[³H]-5-carboxamidotryptamine labels 5-HT_{1D} binding sites in bovine substantia nigra

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1 [³H]-5-hydroxytryptamine (5-HT) has been shown to radiolabel at least five types of 5-HT binding sites in mammalian brain tissue, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_{1E} (Frazer *et al.*, 1990). Selective masking of 5-HT_{1A} and 5-HT_{1C} receptors, has uncovered binding sites which display both high (5-HT_{1D}) and low (5-HT_{1E}) affinity for 5-carboxamidotryptamine (5-CT). By utilizing [³H]-5-CT we have eliminated a portion of the complex binding (5-HT_{1E}) seen when [³H]-5-HT is used as a radioligand.

2 [³H]-5-CT binding to 5-HT_{1D} sites in bovine substantia nigra was rapid, reversible and saturable, displaying high affinity ($K_d = 0.38 \pm 0.04$ nM) and low non-specific binding (>90% specific binding).

3 In bovine substantia nigra, [³H]-5-CT labelled an equivalent number of binding sites to [³H]-5-CT (403 ± 18 and 362 ± 20 fmol mg⁻¹ protein, respectively) and binding was sensitive to guanine nucleotides.

4 A linear correlation ($r^2 = 0.99$) existed between the potency of compounds to displace [³H]-5-HT and [³H]-5-CT in bovine substantia nigra.

5 Therefore, [³H]-5-CT is a novel radioligand for the examination of 5-HT₁-like binding sites, which under proper experimental conditions can be used to radiolabel selectively 5-HT_{1D}-like binding sites.

Keywords: 5-HT_{1D} receptors; 5-carboxamidotryptamine; substantia nigra; [³H]-5-carboxamidotryptamine; bovine brain; 5-hydroxytryptamine

Introduction

Pharmacological and molecular biological techniques have demonstrated the existence of a large family of 5-hydroxytryptamine (5-HT) receptors which have been grouped into several distinct receptor subtypes (5-HT₁, 5-HT₂, 5-HT₃, and 5-HT₄, Frazer *et al.*, 1990). While the endogenous ligand 5-HT activates numerous 5-HT receptor types, 5-HT₁-like receptors alone are suggested to display an equally high affinity for the agonist, 5-carboxamidotryptamine (5-CT, Bradley *et al.*, 1986). Pharmacological approaches have suggested that 5-HT₁-like receptors are a heterogeneous group consisting of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_{1E} receptors (El Mestikawy *et al.*, 1991; Middlemiss & Hutson, 1990; Hoyer, 1988; Waeber *et al.*, 1990; Leonhardt *et al.*, 1989).

The 5-HT_{1D} receptor has been defined as the [³H]-5-HT binding remaining in the presence of 100 nM (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) and 100 nM mesulergine, which selectively masks the 5-HT_{1A} and 5-HT_{1C} receptors, respectively (Heuring & Peroutka, 1987). However, [³H]-5-HT in the presence of the above masking ligands has been shown to recognize a heterogeneous population of binding sites in porcine caudate (Sumner & Humphrey, 1989) and human cortex (Leonhardt *et al.*, 1989). It has also been demonstrated that in the presence of 100 nM cyanopindolol (to mask 5-HT_{1A} and 5-HT_{1B} sites) and 100 nM mesulergine, 5-CT and sumatriptan produced biphasic displacement of [³H]-5-HT binding in the cortex and/or caudate of dog, guinea-pig, rabbit, pig, man, hamster, and calf (Beer *et al.*, 1992). This binding could be separated into two separate components, displaying high (5-HT_{1D}) and low (5-HT_{1E}) affinity for 5-CT. Molecular cloning has confirmed the existence of at least two subtypes of the 5-HT_{1D} receptor, each of which display high affinity for 5-CT (Hartig *et al.*, 1992), as well as the 5-HT_{1E} receptor which displays significantly lower affinity for 5-CT (McAllister *et al.*, 1992).

We have taken advantage of the fact that 5-CT can discriminate between 5-HT_{1D} and 5-HT_{1E} receptors and have radiolabelled 5-CT (α-ditritio 5-CT) to obtain a radioligand for investigating the multiplicity in 5-HT_{1D}-like binding sites (Mahle *et al.*, 1991). Here we describe the binding characteristics of [³H]-5-CT in comparison with that of the widely used radioligand [³H]-5-HT, in bovine substantia nigra, a region appearing homogeneous with respect to 5-HT_{1D}-like binding sites.

Methods

Radioligand binding assay

Bovine brain was obtained from a local slaughterhouse and the substantia nigra dissected, frozen on dry ice and stored at -70°C until assayed, generally less than one week. The tissue was homogenized (1:80 wt/vol) with a polytron (Brinkmann Instruments, Westbury, NY, U.S.A.) in wash buffer (50 mM HEPES, 2.5 mM MgCl₂ and 2.0 mM ethylene glycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), pH adjusted to 7.4 at room temperature, with a saturated Tris base solution). The homogenate was washed twice in wash buffer via centrifugation at 39,000 g (10 min, 4°C) with a resuspension between centrifugations, then incubated in wash buffer at 37°C for 10 min to remove endogenous 5-HT (Nelson *et al.*, 1978), and centrifuged as above. The final pellet was resuspended in the initial volume of assay buffer (wash buffer containing 0.1% ascorbic acid and 10 μM pargyline) and stored on ice till assayed.

Radioligand binding assays ([³H]-5-HT, [³H]-5-CT) were carried out in duplicate in a final volume of 1.0 ml consisting of 100 μl radioligand, 50 μl each of 8-OH-DPAT and mesulergine (100 nM final concentration), to inhibit binding to 5-HT_{1A} or 5-HT_{1C} receptors, respectively (Heuring & Peroutka, 1987), 100 μl assay buffer (or guanine nucleotide, competitor, or 5-HT), and 700 μl membrane homogenate (approximately 0.5 mg protein ml⁻¹). Incubations were in-

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initiated by the addition of the membrane preparation and were conducted for 60 min at 25°C. Saturation analyses were performed with 0.05–10 nM radioligand. Nonspecific binding was defined by 10 μ M 5-HT. Competition experiments were performed with 0.5 nM [³H]-5-HT and [³H]-5-CT, resulting in approximately 70% and 95% specific binding, respectively. To obtain kinetic rate constants, [³H]-5-CT (total and non-specific binding) was assayed at various time points during association and dissociation. Dissociation was initiated by the addition of 10 μ M unlabelled 5-CT.

Bound radioactivity was determined after rapid filtration through Whatman GF/B filter strips, using a Brandel cell harvester (Gaithersburg, MD, U.S.A.), and washing with three washes (5 ml each) of ice-cold wash buffer. Filters were counted by liquid scintillation spectrometry in Atomlight (New England Nuclear) at approximately 47% efficiency. Protein was determined by the method of Bradford (1976), with bovine serum albumin used as the standard.

Chemicals

[³H]-5-HT was purchased from New England Nuclear (Boston, MA, U.S.A.). [³H]-5-CT was developed through custom synthesis by Dr Ron Mattson (Bristol-Myers Squibb) and Dr Stephen Hurt (New England Nuclear, Boston, MA, U.S.A.). Dr John Macor (Pfizer) kindly provided 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one (CP-93,129). Guanyl and adenylyl nucleotides were obtained from Sigma Chemical Co (St. Louis, MO, U.S.A.), while other drugs used in competition studies were purchased from Sigma Chemical Co., Research Biochemicals (Natick, MA, U.S.A.), or synthesized locally.

Data analysis

Data were analysed with the non-linear regression analysis programme LIGAND (Munson & Rodbard, 1980), where data were best fitted to a one-site model yielding for saturation studies, the equilibrium dissociation constant (K_d), the maximal number of binding sites (B_{max}), and Hill coefficient (nH). For competition studies, all data were analysed by non-linear regression analysis, LIGAND (Munson & Rodbard, 1980), initially assuming a one-site model of [³H]-5-CT or [³H]-5-HT binding, followed by analysis according to a two-site model. An *F* test ($P \leq 0.01$) was then used to determine whether the data were best described by the two-site model. Kinetic association and dissociation rate constants were obtained with the data analysis programme ENZFITTER (Elsevier Biosoft, Ferguson, MO, U.S.A.), using a first order rate equation or a single exponential decay, respectively. Guanine nucleotide inhibition of ³H-labelled agonist binding was fitted to a four parameter logistic equation to obtain response parameters (IC_{50} , E_{max} and slope index).

Results

As demonstrated in Figure 1, [³H]-5-CT binding, performed under conditions designed to radiolabel 5-HT_{1D} binding sites (Heuring & Peroutka, 1987) rapidly reached equilibrium and was reversible. Association and dissociation rate constants were determined for specific [³H]-5-CT binding (0.5 nM) to bovine substantia nigra membrane homogenates (Figure 1). The association time study yielded a K_{obs} value of $0.17 \pm 0.02 \text{ min}^{-1}$ which converted to a K_1 value of $1.56 \times 10^8 \text{ M}^{-1} \text{ min}^{-1}$. The dissociation time study yielded a K_{-1} value of $0.093 \pm 0.005 \text{ min}^{-1}$. The resulting K_d value was $0.60 \pm 0.07 \text{ nM}$.

[³H]-5-CT binding (under 5-HT_{1D} conditions) in bovine substantia nigra membrane homogenates was homogeneous (nH = 0.99 ± 0.003), saturable and demonstrated high affinity and low non-specific binding. Saturation analysis and the resulting Scatchard transformation (Figure 2) indicated a K_d

Figure 1 Kinetics of association and dissociation of [³H]-5-carboxamidotryptamine ([³H]-5-CT) binding in bovine substantia nigra membrane homogenates under 5-HT_{1D} conditions. The tissue homogenate (approximately 0.5 mg protein ml⁻¹) was incubated with 0.5 nM [³H]-5-CT and radioactivity measured for 60 min. After that period, an excess of unlabelled 5-CT (1 μ M) was added with radioactivity measured for an additional 60 min. Using a first order rate equation (see Methods) and single exponential decay produced a dissociation rate constant of 8.99×10^{-2} and a K_{obs} of 1.58×10^{-1} , which yielded a K_d value of 0.64 nM. Data points shown reflect specific binding. This experiment was performed twice more yielding similar results.

Figure 2 Representative saturation analysis (a, total binding (●); non-specific binding (○) and specific binding (◆)) and Scatchard transformation (b) of [³H]-5-carboxamidotryptamine ([³H]-5-CT) binding in bovine substantia nigra membrane homogenates. Non-specific binding was defined by 10 μ M 5-hydroxytryptamine. Data points represent a single experiment performed in duplicate, in the same membrane preparation as used for Figure 3. Values for three separate experiments are described in the text. Non-linear regression analysis of the illustrated experiment yielded the following response parameters: $K_d = 0.4 \text{ nM}$, $B_{max} = 367 \text{ fmol mg}^{-1} \text{ protein}$, and nH = 1.00.

value of 0.38 ± 0.04 nM, which was quite similar to that obtained in the kinetic studies (0.60 ± 0.07 nM) and a $B_{\max}$ value of 403 ± 32 fmol mg^{-1} protein. In parallel experiments using the same membrane homogenates, [^3H]-5-HT saturation analysis (Figure 3, $n\text{H} = 0.97 \pm 0.03$, $K_d = 1.26 \pm 0.05$ nM), while generating significantly greater non-specific binding, yielded roughly an equivalent number of binding sites ($B_{\max} = 362 \pm 20$ fmol mg^{-1} protein) as obtained with [^3H]-5-CT.

When unlabelled 5-CT competed for [^3H]-5-HT binding in guinea-pig striatal membrane homogenates (Figure 4), a region which possesses both 5-HT_{1D} and 5-HT_{1E} receptors

Figure 3 Representative saturation analysis (a, total binding (●); non-specific binding (○) and specific binding (◆)) and Scatchard transformation (b) of [^3H]-5-hydroxytryptamine ([^3H]-5-HT) binding in bovine substantia nigra membrane homogenates. Non-specific binding was defined by $10 \mu\text{M}$ 5-HT. Data points represent a single experiment performed in duplicate. Values for three separate experiments are described in the text. Non-linear regression analysis of the illustrated experiment yielded the following response parameters: $K_d = 1.29$ nM, $B_{\max} = 338$ fmol mg^{-1} protein, and $n\text{H} = 1.02$.

Figure 4 Representative displacement curves of 0.5 nM [^3H]-5-hydroxytryptamine ([^3H]-5-HT) (●), and [^3H]-5-carboxamidotryptamine ([^3H]-5-CT) (○) binding by varying concentrations of unlabelled 5-CT in guinea-pig striatal membrane homogenates. Data points represent duplicate determinations with a range of less than 5%. Each experiment was performed an additional two times yielding similar results. Data were analysed by non-linear regression analysis as described in the Methods section, where 5-CT produced a biphasic displacement of [^3H]-5-HT: K_H , 0.36 nM (64%) and K_L , 215 nM (36%) and monophasic displacement of [^3H]-5-CT: K_i , 0.4 nM.

(Beer *et al.*, 1992), a biphasic displacement curve was obtained (slope index 0.36 ± 0.04), which could be separated into a high ($K_i = 0.37 \pm 0.01$ nM, 64%) and a low ($K_i = 190 \pm 36$ nM) affinity component ($P \leq 0.01$). However, unlabelled 5-CT competed for [^3H]-5-CT in a monophasic manner (slope index not significantly different from unity) producing a single affinity constant ($K_i = 0.68 \pm 0.04$ nM, slope index 0.91 ± 0.02).

Composite competition curves ($n = 3$) for both [^3H]-5-HT and [^3H]-5-CT binding (under 5-HT_{1D} conditions) in bovine substantia nigra membrane homogenates are displayed in Figure 5a and b. With the exception of methiothepin, all compounds produced monophasic displacement ($P \leq 0.01$) of both [^3H]-5-HT and [^3H]-5-CT binding. Methiothepin inhibition of both [^3H]-5-HT and [^3H]-5-CT binding produced shallow displacement curves (slope index approximately 0.6), which could be separated into two (nanomolar and micromolar) components. As responses mediated through 5-HT₁-like receptors should be antagonized by methiothepin (Bradley *et al.*, 1986), and such responses are antagonized by nanomolar concentrations of methiothepin (Engel *et al.*, 1986), the nanomolar component of agonist displacement was used for the correlation analysis. Table 1 lists the respective K_i values determined by non-linear regression analysis, from both sets of competition curves. A linear correlation ($r = 0.99$) exists between the affinities of drugs for specific [^3H]-5-HT and [^3H]-5-CT binding.

A non-hydrolyzable guanine nucleotide (guanosine 5'- β , γ -imidotriphosphate, Gpp(NH)p), caused a concentration dependent attenuation of both [^3H]-5-HT and [^3H]-5-CT binding in bovine substantia nigra homogenates (Figure 6).

Figure 5 (a) Displacement of 0.5 nM [^3H]-5-hydroxytryptamine ([^3H]-5-HT) and (b) [^3H]-5-carboxamidotryptamine ([^3H]-5-CT) binding in bovine substantia nigra membrane homogenates. Competing drugs are denoted by the following symbols: 5-CT (○); 5-HT (●); dihydroergotamine (□); sumatriptan (■); yohimbine (◆); methiothepin (+); DPAT (Δ); CP-93,129 (▲) and buspirone (◇). Data points are the mean $\pm$ s.e. for three separate experiments each performed in duplicate. Data were analysed using non-linear regression analysis as described in the Methods section. Values for the respective concentration-effect curves are illustrated in Table 1.

Table 1 Parameters for the displacement of [³H]-5-hydroxytryptamine ([³H]-5-HT) and [³H]-carboxamidotryptamine ([³H]-5-CT) from 5-HT_{1D} binding sites in bovine substantia nigra membrane homogenates

Drug	[³ H]-5-HT		[³ H]-5-CT	
	K _i (nM)	Slope index (nH)	K _i (nM)	Slope index (nH)
5-CT	0.59 ± 0.11	0.81 ± 0.06	0.51 ± 0.03	0.97 ± 0.02
5-HT	1.25 ± 0.16	1.04 ± 0.08	1.61 ± 0.11	0.90 ± 0.01
Dihydro-ergotamine	7.56 ± 0.94	1.22 ± 0.05	4.71 ± 0.21	1.17 ± 0.12
Sumatriptan	15 ± 4	0.87 ± 0.02	11 ± 2	0.89 ± 0.04
Yohimbine	34 ± 6	0.85 ± 0.06	31 ± 0.6	0.87 ± 0.02
Methiothepin	22 ± 7	0.57 ± 0.04	24 ± 4	0.65 ± 0.02
	(69%)		(78%)	
	1760 ± 1081		726 ± 29.12	
	(31%)		(22%)	
8-OH-DPAT	406 ± 47	0.92 ± 0.02	401 ± 10	0.98 ± 0.03
CP-93,129	1240 ± 125	0.98 ± 0.14	861 ± 26	0.94 ± 0.09
Buspirone	91649 ± 34436	0.82 ± 0.31	59057 ± 3223	1.17 ± 0.02

Values obtained by non-linear regression analysis (LIGAND).

Mean ± s.e.mean.

n = 3 individual repetitions in a common membrane homogenate.

Figure 6 Displacement of 0.5 nM [³H]-5-hydroxytryptamine ([³H]-5-HT) (●) and [³H]-5-carboxamidotryptamine ([³H]-5-CT) (○) binding by varying concentrations of Gpp (NH)p in bovine substantia nigra membrane homogenates. Data points are the mean ± s.e. for three separate experiments each performed in duplicate. Data were fitted by a four parameter logistic equation which generated the following parameters 5-HT and 5-CT, respectively: IC₅₀ (nM): 1.02 ± 0.12, 1.40 ± 0.03; E_{max} (%): 53 ± 2, 49 ± 2; and slope index 0.59 ± 0.07, 0.64 ± 0.06.

Gpp(NH)p affected both [³H]-5-HT and [³H]-5-CT binding in a qualitatively and quantitatively similar manner; IC₅₀ (nM): 1.02 ± 0.12, 1.40 ± 0.03; E_{max} (%): 53 ± 2, 49 ± 2; and slope index 0.59 ± 0.07, 0.64 ± 0.06, respectively. The adenine nucleotides (adenosine 5'-β,γ-imido)triphosphate, App(NH)p and adenosine 5'-O-(thiotriphosphate, ATPγS) produced a negligible effect on both [³H]-5-HT and [³H]-5-CT binding at a concentration-range from 1 nM to 10 μM (data not shown).

Discussion

Radioligand binding assays have been critical in the elucidation and pharmacological characterization of subtypes of 5-HT receptors in their native tissue. Similar to classical pharmacological studies involving isolated tissue, a prerequisite for the classification of receptor subtypes is selective radiolabelled antagonists. The emerging data suggests that 5-HT receptors can be classified into one of at least four families of receptors, 5-HT₁-like, 5-HT₂, 5-HT₃, or 5-HT₄ (Zifa & Fillion, 1992). While the heterogeneity of other 5-HT receptor families is only now being fully appreciated (Kursar

et al., 1992), 5-HT₁ receptors have long been recognized as a heterogeneous group (Pedigo *et al.*, 1981; Pazos *et al.*, 1985; Heuring & Peroutka, 1987). As there were no selective antagonists and to allow scope for future modification, Bradley *et al.* (1986) deliberately kept the 5-HT₁-like receptor classification criteria very broad. 5-HT₁-like receptors characteristically possess a high affinity for 5-HT and 5-CT, moderate affinity for methiothepin and methysergide, and low affinity for ketanserin and MDL 72222. However, solely on the basis of radioligand binding techniques in native tissue, with [³H]-5-HT used as the radioligand at least 5 subtypes of 5-HT₁ receptors were distinguished: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_{1E} (Pedigo *et al.*, 1981; Hoyer *et al.*, 1985; Pazos *et al.*, 1985; Heuring & Peroutka, 1987; Leonhardt *et al.*, 1989), which increases the complexity of binding curves and data analysis.

The ability to mask selectively 5-HT_{1A} and 5-HT_{1C} receptors with 8-OH-DPAT and mesulergine, respectively (Heuring & Peroutka, 1987), has decreased the complexity of radioligand binding analysis. The hypothesis that 5-HT_{1B} receptors represent the species homologue of the 5-HT_{1D} receptor (Adham *et al.*, 1992), has also made interpretation of heterogeneous binding curves in porcine and bovine brain considerably less complex. Nonetheless, several compounds, namely 5-CT, ergotamine, and sumatriptan compete for [³H]-5-HT in a biphasic manner generating a high affinity 5-HT_{1D} component and a low affinity 5-HT_{1E} component (Leonhardt *et al.*, 1989; Weisberg & Teitler, 1992; Beer *et al.*, 1992).

Molecular cloning has substantiated in human brain what radioligand binding first predicted in bovine brain, with the cloning and sequencing of genes encoding a 5-HT_{1D} receptor (Hamblin & Metcalf, 1991) and a 5-HT_{1E} receptor (McAllister *et al.*, 1992). Also as predicted on the basis of radioligand binding assays performed in brain membrane homogenates from numerous mammalian species (Leonhardt *et al.*, 1989; Beer *et al.*, 1992), the human 5-HT_{1E} receptor as opposed to the 5-HT_{1D} receptor, has been shown to have a low affinity for 5-CT, ergotamine and sumatriptan. Recently, based upon sequence homology, another new member of the 5-HT₁ receptor family has been described (Adham *et al.*, 1993). While this receptor bears the greatest sequence homology with the 5-HT_{1E} receptor, it also shares the pharmacological property of displaying low affinity for 5-CT (approximately 1 μM). Thus this site would not be radiolabelled by low concentrations of [³H]-5-CT. Therefore, 5-CT in the presence of 8-OH-DPAT and mesulergine should selectively bind to 5-HT_{1D} receptor subtypes. Thus in guinea-pig striatum (Figure 4), 5-CT displaces [³H]-5-HT in a biphasic

manner demonstrating a high (5-HT_{1D}) and a low (5-HT_{1E}) affinity component. However, a similar displacement of [³H]-5-CT yielded a monophasic 5-HT_{1D} component.

On this supposition, we have tritiated 5-CT to obtain a high affinity radioligand which would be useful in evaluation of the multiplicity of 5-HT_{1D}-like receptors. We have confirmed our hypothesis (Mahle *et al.*, 1991), in that the radioligand [³H]-5-CT was found to label multiple high affinity 5-HT_{1D}-like binding sites in guinea-pig brain which occurred in a regionally disproportionate manner. However, to validate this compound as a useful radioligand, necessitated conducting parallel studies with [³H]-5-HT in a region where the density of 5-HT_{1E} binding sites would make a negligible contribution. Therefore, bovine substantia nigra was chosen, because in this region 5-CT competed for [³H]-5-HT in a monophasic manner (Figure 5b), suggesting a relative lack of 5-HT_{1E} receptors, as compared to that described in guinea-pig striatum (Figure 4). Another factor supporting the relative homogeneity of bovine substantia nigra is that [³H]-5-HT and [³H]-5-CT also label a nearly equivalent density of binding sites. If an appreciable density of 5-HT_{1E} sites had been present, one would expect the *B*_{max} observed with [³H]-5-HT to be significantly higher. This was not the case. However, the density observed with [³H]-5-CT was slightly higher (approximately 10–15%), which may indicate the existence of sites which display a greater affinity for 5-CT than for 5-HT (Plassat *et al.*, 1992).

Yet, pharmacologically the binding sites labelled by [³H]-5-HT appeared similar to those labelled by [³H]-5-CT. A series of nine compounds displayed similar affinities regardless of the radioligand used. While typically, antagonists cause

monophasic displacement of radiolabelled agonists (De Lean *et al.*, 1980), methiothepin, a 5-HT₁-like antagonist (Bradley *et al.*, 1986), displaced both radioligands in a shallow manner, suggesting a complexity in binding. This however cannot be explained by a heterogeneity of 5-HT_{1D}-like binding sites in bovine substantia nigra, as methiothepin displacement of [³H]-5-CT from the 5-HT_{1D} receptor expressed in COS cells also generated a shallow slope index (data not shown). It is also highly unlikely, due to the low concentration of agonist radioligand used, that a slight partial agonism of methiothepin is uncovering multiple affinity states of the 5-HT_{1D} receptor. All other agonist compounds competed in a monophasic manner, also suggesting a homogeneity of binding sites and affinity states in bovine substantia nigra.

In conclusion, when 5-CT competed for [³H]-5-HT in guinea-pig striatal membrane homogenates, a biphasic displacement was observed, which consisted of both a 5-HT_{1D} and 5-HT_{1E} component. The low affinity 5-HT_{1E} component was not observed however, when [³H]-5-CT was used as the radioligand, suggesting that [³H]-5-CT (in the presence of 8-OH-DPAT and mesulergine) selectively labels 5-HT_{1D}-like binding sites. Therefore, the high affinity and low non-specific binding coupled with the lack of affinity for 5-HT_{1E} binding sites make [³H]-5-CT a superior radioligand for 5-HT₁-like binding assays. This compound will be useful in examining the heterogeneity of 5-HT_{1D}-like binding sites and will help in the pharmacological evaluation of novel members of the 5-HT receptor family which possess a greater affinity for 5-CT than for the endogenous ligand, 5-HT.

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