guinea-pig striatum; autoradiography

Introduction

Receptors for 5-hydroxytryptamine (5-HT) of the 5-HT₄ type have been identified in a variety of functional preparations both centrally and peripherally (Bockaert *et al.*, 1992). In the central nervous system 5-HT₄ receptors have been shown to mediate an activation of adenylate cyclase (Dumuis *et al.*, 1988; Bockaert *et al.*, 1990). In the periphery, 5-HT₄ receptors produce a variety of responses including relaxation of smooth muscle (Reeves *et al.*, 1991), neuronal cholinergically mediated contraction of guinea-pig ileum and proximal colon (Eglen *et al.*, 1990; Elswood *et al.*, 1991), enhancement of the 'twitch' response in electrically field stimulated guinea-pig ileum (Craig & Clarke, 1990) and induction of chloride secretion in rat distal colon (Bunce *et al.*, 1991). 5-HT₄ receptors have also been implicated in cardiovascular effects in both pig and man (Bom *et al.*, 1988; Villalon *et al.*, 1990; Kaumann *et al.*, 1990).

GR113808 ([1-[2-methylsulphonyl]amino]ethyl]-4-piperidin-yl)methyl 1-methyl-1H-indole-3-carboxylate (Figure 1, Grossman *et al.*, 1993) is a highly potent and competitive antagonist of 5-HT₄ receptor-mediated effects in guinea-pig proximal colon and rat oesophagus (pA_2 9.2; Schild slope 1.1 and pA_2 9.5 Schild slope 0.7 respectively). GR113808 is also highly selective with only weak affinity at 5-HT₃ receptors (pK_i 6.0) and no activity at other 5-HT receptors (up to 10 μ M). GR113808 has been radiolabelled to a specific activity of 83.4 Ci mmol⁻¹ with tritium and we now report on the binding characteristics of this compound.

Methods

Tissue preparation

Dunkin Hartley guinea-pigs (~400 g) were killed by cervical dislocation and the brain removed and dissected. Tissue was used immediately.

For binding, pooled striatal or hippocampal brain tissue was placed in 15 volumes of HEPES buffer (50 mM pH 7.4, 4°C). This was homogenized in an Ultra-Turrax homogenizer for 12 s and subsequently centrifuged at 48,000 *g* and 4°C in a Sorvall RC5C centrifuge. The resulting pellet was resuspended in HEPES buffer to make a homogenate at 30 mg ml⁻¹ for striatum and 50 mg ml⁻¹ for hippocampus.

Homogenate binding

All determinations were performed in triplicate. Assay tubes contained: 300 μ l HEPES buffer at pH 7.4, 200 μ l of a solution of either a competing agent (for drug competition studies), 5-HT to give a final concentration of 30 μ M (to determine non-specific binding) or buffer (for determination of total binding), 400 μ l of [³H]-GR113808 in HEPES buffer

Figure 1 Structure of GR113808. *Indicates the site at which the compound has been radiolabelled with tritium.

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to give a final concentration of 0.1 nM and 100 μ l of tissue preparation. Assay tubes were incubated at 37°C.

To determine the rate of association, reactions were stopped 0, 0.5, 1, 2, 3, 4, 5, 10, 15, 20 min after addition of the tissue and specific binding was determined. For dissociation, 5-HT (30 μ M) was added to the assay tube for the same time periods after the reaction had proceeded for 30 min. The reaction was terminated by rapid vacuum filtration and washing (1 $\times$ 4 ml; see Results) with ice-cold buffer through Whatman GF/B filter paper using a Brandel Cell Harvester. Filters were presoaked in a solution of polyethylenimine (~0.1%) to reduce filter binding. For saturation analysis assay tubes were set up as above with the exception that 15 concentrations of [³H]-GR113808 in HEPES buffer were used to give final concentrations of 0.004–2 nM. For both saturation analysis and drug competition studies, assay tubes were incubated at 37°C for 30 min and the reaction terminated as above. Filters were placed in 8 ml of Packard Emulsifier Safe scintillation cocktail overnight before scintillation counting.

Protein assays

Tissue protein was assayed by the Bio-Rad Protein Assay (Bradford, 1976). Bovine serum albumin was used as the standard.

Data calculation

Equilibrium saturation parameters and pK_i values were calculated by use of the computer programme 'Ligand' (Munson & Rodbard, 1980). Hill coefficients were obtained with the computer programme 'Allfit' (DeLean *et al.*, 1977). Significance testing was carried out with an unpaired Student's *t* test. Values were considered significantly different when $P < 0.05$.

Autoradiography

Lister hooded rats or Dunkin Hartley guinea-pigs were killed and the brain removed. Slide mounted sections of the brain were prepared as described by Kilpatrick *et al.* (1988). Briefly, rat and guinea-pig brains were mounted in O.C.T. embedding compound, frozen in liquid nitrogen and 20 μ m sections cut using a cryostat. Sections were taken alternately onto separate slides for determination of total and non-specific binding. The slide-mounted sections were incubated in HEPES buffer containing 0.1 nM [³H]-GR113808 at 37°C for 30 min. Non-specific binding was determined by the addition of 5-HT to the HEPES buffer to give a final concentration of 30 μ M. At the end of the 30 min incubation period slides were washed in ice-cold buffer for 2 $\times$ 5 s, dipped in ice-cold water to remove the buffer and then allowed to dry in a stream of air. Slides were exposed to autoradiographic film (Hyperfilm - ³H; Amersham International) for 6 weeks prior to development. Quantitative measurements of specific binding were made with a Cambridge Instruments Quantimet 970.

Drugs and solutions

[³H]-GR113808 had a specific activity of 83.4 Ci mmol⁻¹ and was synthesized by Amersham International. The following compounds (source) were used: 5-hydroxytryptamine creatinine sulphate (Sigma), 5-methoxytryptamine hydrochloride (Sigma), metoclopramide hydrochloride (Sigma), dopamine (Sigma), γ -aminobutyric acid (Sigma), atropine sulphate (Sigma), tropisetron (Research Biochemicals Inc), ketanserin tartrate (Janssen), spiperone (Janssen) methysergide maleate (Sandoz), histamine phosphate (BDH), granisetron hydrochloride (Glaxo), ondansetron hydrochloride (Glaxo), α -methyl-5-hydroxytryptamine maleate (α -Me-5-HT; Glaxo), 2-methyl-5-hydroxytryptamine HCl.H₂O (2-Me-5-HT; Glaxo), 5-carboxamidotryptamine maleate (5-CT; Glaxo), 8-hydroxy-

2-(di-n-propylamino)tetralin (8-OH-DPAT, Glaxo), 5-OH-indalpine maleate (Glaxo), cisapride (Janssen; made into tartrate at Glaxo), renzapride (Glaxo), R,S-zacopride, R-zacopride, S-zacopride hydrochloride (Glaxo), SDZ205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester; Glaxo), BIMU1 (endo-N-(8-methyl-8-azabicyclo-[3.2.1] oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxamide; Glaxo), BIMU8 (endo-N-(8-methyl-8-azabicyclo-[3.2.1] oct-3-yl)-2,3-dihydro-(1-methyl) ethyl-2-oxo-1H-benzimidazole-1-carboxamide; Glaxo), GR113808 maleate (Glaxo).

Results

Establishment of suitable preparation

In washed crude homogenates of guinea-pig striatum 5-HT (30 μ M) inhibited 70–80% of total [³H]-GR113808 (0.1 nM) binding. In guinea-pig hippocampus specific binding was slightly lower (50–70%) (specific binding = total binding minus binding in the presence of 5-HT (30 μ M)). The specific binding in both tissues was linear over a wide range of tissue concentration (1–10 mg, per assay tube; data not shown).

Association and dissociation of specific [³H]-GR113808 binding

The rates of association and dissociation of specific [³H]-GR113808 binding were investigated. Results indicate that, in

Figure 2 (a) Saturation analysis of [³H]-GR113808 (0.004–2 nM) binding in guinea-pig striatum where (○) is total binding; (●) is specific binding; and (▲) is non-specific binding determined by the addition of 30 μ M 5-HT. (b) Scatchard transformation of specific [³H]-GR113808 (0.004–2 nM) binding to homogenates of guinea-pig striatum (●) and guinea-pig hippocampus (○). Results are from a single experiment but are representative of three such experiments.

both hippocampus and striatum, association and dissociation occur very rapidly such that the ligand is either fully associated or dissociated within 3 min. From a number of studies using longer association periods it was found a steady-state of specific binding existed for at least 1 h (data not shown). As the ligand was half saturated at time 0 min it was not possible to calculate rate constants accurately for association and dissociation. Because of this fast dissociation rate, samples were only washed once during the filtration stage of the assay to minimize dissociation of ligand from the receptor.

Saturation analysis

Equilibration saturation studies of specific binding of [3 H]-GR113808 (0.004–2 nM) revealed a single saturable site of high affinity in both tissues (striatum; $K_d = 0.20 \pm 0.02$ nM, $B_{max} = 92.7 \pm 7.1$ fmol mg $^{-1}$ protein; hippocampus: $K_d = 0.13 \pm 0.01$ nM, $B_{max} = 40.0 \pm 1.1$ fmol mg $^{-1}$ protein; values are $\pm$ s.e.mean, $n = 3$) (Figure 2). Non-specific binding increased linearly with increasing ligand concentration in both tissues.

Drug competition studies

A range of 5-HT $_4$ receptor agonists and antagonists completely inhibited the specific binding of [3 H]-GR113808. Inhibition curves were apparently monophasic (Figure 3). Affinity values and Hill slopes derived from these curves using computer analysis are presented in Table 1. Of the agonists tested, only those active in 5-HT $_4$ receptor containing preparations (5-HT, 5-methoxytryptamine, 5-CT, α -Me-5-HT, BIMU1, BIMU8) potently inhibited [3 H]-GR113808 binding. By contrast, agonists for other 5-HT receptors, for example the 5-HT $_{1A}$ agonist, 8-OH-DPAT, the so called 5-HT $_{1P}$ receptor agonist, 5-OH-indalpine, the 5-HT $_{1D}$ receptor agonist, sumatriptan and the 5-HT $_3$ receptor agonist, 2-Me-5-HT, had no effect on [3 H]-GR113808 binding at concentrations up to 10 μ M. The substituted benzamides, metoclopramide, cisapride, renzapride and zacopride, partial agonists at the 5-HT $_4$ receptor all potently inhibited [3 H]-GR113808 binding. Specific [3 H]-GR113808 binding was also inhibited by the 5-HT $_4$ receptor antagonists, tropisetron, SDZ205-557 and unlabelled GR113808. Hill slopes for the inhibition of [3 H]-GR113808 binding by 5-HT $_4$ receptor agonists and antagonists were not significantly different from unity (95% confidence limits overlapped unity), with the exception of 5-methoxytryptamine, 5-CT, α -Me-5-HT, renzapride and tropisetron in guinea-pig striatum and BIMU1 in guinea-pig hippocampus.

Several antagonists at other 5-HT receptors and ligands for other neurotransmitters were also tested. The 5-HT $_1$ -like receptor antagonists, spiperone and methysergide, the 5-HT $_2$ receptor antagonist, ketanserin, and the 5-HT $_3$ receptor antagonists, ondansetron and granisetron had no effect on the binding of [3 H]-GR113808 up to 10 μ M. Similarly atropine, histamine, dopamine and GABA had no effect on [3 H]-GR113808 binding when tested at 10 μ M.

The effect of GTP and the non hydrolysable analogue GppNHp on 5-HT affinity was determined in both the hippocampus and the striatum. In guinea-pig hippocampus the addition of GppNHp (100 μ M) and Mg $^{2+}$ (100 μ M) had little effect on the ability of 5-HT to displace [3 H]-GR113808 binding lowering the pK $_i$ value from 7.1 to 6.9 ($n = 2$). However, in guinea-pig striatum, GTP (100 μ M) reduced the affinity of 5-HT from pK $_i = 7.3 \pm 0.1$ to 6.7 ± 0.2 ($n = 4$). The Hill coefficient was slightly increased from 0.77 to 0.83. Similarly, GppNHp also reduced the affinity of 5-HT from pK $_i = 7.3 \pm 0.1$ to 6.6 ± 0.1 ($n = 3$). In binding studies using homogenates of striatum it was found that the addition of Mg $^{2+}$ (100 μ M) completely inhibited specific binding of [3 H]-GR113808 and therefore this was omitted from the assay.

Figure 3 Inhibition of 0.1 nM [3 H]-GR113808 binding in homogenates of guinea-pig striatum by 5-HT $_4$ receptor agonists and antagonists. (a) Agonists: (■) BIMU1; (○) 5-HT; (□) 5-methoxytryptamine; (●) α -Me-5-HT; (▲) 5-carboxamidotryptamine. (b) Antagonists: (□) GR113808; (●) SDZ205-557; (○) tropisetron. Results are presented as a percentage of the total binding in the absence of a competing agent and are the means $\pm$ s.e.mean of 3 separate observations. For abbreviations, see text.

Autoradiographic analysis of 5-HT $_4$ receptor distribution in guinea-pig and rat brain

Autoradiographic analysis of specific [3 H]-GR113808 binding indicated that it was differentially localized throughout the guinea-pig and rat brain ($n = 4$; Table 2, Figure 4). The areas showing the highest levels of specific binding included the striatum, olfactory tubercle, globus pallidus, nucleus accumbens and substantia nigra in both species. The interpeduncular nucleus (IPN) had the highest levels of binding in the rat (Table 2). In both the guinea-pig and the rat, neither the frontal cortex nor the cerebellum showed measurable specific binding of [3 H]-GR113808. Non-specific binding of the [3 H]-GR113808 was uniform throughout the brain of both guinea-pig and rat.

Discussion

Specific [3 H]-GR113808 binding was detected in homogenates of guinea-pig striatum and hippocampus. Because functional responses are not measured in a binding assay, it is important that certain criteria are met before concluding that this represents the 5-HT $_4$ receptor rather than a non-receptor site (Burt, 1978). The characteristics of specific [3 H]-GR113808 binding in relation to these criteria are discussed below.

Table 1 The affinities of various compounds that compete for 0.1 nM [³H]-GR113808 binding in homogenates of guinea-pig striatum and hippocampus.

Compound	Guinea-pig striatum		Guinea-pig hippocampus	
	pK _i †	Hill coefficient††	pK _i †	Hill coefficient††
Agonist‡				
5-HT	7.3	0.77	7.1	0.82
5-methoxytryptamine	6.5	0.65	6.2	0.90
α-Me-5-HT	5.8	0.58	5.8	0.90
5-carboxamidotryptamine	5.2	0.71	4.9	0.76
BIMU8	7.9*	0.84*	7.6*	0.87*
Partial agonists**				
BIMU1	7.8	0.89	7.8	0.84
Metoclopramide	6.3	1.00	6.2	0.90
Cisapride	7.5	0.93	7.2	0.80
Renzapride	7.0	0.84	6.9	0.75
R,S-zacopride	6.8	0.77	6.7	0.83
S-zacopride	6.7	0.87	6.6	0.80
R-zacopride	6.3	0.62	6.0	0.75
Antagonists***				
Tropisetron	7.2	0.81	7.2	0.88
SDZ205-557	8.2	1.01	8.1	0.69
GR113808	9.5	0.94	9.6	0.89

Affinity estimates are given as pK_i values determined using the computer programme 'Ligand'. Values are the mean of three determinations except * where only one value was obtained. Hill coefficients are obtained from the curve fitting programme 'Allfit'.

†s.e.mean ≤ 0.2.

††s.e.mean ≤ 0.23.

‡Elswood *et al.*, 1991; Turconi *et al.*, 1991.

**Eglen *et al.*, 1990; Turconi *et al.*, 1991.

***Dumuis *et al.*, 1988; Buchheit *et al.*, 1992; Grossman *et al.*, 1993.

Table 2 Distribution of 0.1 nM [³H]-GR113808 binding assessed by autoradiographic analysis of sections of guinea-pig and rat brain

Brain area	Specific binding (nCi mg ⁻¹ tissue)	
	Guinea-pig	Rat
Olfactory tubercle	1.14 ± 0.19	0.81 ± 0.11
N. accumbens	0.89 ± 0.12	0.28 ± 0.03
Striatum	1.02 ± 0.09	0.37 ± 0.03
Frontal cortex	0.02 ± 0.05	0.04 ± 0.03
Globus pallidus	1.08 ± 0.15	0.56 ± 0.08
Septum	0.22 ± 0.04	0.07 ± 0.07
Substantia nigra	0.99 ± 0.13	0.36 ± 0.08
S. colliculus	0.18 ± 0.06	0.27 ± 0.09
Hippocampus (dorsal)	0.31 ± 0.09	0.09 ± 0.11
Hippocampus (medial)	0.26 ± 0.05	0.18 ± 0.03
Hippocampus (ventral)	0.29 ± 0.08	0.14 ± 0.11
Cerebellum	0.04 ± 0.02	– 0.05 ± 0.03
IPN	–	0.93 ± 0.14

Values are presented as the mean and s.e.mean of measurements from four animals.

In functional studies in the rat oesophagus, GR113808 elicits surmountable antagonism at the 5-HT₄ receptor (Grossman *et al.*, 1993). Consistent with this, specific [³H]-GR113808 binding was also reversible. Indeed specific [³H]-GR113808 binding displayed very rapid kinetics such that the association and dissociation of the ligand was complete within 3 min. Due to this very rapid rate it was not possible to determine accurately association and dissociation rate constants.

Functional receptors exist in finite numbers and so it is important to show that specific binding is saturable. Specific [³H]-GR113808 binding readily saturated (Figure 2). Scatchard transformation of specific binding in both the guinea-pig striatum and the hippocampus revealed the involvement of a single site. Curve fitting analysis revealed an equilibrium dissociation constant for [³H]-GR113808 (0.20 nM and

0.13 nM for striatum and hippocampus respectively) very close to the affinity estimates derived from functional receptor assays in guinea-pig proximal colon and rat oesophagus (pA₂ 9.2–9.5).

The most important evidence that a binding site represents a functional receptor is that agonists and antagonists which are active at the receptor inhibit binding with the expected activity. Of the indole agonists tested, all those that are active in functional studies, 5-methoxytryptamine, α-Me-5-HT and 5-CT, potently inhibited [³H]-GR113808 binding whilst agonists that are inactive in functional studies, 2-Me-5-HT, 8-OH-DPAT, 5-OH-indalpine and sumatriptan, had no effect on [³H]-GR113808 binding. The benzimidazolone compounds, BIMU1 and BIMU8 have been shown to be partial agonists in 5-HT₄ receptor containing preparations, including the mouse colliculi and guinea-pig ileum (both contractions of unstimulated preparations and enhancement of twitch in field stimulated preparations) (Turconi *et al.*, 1991) as well as rat oesophageal tunica muscularis mucosae (Baxter & Clarke, 1992). Both compounds potently inhibited [³H]-GR113808 binding with pK_i values of 7.3 and 7.9 in the striatum for BIMU1 and BIMU8 respectively.

Similarly, benzamides which are partial agonists in a wide range of 5-HT₄ receptor containing preparations, including the guinea-pig proximal colon (Elswood *et al.*, 1991), electrical field stimulated guinea-pig ileum (Craig & Clarke, 1990) and guinea-pig hippocampus (Bockaert *et al.*, 1990), also potently inhibited specific [³H]-GR113808 binding. In functional studies in rat oesophagus and guinea-pig colon, renzapride and R,S-zacopride have very similar activity with renzapride being fractionally more potent (Reeves *et al.*, 1991; Elswood *et al.*, 1991). In the present binding study, renzapride also revealed a slightly higher affinity than R,S-zacopride (pK_i = 7.0 and 6.8 respectively in the striatum and 6.9 and 6.7 in the hippocampus). In guinea-pig colon, cisapride and metoclopramide have been tested as antagonists, with cisapride some 10 fold more potent than metoclopramide. This result was mirrored in the present binding study where pK_i estimates for cisapride and metoclopramide

Figure 4 Autoradiographs of 0.1 nM [3 H]-GR113808 binding in sections of guinea-pig brain. Sections in the left hand column show total binding and sections in the right hand column show non-specific binding defined by the inclusion of 5-HT (30 μ M). Regions of high levels of binding are indicated; a, striatum; b, olfactory tubercle; c, striatum; d, globus pallidus; e, hippocampus; f, substantia nigra.

were 7.5 and 6.3 respectively in the striatum and 7.2 and 6.2 in the hippocampus. The use of the enantiomers of zacopride has indicated small but significant differences in the affinities of R- and S-zacopride for the [3 H]-GR113808 binding site in homogenates of both guinea-pig hippocampus and striatum ($P=0.01$ and 0.04 respectively). This compares well with functional data in guinea-pig ileum where S-zacopride has been shown to be a full agonist and R-zacopride a weak partial agonist (Eglen *et al.*, 1990). This indicates a stereoselectivity for the [3 H]-GR113808 binding site.

The affinity of 5-HT $_4$ receptor antagonists in the present binding study compares well with functional data for these compounds. Tropisetron, the first 5-HT $_4$ receptor antagonist identified, has affinity values in functional studies ranging from 6.0–6.7 (Dumuis *et al.*, 1988; Craig & Clarke, 1990;

Eglen *et al.*, 1990; Elsworth *et al.*, 1991; Reeves *et al.*, 1991) in both the guinea-pig hippocampus and the striatum pK_i values of 7.2 were obtained. Thus, in binding studies, tropisetron seems to have between 3 and 10 fold higher affinity than in functional studies. The more recently identified 5-HT $_4$ receptor antagonist, SDZ205-557 (Buchheit *et al.*, 1992) inhibited [3 H]-GR113808 binding with pK_i values of 8.0 and 8.2 in guinea-pig hippocampus and striatum respectively. As with tropisetron, the affinity estimate obtained in the binding assay is higher than that obtained in functional studies. Unlabelled GR113808 also inhibited [3 H]-GR113808 binding with a pK_i value of 9.5 in guinea-pig striatum and 9.6 in guinea-pig hippocampus. Again this value compares closely with affinity estimates from functional assays in guinea-pig colon and rat oesophagus where

GR113808 has pA₂ values of 9.2 and 9.5 respectively (Grossman *et al.*, 1993).

Hill coefficients for 5-HT are slightly increased by the addition of GTP, however, values obtained both in the presence and absence of GTP are not significantly different from unity. In guinea-pig striatum some agonists had Hill coefficients significantly lower than unity although this did not appear to be the case in the hippocampus. In future experiments it would be interesting to determine the effect of GTP on agonist Hill coefficients in guinea-pig striatum. The Hill coefficient for inhibition of [³H]-GR113808 binding by tropisetron is also lower than unity and thus contrasts with that observed for the other antagonists tested. The reason for this is, however, now known.

5-HT₄ receptors are thought to be coupled to adenylate cyclase as 5-HT₄ receptor stimulation leads to an increase in cyclic AMP levels in a number of preparations both centrally (Dumuis *et al.*, 1988; Bockaert *et al.*, 1990) and peripherally (Kaumann *et al.*, 1990; Ford *et al.*, 1992). However, the addition of the nonhydrolysable analogue of GTP, GppNHP, to homogenates of guinea-pig hippocampus had little effect on the binding of 5-HT, which may suggest little coupling of the 5-HT₄ receptor to a G-protein. By contrast, in guinea-pig striatum, the addition of GTP or GppNHP lowered the affinity of 5-HT by approximately 3 fold. This difference suggests that the addition of GTP results in the conversion of the 5-HT₄ receptor to a lower affinity state which is indicative of a G-protein coupled receptor (Houslay, 1990). The variation in the effects of the addition of GTP to hippocampus and striatum may suggest tissue-specific differences in the coupling of the receptor with a G-protein such that coupling efficiency is higher in the striatum.

It is unlikely that a functional receptor would be uniformly distributed throughout the brain. Autoradiographic analysis of 5-HT₄ receptor distribution in both the guinea-pig and the rat indicated that 5-HT₄ receptor distribution is primarily localized to two anatomically and functionally distinct systems both of which employ dopamine as a neurotransmitter. Firstly the extrapyramidal motor system (striatum, globus pallidus and substantia nigra) and secondly the mesolimbic system (nucleus accumbens and olfactory tubercle). Although the distribution of 5-HT₄ receptors appears to be essentially the same in both the rat and the guinea-pig, the density of 5-HT₄ receptors appears to be considerably higher in the guinea-pig. Also, in the rat, the highest level of binding is in the interpeduncular nucleus which is unlabelled in the guinea-pig.

In conclusion, the specific binding of [³H]-GR113808 meets the criteria for the identification of the 5-HT₄ receptor. As such, the binding is reversible, saturable, of high affinity, discretely localized and inhibited by 5-HT₄ receptor agonists and antagonists with affinities close to those expected from functional assays.

The development of a radioligand for the 5-HT₄ receptor provides an opportunity to determine the distribution of this receptor in human brain and peripheral tissues. Such a compound will also allow more detailed studies of the receptor including investigation of regulation mechanisms, its ontogeny and may also ultimately aid receptor purification.

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