The Journal of Neuroscience, March 1987, 7(3): 894–903

Characterization of a Novel ^{3}H-5-Hydroxytryptamine Binding Site Subtype in Bovine Brain Membranes

Richard E. Heuring and Stephen J. Peroutka

Departments of Neurology and Pharmacology, Stanford University Medical Center, Stanford, California 94305

^{3}H-5-Hydroxytryptamine (5-HT) binding sites were analyzed in bovine brain membranes. The addition of either the 5-HT_{1A}-selective drug 8-OH-DPAT (100 nM) or the 5-HT_{1C}-selective drug mesulergine (100 nM) to the assay resulted in a 5–10% decrease in specific ^{3}H-5-HT binding. Scatchard analysis revealed that the simultaneous addition of both drugs decreased the B_{max} of ^{3}H-5-HT binding by 10–15% without affecting the K_D value (1.8 ± 0.3 nM). Competition studies using a series of pharmacologic agents revealed that the sites labeled by ^{3}H-5-HT in bovine caudate in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine appear to be homogeneous. 5-HT_{1A} selective agents such as 8-OH-DPAT, ipsapirone, and buspirone display micromolar affinities for these sites. RU 24969 and (−)pindolol are approximately 2 orders of magnitude less potent at these sites than at 5-HT_{1B} sites which have been identified in rat brain. Agents displaying nanomolar potencies for 5-HT_{1C} sites such as mianserin and mesulergine are 2–3 orders of magnitude less potent at the ^{3}H-5-HT binding sites in bovine caudate. In addition, both 5-HT_2- and 5-HT_3-selective agents are essentially inactive at these binding sites. These ^{3}H-5-HT sites display nanomolar affinity for 5-carboxyamidotryptamine, 5-methoxytryptamine, metergoline, and 5-HT. Apparent K_i values of 10–100 nM are obtained for *d*-LSD, RU 24969, methiothepin, tryptamine, methysergide, and yohimbine, whereas *l*-LSD and corynanthine are significantly less potent. In addition, these ^{3}H-5-HT labeled sites are regulated by guanine nucleotides and calcium. Regional studies indicate that this class of sites is most dense in the basal ganglia but exists in all regions of bovine brain. These data therefore demonstrate the presence of a homogeneous class of 5-HT_1 binding sites in bovine caudate that is pharmacologically distinct from previously defined 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2, and 5-HT_3 receptor subtypes. We therefore suggest that this class of sites be designated the 5-HT_{1D} subtype of binding sites labeled by ^{3}H-5-HT.

Radioligand binding studies have identified multiple 5-hydroxytryptamine (5-HT) receptors in the mammalian CNS. Initially, 2 main types of central 5-HT binding sites were characterized: 5-HT_1 sites labeled by ^{3}H-5-HT and 5-HT_2 sites labeled by ^{3}H-spiperone (Peroutka and Snyder, 1979). More recently, 5-HT_1 binding sites labeled by ^{3}H-5-HT have been shown to be heterogeneous (Pedigo et al., 1981; Schnellman et al., 1984; Asarch et al., 1985; Hoyer et al., 1985b; Peroutka, 1986). To date, 3 distinct subtypes of the 5-HT_1 binding site have been identified. 5-HT_{1A} sites can be directly labeled with ^{3}H-8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin (8-OH-DPAT) (Gozlan et al., 1983; Hall et al., 1985; Peroutka, 1985, 1986), ^{3}H-ipsapirone (Dompert et al., 1985) or ^{3}H-1-(2-(4-aminophenyl)ethyl)-4-(3-trifluoromethylphenyl)piperazine (PAPP) (Ransom et al., 1986) and display nanomolar affinity for 8-OH-DPAT, ipsapirone, and buspirone. The 5-HT_{1B} site appears to be limited to rat and mouse brain and displays high affinity for RU 24969 and (−)pindolol (Hoyer et al., 1985a; Heuring et al., 1986). The 5-HT_{1C} site is densely present in the choroid plexus but has also been identified in rat and porcine cortex (Pazos et al., 1984; Yagaloff and Hartig, 1985; Peroutka, 1986). This site displays relatively high affinity for mesulergine, mianserin, and methysergide. Although all 5-HT_1 binding sites can be labeled by ^{3}H-5-HT, each of the known subtypes displays a unique pharmacological profile.

The bovine caudate contains a high density of ^{3}H-5-HT binding sites (Whitaker and Seeman, 1978; Peroutka and Snyder, 1981). Preliminary studies in this laboratory indicated that the specific ^{3}H-5-HT binding in this region contained non-5-HT_{1A} binding sites (Peroutka, 1985). Therefore, the present study was performed in order to characterize these sites more extensively. The data show that the majority of ^{3}H-5-HT binding sites in bovine caudate are homogeneous and distinct from the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} sites that have been described previously. Moreover, the site appears to exist in all bovine brain regions and is the most common 5-HT_1 binding site subtype in bovine brain.

Materials and Methods

Receptor binding assays were performed according to the methods of Peroutka (1985). Briefly, adult bovine brains were obtained from a local slaughterhouse, dissected, immediately frozen, and stored at −70°C until needed. On the day of study, the tissue was defrosted and homogenized in 20 vol of 50 mM Tris-HCl (pH 7.7 at 25°C) using a Brinkmann Polytron and then centrifuged in an IEC B20A centrifuge at 49,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in the same volume of Tris-HCl buffer and incubated at 37°C for 10 min prior to a second centrifugation at 49,000 × *g* for 10 min. The final pellet was resuspended in 80 vol of Tris-HCl buffer containing 10 μM pargyline, 4 mM calcium chloride and 0.1% ascorbic acid. For experiments concerning the effects of Ca^{2+}, membranes were suspended in Ca^{2+}-free buffer. Ascorbic acid was added to all assays because of its recently demonstrated effects on ^{3}H-5-HT stability (Peroutka et al., 1986c). The suspensions were immediately used in the

Received June 16, 1986; revised Sept. 8, 1986; accepted Sept. 25, 1986.

This work was supported in part by the John A. Hartford Foundation, the Alfred P. Sloan Foundation, and NIH Grant NS 12151-11. We would like to thank Faith Smith for help in preparation of the manuscript, Christina M. Demopulos for excellent technical assistance, and Dr. Charles Weitz for assistance with HPLC experiments.

Correspondence should be addressed to Stephen J. Peroutka at the above address.

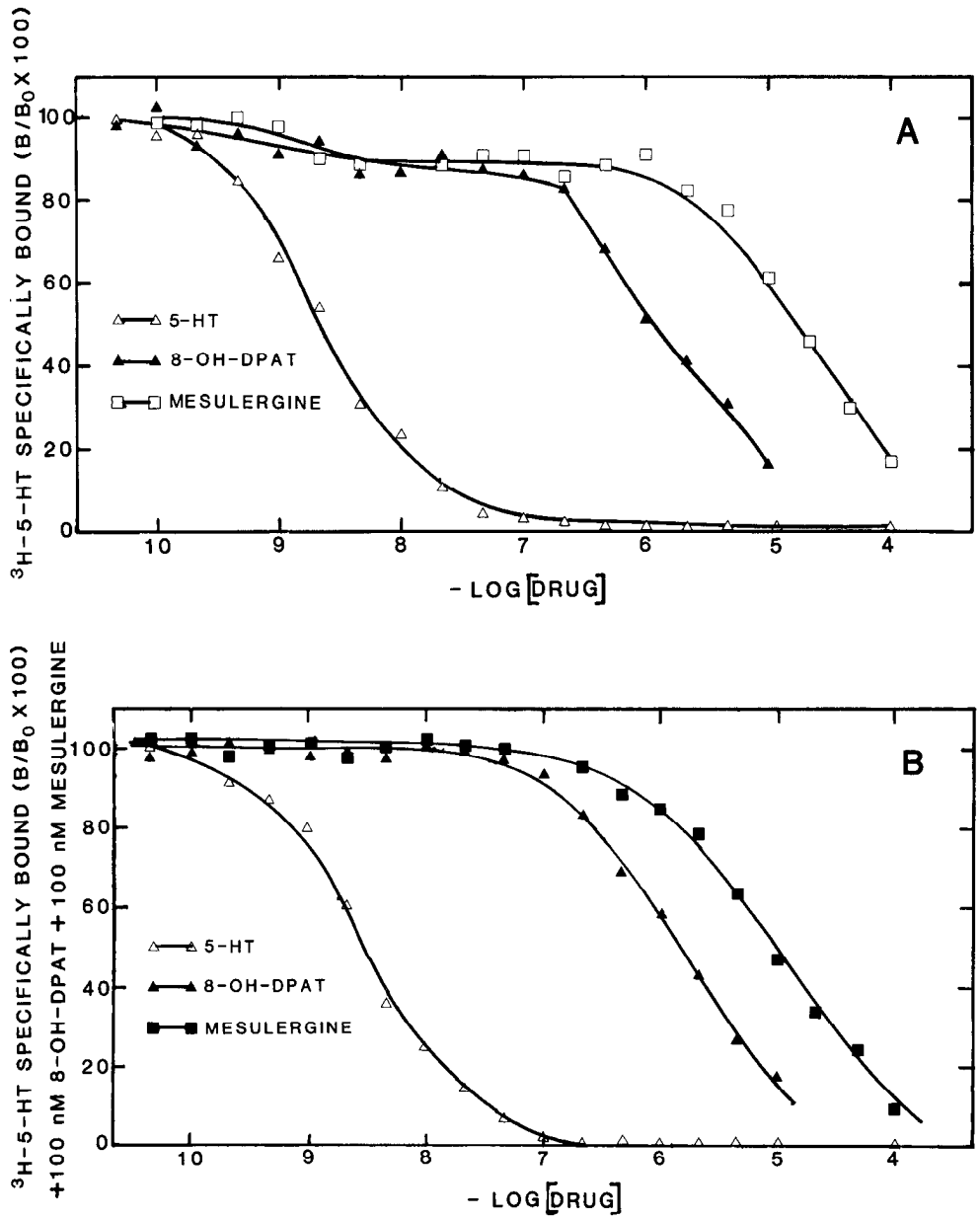

Figure 1. 5-HT, 8-OH-DPAT and mesulergine competition studies with ^{3}H-5-HT binding in bovine caudate. Increasing concentrations of 5-HT, 8-OH-DPAT, or mesulergine were incubated with 2.0 nM ^{3}H-5-HT and bovine caudate membranes as described in Materials and Methods. Data shown are the results of a single experiment performed in triplicate. Each experiment was repeated 3–6 times. *A,* Competition curves under standard assay conditions. *B,* Competition curves in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine.

binding assay. In addition, the stability of the ^{3}H-5-HT (i.e., >98% recovery) under standard assay conditions was documented by high-pressure liquid chromatographic analysis as described previously (Peroutka et al., 1986c).

Binding assays consisted of 0.1 ml ^{3}H-ligand (final concentration, 1.6–2.0 nM ^{3}H-5-HT), 0.1 ml buffer or displacing drug and 0.8 ml tissue suspension. Following incubation at 25°C for 30 min, the assays were rapidly filtered under vacuum through #32 glass fiber filters (Schleicher and Schuell; Keene, NH) with two 5 ml washes using 50 mM Tris-HCl buffer (pH 7.4). Radioactivity was measured by liquid scintillation spectroscopy in 5 ml of 3a70 scintillation cocktail (Research Products International, Mount Prospect, IL) at 56% efficiency. Specific binding was defined using 10 μM 5-HT for all experiments and represented 70–80% of total binding for ^{3}H-5-HT.

Drugs were obtained from the following sources: ^{3}H-5-HT (19.8 Ci/mmol; Amersham, Arlington Heights, IL); 8-OH-DPAT, 1-(*m*-trifluoromethylphenyl)piperazine (TFMPP) (Research Biochemicals, Inc., Waltham, MA); spiperone, ketanserin (Janssen Pharmaceuticals; Beerse); cyproheptadine, 5-methoxytryptamine, corynanthine, metoclopramide, 5-HT, tryptamine, *N,N*-dimethyltryptamine (*N,N*-DMT), GTP, GDP, GMP, ATP, dopamine, (−)epinephrine (Sigma Chemical Co., St. Louis, MO); quipazine (Miles Scientific, Naperville, IL); RU 24969 (Roussel Uclaf, Paris); ipsapirone (Troponwerke, Cologne); methysergide, mesulergine, (−)pindolol (Sandoz, East Hanover, NJ); mianserin (Organon, West Orange, NJ); (−)propranolol (Ayerst, New York, NY); *d*-LSD, *l*-LSD (National Institute on Drug Abuse, Bethesda, MD); *m*-chlorophenylpiperazine (mCPP) (Aldrich, Milwaukee, WI); metergoline (Farmitalia, Milan); buspirone (Bristol Myers, Evansville, IN); haloperidol (McNeil Pharmaceuticals, Spring House, PA); chlorimipramine (Ciba-Geigy, Summit, NJ); prazosin (Pfizer, New York, NY); GppNHp (Pharmacia, Piscataway, NJ); MDL 72222 (Merrel-Dow, Strasburg, France); methiothepin (Hoffmann-La Roche, Nutley, NJ). WB 4101 was the gift of Dr. David A. Greenberg, 5-carboxyamidotryptamine (5-CT) was the gift of Dr. Roger Whiting, and yohimbine was the gift of Dr. Julian Davidson.

Drug displacement studies were analyzed by computer-assisted nonlinear regression analysis based on the methods of Munson and Rodbard (1980) and McPherson (1983). Drug concentrations ranged from 10^{-11} to 10^{-4} M. All data were initially analyzed based on an assumption of a 1-site model of ^{3}H-ligand binding. The data were then analyzed according to a 2-site model, and the results of the curve fitting were statistically compared to the 1-site study by an *F* test. The 2-site model

Table 1. Computer-assisted nonlinear regression analysis of 5-HT, 8-OH-DPAT, and mesulergine interactions with ^{3}H-5-HT binding in bovine caudate

	K_i (nM)		
	^{3}H-5-HT		^{3}H-5-HT + 100 nM 8-OH-DPAT + 100 nM mesulergine
Drug	K_1	K_2	K_1
5-HT	2.9 ± 2 (100%)	–	3.2 ± 2
8-OH-DPAT	1.7 ± 0.9 (7%)	1100 ± 200 (93%)	700 ± 80
Mesulergine	0.91 ± 0.1 (8%)	10,000 ± 2000 (92%)	6500 ± 500

Competition studies were performed as described in Materials and Methods using 2 nM ^{3}H-5-HT. Computer-assisted analysis of the competition curves was performed according to the method of Munson and Rodbard (1980) and McPherson (1983) and was used to determine K_i values and relative concentrations of each binding site. Each curve was fit according to both a 1-site and a 2-site model. When the 2-site model gave a statistically better fit ($p < 0.05$), 2 K_i values are given, with the percentage of each site reported in parentheses. Data given are the means ± SE of 3 separate experiments, each performed in triplicate.

was accepted if the observed "fit" was significantly better ($p < 0.05$) than the "fit" observed using a 1-site model of ^{3}H-ligand binding. All other statistical comparisons were made using the standard t test.

Results

5-HT, 8-OH-DPAT, and mesulergine competition studies with ^{3}H-5-HT in bovine caudate

Drug competition studies with ^{3}H-5-HT were performed in bovine caudate as shown in Figure 1*A*. 5-HT competes for specific ^{3}H-5-HT binding in a monophasic manner. It begins displacing specific ^{3}H-5-HT binding at concentrations above 10^{-10} M and displaces 50% of binding at approximately 3×10^{-9} M. All remaining specific binding is displaced by a concentration of 3×10^{-7} M, and no further displacement is observed at concentrations as high as 10^{-4} M. Therefore, 10^{-5} M 5-HT was used to define nonspecific binding in all subsequent experiments. Computer-assisted nonlinear regression analysis of the data shows that the 5-HT competition curve is adequately described by a 1-site model with a K_i value of 2.9 ± 1 nM (Table 1).

In contrast, the 5-HT$_{1A}$-selective drug 8-OH-DPAT and the 5-HT$_{1C}$-selective drug mesulergine compete with ^{3}H-5-HT in a biphasic manner (Fig. 1*A*). Each drug competes for a small percentage of sites at nanomolar concentrations with a plateau observed at concentrations between 10^{-8} and 2×10^{-7} M. 8-OH-DPAT displaces remaining specific ^{3}H-5-HT binding at concentrations above 2×10^{-7} M. Similarly, mesulergine competes for all but approximately 10% of specific ^{3}H-5-HT binding at concentrations as high as 10^{-4} M. Computer-assisted iterative curve-fitting analysis confirms the biphasic nature of the curves by best fitting each with a 2-site model ($p < 0.01$). 8-OH-DPAT recognizes 7 ± 3% of total ^{3}H-5-HT sites with high affinity (K_i = 1.7 ± 1 nM) and the remaining 93 ± 3% of sites with relatively low affinity (K_i = 1100 ± 200 nM). Similarly, mesulergine recognizes a low percentage (8 ± 2%) of high-affinity sites (K_i = 0.91 ± 0.1 nM) and the remaining sites with relatively low affinity (K_i = 10,000 ± 2000 nM). Since these data suggest a low percentage of 5-HT$_{1A}$ and 5-HT$_{1C}$ sites in bovine caudate, all subsequent competition experiments were performed in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. These drug concentrations are able to selectively block the low percentage of putative 5-HT$_{1A}$ and 5-HT$_{1C}$ sites without significantly affecting the remaining ^{3}H-5-HT-labeled sites.

Under this condition, each of the 3 drugs appears to recognize a homogeneous population of specific ^{3}H-5-HT binding sites (Fig. 1*B*) in bovine caudate. The competition curve for 5-HT is virtually identical to the curve observed under standard conditions (Fig. 1*A*). By contrast, neither 8-OH-DPAT nor mesulergine displaced any specific ^{3}H-5-HT binding at concentrations below 10^{-7} M. Above concentrations of 2×10^{-7} M, monophasic competition curves were observed with both drugs. Computer-assisted iterative curve fitting analysis confirmed that all 3 drug competition curves are adequately described by a 1-site model of ^{3}H-5-HT binding (Table 1).

Saturation analysis of ^{3}H-5-HT binding in bovine caudate

Saturation studies were performed with ^{3}H-5-HT under standard assay conditions or in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. ^{3}H-5-HT displayed monophasic saturation in both the absence and presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. For the experiment shown in Figure 2*A*, Scatchard analysis demonstrated a single high-affinity (K_D = 1.1 nM) component of ^{3}H-5-HT binding (Fig. 2*B*). However, in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine, ^{3}H-5-HT labeled approximately 10% fewer binding sites (B_{max} = 8.2 pmol/g tissue) than under standard assay conditions (9.0 pmol/g tissue), despite the fact that the K_D value (1.5 nM) was not significantly different. Based on an analysis of 4 independent saturation studies in bovine caudate, ^{3}H-5-HT displayed a K_D value of 1.5 ± 0.2 nM and a B_{max} of 8.7 ± 0.3 pmol/g tissue under standard assay conditions. In the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine, the K_D was essentially unchanged (1.8 ± 0.5 nM). However, the B_{max} value (7.7 ± 0.3 pmol/g tissue) was reduced significantly compared with control values ($p < 0.05$, t test). This 11% decrease in B_{max} is consistent with the competition studies shown in Figure 1*A*, which suggest that 10–15% of ^{3}H-5-HT labeled sites in bovine caudate are displaced by 100 nM 8-OH-DPAT + 100 nM mesulergine.

Effect of nucleotides on ^{3}H-5-HT binding in bovine caudate

Nucleotide [GTP, GDP, Gpp(NH)p, GMP, ATP] interactions with specific ^{3}H-5-HT binding in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine were examined in bovine caudate membranes. GTP, GDP, and the nonmetabolizable GTP analog, Gpp(NH)p, significantly decreased specific ^{3}H-5-HT binding ($p < 0.01$) at a concentration of 10^{-3} M. Each nucleotide displaced 40–42% of specific ^{3}H-5-HT binding in the bovine caudate. By contrast, 10^{-3} M ATP and GMP had no significant effect on specific ^{3}H-5-HT binding. A statistically significant difference exists between the effect ATP and those of GTP, GDP, or GppNHp ($p < 0.01$ for each comparison). Thus, modulation

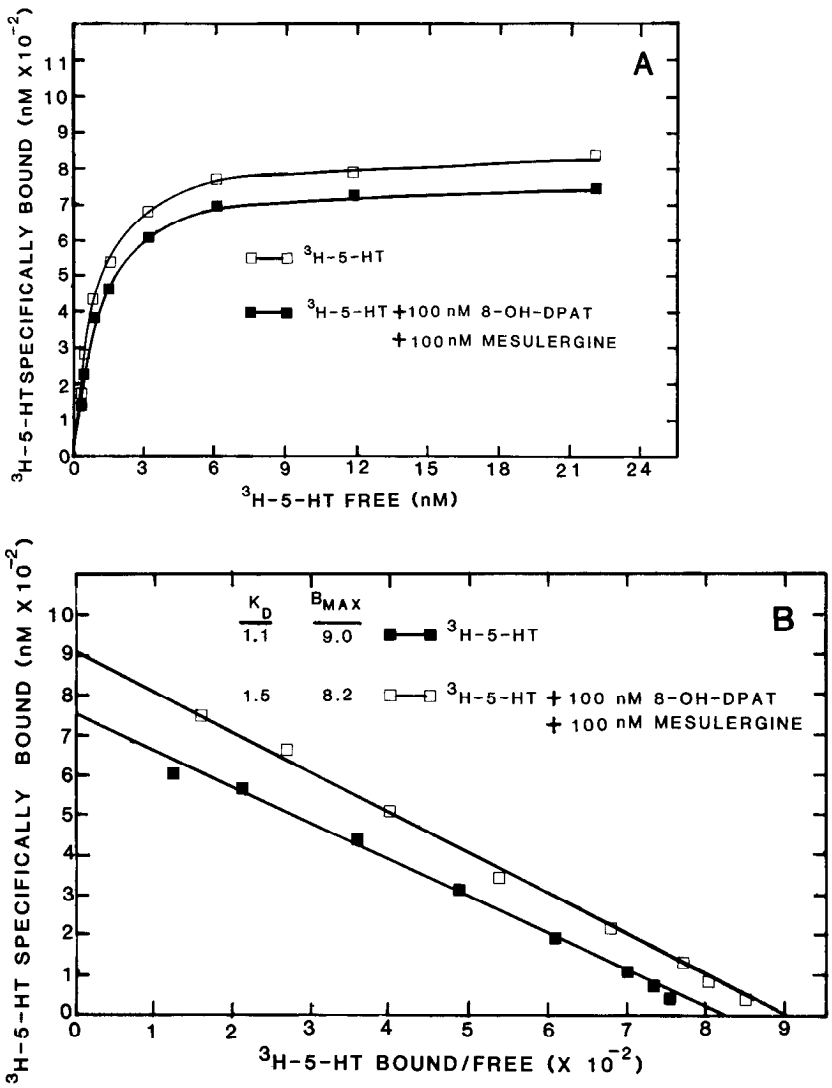

Figure 2. ^{3}H-5-HT saturation studies in bovine caudate. Increasing concentrations (0.95–46 nM) of ^{3}H-5-HT were incubated with bovine caudate membranes under standard assay (□) conditions or in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine (■) as described in Materials and Methods. Data shown are the results of a single experiment performed in triplicate. Each experiment was repeated 4 times. Scatchard data were analyzed by linear-regression analysis. The K_D and B_{max} values given are for this single experiment, which was performed in triplicate. *A*, Specific ^{3}H-5-HT binding. *B*, Scatchard analysis of data shown in *A*.

of specific ^{3}H-5-HT binding sites in bovine caudate shows a rank order of sensitivity as previously demonstrated with other 5-HT binding site subtypes: GppNHp = GTP = GDP > GMP = ATP (Peroutka et al., 1979; Mallat and Hamon, 1982; Battaglia et al., 1984; Schlegel and Peroutka, 1986).

Effect of 4 mM Ca^{2+} on specific ^{3}H-5-HT binding in bovine caudate

In the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine in bovine caudate membranes, 4 mM Ca^{2+} modulated specific ^{3}H-5-HT binding. At half-maximal ^{3}H-5-HT concentrations (2.0 nM), the addition of 4 mM Ca^{2+} to the binding assay caused an increase in total binding and a decrease in nonspecific binding, yielding an increase in specific ^{3}H-5-HT binding. For example, 4 mM Ca^{2+} increased total binding by 7 ± 1% and decreased nonspecific binding by 23 ± 1% compared with values obtained using a Ca^{2+}-free buffer. The net result was a significant increase (17 ± 1%; $p < 0.01$) in the specific binding of ^{3}H-5-HT to bovine caudate membranes in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. In addition, the sensitivity of ^{3}H-5-HT binding to 10^{-3} M GTP was significantly increased in the absence of Ca^{2+}. For example, 10^{-3} M GTP decreased ^{3}H-5-HT binding to 48 + 4% of control values in the absence of Ca^{2+}. This effect was significantly greater ($p < 0.01$) than the effect observed in the presence of 4 mM Ca^{2+} (i.e., 60 ± 1% of control values). By contrast, 10^{-3} M ATP effects on ^{3}H-5-HT binding were unaltered in the absence of Ca^{2+} (100 + 3% of control values). This pattern of Ca^{2+} sensitivity has been described previously for other 5-HT binding site subtypes (Mallat and Hamon, 1982; Hall et al., 1985; Blurton and Wood, 1986).

Regional analysis of ^{3}H-5-HT binding in the absence or presence of 100 nM 8-OH-DPAT and 100 nM mesulergine

A concentration of 2.0 nM ^{3}H-5-HT was used to define regional differences in total ^{3}H-5-HT binding versus ^{3}H-5-HT binding in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. As noted in previous studies (Peroutka and Snyder, 1981), total ^{3}H-5-HT binding was most dense in the substantia nigra, caudate, hippocampus, globus pallidus, and putamen (Table 2). However, a distinct difference existed between regions in the amount of specific ^{3}H-5-HT binding that remained in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. In both caudate and globus pallidus, approximately 80% of specific ^{3}H-5-HT binding remained under this condition. By contrast, the majority of specific ^{3}H-5-HT binding in hippocampus was displaced by 100 nM 8-OH-DPAT + 100 nM mesulergine, with

Table 2. Regional distribution of specific ^{3}H-5-HT binding in the absence or presence of 100 nM 8-OH-DPAT and 100 nM mesulergine

Region	Specific ^{3}H-5-HT binding (100 = 3.8 ± 0.3 pmol/g tissue)		Percentage of total binding remaining
	^{3}H-5-HT (total)	^{3}H-5-HT + 100 nM 8-OH-DPAT + 100 nM mesulergine	
Substantia nigra	129 ± 20	85 ± 5	66
Caudate	100 ± 9	83 ± 0.3	83
Hippocampus	93 ± 10	28 ± 4	30
Globus pallidus	88 ± 10	70 ± 10	80
Occipital cortex	83 ± 1	50 ± 2	60
Putamen	81 ± 6	54 ± 4	67
Superior colliculus	78 ± 20	61 ± 10	78
Inferior colliculus	76 ± 10	60 ± 9	79
Frontal cortex	75 ± 4	58 ± 8	77
Temporal cortex	72 ± 4	41 ± 2	57
Cingulate cortex	63 ± 10	40 ± 1	63
Thalamus	59 ± 6	49 ± 2	83
Hypothalamus	55 ± 6	34 ± 4	62
Pulvinar	29 ± 1	26 ± 1	90
Choroid plexus	24 ± 2	11 ± 1	46
Pons	21 ± 4	19 ± 4	90
Cerebellar nuclei	16 ± 4	16 ± 4	100
Medulla oblongata	15 ± 4	5 ± 0.4	33
Cerebellar cortex	7 ± 2	8 ± 2	110

Binding assays were performed as described in Materials and Methods. Values given are the mean ± SE of triplicate assays. Each experiment was repeated 3–4 times. Data are described as the percent of total specific binding observed using 2.0 nM ^{3}H-5-HT, where 100% = 3.8 ± 0.3 pmol/g wet weight tissue.

only 30% of the original amount of specific ^{3}H-5-HT binding remaining.

In cortical areas, total specific ^{3}H-5-HT binding ranged from 63 to 83% of caudate values. The amount of specific binding remaining in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine ranged from 57% in the temporal cortex to 77% in the frontal cortex. While the midbrain, brain stem, and cerebellar regions contained relatively low densities of total ^{3}H-5-HT binding sites, they were similar in that they contained a very high percentage of sites not displaced by 100 nM 8-OH-DPAT + 100 nM mesulergine. The single exception was the medulla oblongata, in which only 35% of the specific ^{3}H-5-HT binding remained in the presence of 8-OH-DPAT and mesulergine.

The choroid plexus was the only other brain region in which the majority of ^{3}H-5-HT binding was displaced by 100 nM 8-OH-DPAT + 100 nM mesulergine. This finding is of interest since the choroid plexus is the region in which 5-HT$_{1C}$ binding R sites were first identified and characterized (Pazos et al., 1984; Yagaloff and Hartig, 1985).

Drug interactions with ^{3}H-5-HT binding sites in bovine caudate in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine

To further characterize specific ^{3}H-5-HT binding sites in bovine caudate, the interactions of a series of drugs were determined in competition studies with ^{3}H-5-HT in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. Figure 3*A* shows that several potent 5-HT$_{1A}$-selective drugs are all relatively weak in competing for ^{3}H-5-HT binding in bovine caudate. Although WB 4101, buspirone and ipsapirone display nanomolar affinity for 5-HT$_{1A}$ sites in rat frontal cortex (Peroutka, 1986), they compete for specific ^{3}H-5-HT binding in bovine caudate at micromolar concentrations. Moreover, all 3 competition curves are monophasic below concentrations of 10^{-4} M. The Hill slope of WB 4101 competition with ^{3}H-5-HT binding under these conditions was 1.1 ± 0.02. K_i values for each agent are presented in Table 3.

By contrast, certain 5-HT$_{1B}$-selective drugs were more potent competitors of ^{3}H-5-HT binding sites in bovine caudate (Fig. 3*B*). Computer analysis of RU 24969 interactions with the specific ^{3}H-5-HT binding indicates that the data are adequately explained by a single population of binding sites with a K_i value of 25 ± 1 nM. The piperazines TFMPP and mCPP also displaced ^{3}H-5-HT in a monophasic manner, with Hill slopes of 0.90 ± 0.1 and 0.99 ± 0.03, respectively. In addition, 2 relatively potent inhibitors of 5-HT$_{1B}$ sites in rat brain (Hoyer et al., 1985), (−)pindolol and (−)propranolol, were more than 100-fold less potent at these ^{3}H-5-HT binding sites in bovine brain (Table 3).

Similarly, drugs reported to display relatively high affinity for the 5-HT$_{1C}$ binding site subtype (Pazos et al., 1984; Hoyer et al., 1985b) were markedly less potent in competing for ^{3}H-5-HT binding in bovine caudate (Fig. 3*C*). Methysergide is the most potent of the 5-HT$_{1C}$-selective agents, with an IC$_{50}$ value of approximately 100 nM and a Hill slope of 0.92 ± 0.06. Mianserin was slightly less potent (K_i = 480 nM; Hill slope = 1.04 ± 0.03), while ketanserin and mesulergine were essentially inactive at the ^{3}H-5-HT binding sites in bovine caudate.

The most potent agents at this class of ^{3}H-5-HT binding sites

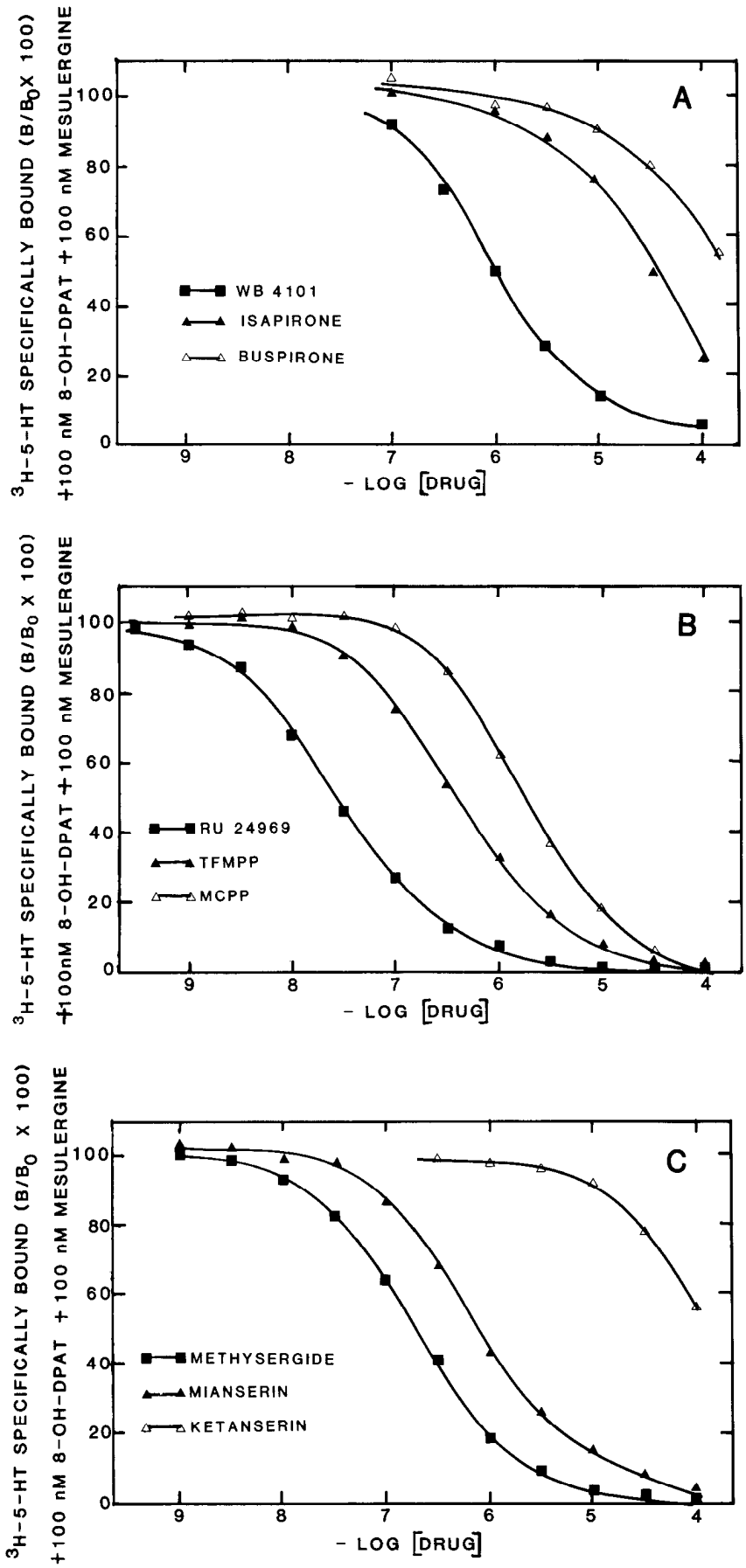

Figure 3. Competition studies of 5-HT$_{1A}$-, 5-HT$_{1B}$-, and 5-HT$_{1C}$-selective drugs with ^{3}H-5-HT binding in bovine caudate membranes in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine. Competition studies with ^{3}H-5-HT (1.6–2.0 nM) binding in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine were performed as described in Materials and Methods. The data given are the results of single experiments performed in triplicate. Each experiment was repeated 3–6 times. *A,* Competition studies with 5-HT$_{1A}$-selective drugs. *B,* Competition studies with 5-HT$_{1B}$-selective drugs. *C,* Competition studies with 5-HT$_{1C}$-selective drugs.

Table 3. Drug interactions with ^{3}H-5-HT binding in bovine caudate membranes

Drug	K_i (nM)	Drug	K_i (nM)
5-HT$_{1A}$ selective drugs		Tryptamines	
WB 4101	590 ± 60	5-CT	0.75 ± 0.1
8-OH-DPAT	700 ± 50	5-Methoxytryptamine	2.5 ± 1
Ipsapirone	16,000 ± 2000	5-HT	3.2 ± 1
Buspirone	>100,000	Tryptamine	36 ± 6
5-HT$_{1B}$ selective drugs		*N,N*-DMT	190 ± 10
RU 24969	25 ± 1	5-HT$_2$ selective drugs	
TFMPP	280 ± 30	Cyproheptadine	1200 ± 100
mCPP	1400 ± 200	Haloperidol	8200 ± 600
(−)Pindolol	7000 ± 700	Spiperone	>100,000
(−)Propranolol	7200 ± 5000	5-HT$_3$ selective drugs	
5-HT$_{1C}$ selective drugs		Metoclopramide	9000 ± 300
Methysergide	120 ± 20	MDL 72222	>100,000
Mianserin	480 ± 20	Others	
Mesulergine	6500 ± 500	Methiothepin	36 ± 6
Ketanserin	53,000 ± 10,000	Yohimbine	59 ± 6
Ergot derivatives		Quipazine	800 ± 100
Metergoline	4.2 ± 1	Corynanthine	23,000 ± 1000
d-LSD	24 ± 2	Dopamine	36,000 ± 5000
l-LSD	>100,000	Epinephrine	>100,000
		Prazosin	>100,000

Binding assays using 1.6–2.0 nM ^{3}H-5-HT in the presence of 100 nM 8-OH-DPAT + 100 nM mesulergine were performed as described in Materials and Methods. IC_{50} values were determined by log-logit analysis. K_i values were calculated according to the equation $K_i = IC_{50}/(1 + [^3H\text{-}5\text{-}HT]/K_D)$. The K_D value for ^{3}H-5-HT (1.8 nM) was derived from saturation experiments. Data given are means K_i ± SE of 3–6 experiments, each performed in triplicate.

were tryptamine derivatives (Table 3). 5-CT had a K_i of 0.75 ± 0.1 nM, while 5-HT and 5-methoxytryptamine were slightly less potent. Ergot derivatives were also potent agents. Metergoline, *d*-LSD, and methysergide had K_i values of 4.2 ± 1, 24 ± 2, and 120 ± 20 nM, respectively. In addition, yohimbine, a putative alpha$_2$-adrenergic agent, was moderately potent at this class of ^{3}H-5-HT binding sites (K_i = 59 ± 6 nM). The binding site also displayed stereoselectivity, as demonstrated by the inactivity of *l*-LSD and corynanthine. Drugs that are potent agents at the putative 5-HT$_3$ receptor in the periphery (MDL 72222 and metoclopramide) were essentially inactive at this central 5-HT binding site. Finally, the biogenic amine neurotransmitters epinephrine and dopamine were inactive at this binding site.

Discussion

The major finding of the present study is that ^{3}H-5-HT labels a population of binding sites in bovine caudate that is distinct from previously defined 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ binding site subtypes labeled by ^{3}H-5-HT. The binding of ^{3}H-5-HT in bovine caudate is saturable, stereoselective, and displays high affinity (K_D = 1.8 ± 1 nM). Computer-assisted curve-fitting analysis suggests that these sites are homogeneous in bovine caudate. The most potent agents at these sites are tryptamines, ergots, RU 24969, and yohimbine. The sites are modified by guanine nucleotides and calcium. Regional studies suggest that this class of sites is present in all brain regions and that it represents the predominant form of 5-HT$_1$ binding site subtype in bovine brain. Since these pharmacological characteristics are distinct from previously described 5-HT$_1$ binding site subtypes, we suggest that these sites be designated 5-HT$_{1D}$ binding sites.

The initial studies of ^{3}H-5-HT binding suggested that the radioligand labeled a heterogeneous population of binding sites (Bennett and Snyder, 1976; Fillion et al., 1978). Nelson and colleagues were the first to suggest that at least 2 distinct subtypes could be identified using ^{3}H-5-HT (Pedigo et al., 1981; Schnellman et al., 1984). The 5-HT$_{1A}$ site was defined as having high affinity for spiperone, while the 5-HT$_{1B}$ site was defined as being relatively insensitive to this neuroleptic. More recently, the development of highly potent and binding site subtype selective agents has greatly facilitated the analysis of 5-HT$_1$ binding site subtypes (Middlemiss and Fozard, 1983).

To date, the 5-HT$_{1A}$ site has been the most extensively characterized subtype of the 5-HT$_1$ class of binding sites (Table 4). This site can be directly labeled by ^{3}H-8-OH-DPAT (Gozlan et al., 1983; Hall et al., 1985; Peroutka, 1985, 1986), ^{3}H-ipsapirone (Dompert et al., 1985), ^{3}H-PAPP (Ransom et al., 1986), or ^{3}H-WB 4101 (Norman et al., 1985). Unlike the 5-HT$_1$ binding site subtype described in the present report, the 5-HT$_{1A}$ site displays nanomolar affinity for 8-OH-DPAT, WB 4101, and ipsapirone. In addition, the site is densely present in the hippocampus (Pazos and Palacios, 1985).

A number of functional correlates have been proposed for this site. Neurophysiologically, 5-HT and 5-HT$_{1A}$ selective agents inhibit intrinsic raphe cell firing (VanderMaelen and Wilderman, 1984; Sprouse and Aghajanian, 1985; Trulson and Arasteh, 1985) and the population spike generated by hippocampal CA1 cells (Beck and Goldfarb, 1985; Peroutka et al., 1987). In addition, both forskolin-induced stimulation of adenylate

Table 4. Characteristics of 5-HT receptor subtypes

	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}	5-HT_2	5-HT_3
Radiolabeled by	^{3}H-5-HT ^{3}H-8-OH-DPAT ^{3}H-Ipsapirone ^{3}H-WB 4101 ^{3}H-Buspirone ^{3}H-PAPP	^{3}H-5-HT ^{125}I-CYP (Rat and mouse only)	^{3}H-5-HT ^{3}H-Mesulergine ^{125}I-LSD	^{3}H-5-HT	^{3}H-Spiperone ^{3}H-Mesulergine ^{125}I-LSD ^{3}H-Ketanserin ^{3}H-Ritanserin ^{3}H-Mianserin ^{125}I-Methyl-LSD	–
High-density regions	Raphe nuclei Hippocampus	Substantia nigra Globus pallidus	Choroid plexus	Basal ganglia	Layer IV cortex	Peripheral neurons
Drug potencies						
<10 nM	5-CT 8-OH-DPAT 5-HT RU 24969 *d*-LSD	RU 24969 5-CT 5-HT	Mesulergine Meterogoline Methysergide	5-HT 5-CT Metergoline	Spiperone Mesulergine Methysergide Metergoline Mianserin	MDL 72222
10–1000 nM	Metergoline Methysergide Spiperone Mesulergine	Metergoline Methysergide *d*-LSD	Mianserin 5-HT RU 24969 5-CT *d*-LSD	Methysergide Mianserin 8-OH-DPAT *d*-LSD RU 24969	*d*-LSD	5-HT Metoclopramide (−)Cocaine
>1000 nM	Mianserin	Mianserin Spiperone Mesulergine 8-OH-DPAT	Spiperone 8-OH-DPAT	Mesulergine Spiperone	RU 24969 5-HT 8-OH-DPAT	Methysergide Ketanserin
Potential functional correlates	Raphe and hippocampal inhibition Basilar artery contractions	Synaptosomal "autoreceptor"	Phospatidyl-inositide turnover	? Kidney perfusion ? Rat fundus contractions	Head twitches Vascular contractions	Excitation of sympathetic neurons

cyclase in guinea pig hippocampus (De Vivo and Maayani, 1986) and drug-induced contractions of the canine basilar artery (Peroutka et al., 1986b; Taylor et al., 1986) appear to be modulated by 5-HT_{1A} receptors. The 5-HT_{1A} selective agents 8-OH-DPAT and 5-MeDMT produce the "5-HT behavioral syndrome" and at least 3 specific components of the syndrome (reciprocal forepaw treading, head-weaving, and tremor) have been attributed to stimulation of the 5-HT_{1A} receptor (Tricklebank, 1985; Smith and Peroutka, 1986).

The 5-HT_{1B} site has been labeled in rat brain with both ^{3}H-5-HT (Sills et al., 1984; Blurton and Wood, 1986; Peroutka, 1986) and ^{125}I-cyanopindolol (Hoyer et al., 1985a). The 5-HT_{1B} site reportedly displays extremely high affinity (0.38 nM) for RU 24969 and relatively low affinity for *d*-LSD and 8-OH-DPAT (Peroutka, 1986). The site also has relatively high affinity (pK_D values <100 nM) for (−)pindolol and (−)propranolol (Hoyer et al., 1985a), drugs that are more than 100-fold less potent at the sites described in the present report. Conversely, mianserin is more than 2 orders of magnitude less potent at the 5-HT_{1B} site than at the ^{3}H-5-HT site described in the present report (Hoyer et al., 1985a; Peroutka, 1986). The highest densities of 5-HT_{1B} sites in rat brain are found in the caudate, superior colliculus, lateral geniculate, subiculum, and substantia nigra (Pazos and Palacios, 1985). Extensive pharmacological studies have demonstrated that synaptosomal release of 5-HT can be modulated by 5-HT_{1B} receptors in the rat (Engel et al., 1986; Raiteri et al., 1986). Interestingly, the 5-HT_{1B} site appears to be present in only rat and mouse brain and not in other species, such as cow, guinea pig, frog, chicken, turtle, or human (Heuring et al., 1986).

The 5-HT_{1C} site was first characterized in membranes from pig choroid plexus and cortex (Pazos et al., 1984). The site has been labeled by ^{3}H-5-HT, ^{3}H-mesulergine, and ^{125}I-LSD in the rat choroid plexus and may also be present in rat cortex (Pazos et al., 1984; Yagaloff and Hartig, 1985; Blurton and Wood, 1986; Peroutka, 1986). Recent studies using rat choroid plexus have shown that the 5-HT_{1C} site is linked to phosphatidylinositol turnover (Conn et al., 1986). Pharmacologically, the 5-HT_{1C} site displays nanomolar affinity for 5-HT, mesulergine, methysergide, and mianserin. By contrast, the sites described in the present report have significantly lower affinity for mesulergine, methysergide, and mianserin.

Previous investigators have also suggested that other binding sites can be labeled by ^{3}H-5-HT. However, a class of sites with the pharmacological characteristics described in the present report has not yet been reported. For example, Robaut et al. (1986) identified a ^{3}H-5-HT binding site in rat brain with a K_D of 13–

15 nM that was insensitive to ergot derivatives and GTP. Similarly, solubilized 5-HT_1 binding sites apparently do not recognize the isomers of LSD (Allgren et al., 1985).

In addition, 2 other major types of 5-HT receptor have been described (Table 4). The 5-HT_2 receptor was first identified in brain membranes (Leysen et al., 1978) and displays high affinity for classical 5-HT antagonists and many neuroleptics (Peroutka and Snyder, 1979). The receptor mediates head twitches in rats and mice, as well as 5-HT-induced contractions in many vascular systems (Leysen et al., 1984; Peroutka, 1984). Finally, a 5-HT_3 receptor has been identified in the PNS (Bradley et al., 1986). This putative serotonergic site has not been radiolabeled, and no correlate has been identified in the CNS. Nonetheless, the receptor displays high affinity for 5-HT and related analogs in physiological studies, and, unlike previously described 5-HT receptors, it can be blocked potently by drugs such as MDL 72222, metoclopramide, and (−)cocaine (Fozard, 1984).

Thus, the pharmacological characteristics of the homogeneous class of binding sites labeled by ^{3}H-5-HT in bovine caudate are distinct from the previously defined subtypes of the 5-HT_1 class of binding sites, as well as from 5-HT_2 and 5-HT_3 receptors. The ^{3}H-5-HT sites in bovine caudate display high affinity for 5-HT, are saturable, and display stereoselectivity for the isomers of LSD and yohimbine. The most potent agents at these sites are tryptamine and ergot derivatives, as well as RU 24969. When 5-HT_{1A} and 5-HT_{1C} sites are pharmacologically blocked by the addition of 100 nM 8-OH-DPAT + 100 nM mesulergine, this class of sites can be demonstrated in all regions of bovine brain, with highest densities observed in the basal ganglia. The extensive pharmacological characterization of this homogeneous and unique class of binding sites in the bovine caudate enables us to suggest that this class of sites be designated the 5-HT_{1D} subtype of binding sites labeled by ^{3}H-5-HT. Rather than being a relatively localized binding site subtype (as are the 5-HT_{1A} and 5-HT_{1C} sites), this class of sites appears to exist in high density in many brain regions. Indeed, this class of sites is the predominant subtype of 5-HT_1 sites in bovine brain.

To date, 2 specific physiological effects of 5-HT have been described that may correlate with the novel 5-HT binding site described in the present report. In the first system, Charlton et al. (1986) described an inhibitory prejunctional "5-HT_1-like" receptor in the isolated perfused rat kidney. This 5-HT receptor inhibited the stimulus-induced release of ^{3}H-noradrenaline following sympathetic periteronal nerve stimulation to the kidney. 5-CT was the most potent agonist (IC_{30} = 1.8 nM), whereas 5-HT (IC_{30} = 45 nM) and RU 24969 (IC_{30} = 250 nM) were slightly less potent agonists. 8-OH-DPAT was totally inactive in this system. The effects of these agonists could be antagonized by methiothepin, metergoline, and methysergide but not by cyproheptadine, ketanserin, mesulergine, (−)propranolol, or (±)pindolol. The authors concluded that the 5-HT receptor conformed to the general criteria of a "5-HT_1-like" receptor but could not be correlated to either the 5-HT_{1A}, 5-HT_{1B}, or 5-HT_{1C} subtypes. The effect of 5-HT and related agents in the isolated perfused rat kidney system is therefore quite similar to the drug potencies at the putative 5-HT_{1D} binding site described here.

5-HT-induced contraction of the rat stomach fundus is a second physiological effect that does not appear to be mediated by previously described 5-HT receptor subtypes (Leysen and Tollenaere, 1982; Cohen and Wittenauer, 1986). Interestingly, in the first major study of the system (Vane, 1959), it was demonstrated that 5-HT and 5-MT were equipotent agents in the rat stomach fundus, while tryptamine and *N,N*-DMT were 32- and 110-fold, respectively, less active than 5-HT. Similar rank-order potencies exist for these analogs at the putative 5-HT_{1D} site (Table 3). More recently, Clineschmidt et al. (1985) concluded that the fundic 5-HT receptor resembled the "5-HT_1" binding site but that the heterogeneous nature of the radioligand binding precluded a more definitive correlation. Of note is the fact that yohimbine was a potent antagonist in this system, in agreement with its high affinity for the putative 5-HT_{1D} site.

Clearly, more detailed future studies are needed to define functional correlates of the putative 5-HT_{1D} binding site subtype. However, the present data demonstrates that ^{3}H-5-HT labels a fourth subpopulation of binding sites that are distinct from the previously described 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} sites. The characterization of this homogeneous population of ^{3}H-5-HT labeled sites should facilitate the correlation of physiological actions with radioligand binding data. The identification and characterization of all physiologically relevant 5-HT receptors should, ultimately, elucidate the role of 5-HT in the CNS and PNS.

References

Allgren, R. L., M. M. A. Kyncl, and R. D. Ciaranello (1985) Pharmacological characterization of solubilized 5-HT_1 serotonin binding sites from bovine brain. Brain Res. *348:* 77–85.

Asarch, K. B., R. W. Ransom, and J. C. Shih (1985) 5-HT_{1A} and 5-HT_{1B} selectivity of two phenylpiperazine derivatives: Evidence for 5-HT_{1B} heterogeneity. Life Sci. *36:* 1265–1273.

Battaglia, G., M. Shannon, and M. Titeler (1984) Guanyl nucleotide and divalent cation regulation of cortical S_2 receptors. J. Neurochem. *43:* 1213–1218.

Beck, S. G., and J. Goldfarb (1985) Spiperone differentiates multiple 5-hydroxytryptamine responses in rat hippocampal slices in vitro. Eur. J. Pharmacol. *116:* 195–197.

Bennett, J. P., Jr., and S. H. Snyder (1976) Serotonin and lysergic acid diethylamide binding in rat brain membranes: Relationship to postsynaptic serotonin receptors. Mol. Pharmacol. *12:* 373–389.

Blurton, P. A., and M. D. Wood (1986) Identification of multiple binding sites for [^{3}H]5-hydroxytryptamine in the rat CNS. J. Neurochem. *46:* 1392–1398.

Bradley, P. B., G. Engel, W. Feniuk, J. R. Fozard, P. P. A. Humphrey, D. N. Middlemiss, E. J. Mylecharane, B. P. Richardson, and P. R. Saxena (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology *25:* 563–576.

Charlton, K. G., R. A. Bond, and D. E. Clarke (1986) An inhibitory prejunctional 5-HT_1-like receptor in the isolated perfused rat kidney. Naunyn Schmiedebergs Arch. Pharmacol. *332:* 8–15.

Clineschmidt, B. V., D. R. Reiss, D. J. Pettibone, and J. L. Robinson (1985) Characterization of 5-hydroxytryptamine receptors in rat stomach fundus. J. Pharmacol. Exp. Ther. *235:* 696–708.

Cohen, M. L., and L. A. Wittenauer (1986) Further evidence that the serotonin receptor in the rat stomach fundus is not 5-HT_{1A} or 5-HT_{1B}. Life Sci. *38:* 1–5.

Conn, P. J., E. Sanders-Bush, B. J. Hoffman, and P. R. Hartig (1986) A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. Proc. Natl. Acad. Sci. USA *83:* 4086–4088.

De Vivo, M., and S. Maayani (1986) Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther. *238:* 248–253.

Dompert, W. U., T. Glaser, and J. Traber (1985) ^{3}H-TVX Q 7821: Identification of 5-HT_1 binding sites as target for a novel putative anxiolytic. Naunyn Schmiedebergs Arch. Pharmacol. *328:* 467–470.

Engel, G., D. Hoyer, E. Schlicker, and K. Hillenbrand (1986) Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. Naunyn Schmiedebergs Arch. Pharmacol. *332:* 1–7.

Fillion, G. M. B., J. C. Rousselle, M. P. Fillion, D. M. Beaudoin, M. R. Goiny, J. M. Deniau, and J. J. Jacob (1978) High-affinity binding

of [^{3}H]5-hydroxytryptamine to brain synaptosomal membranes: Comparison with [^{3}H]lysergic acid diethylamide binding. Mol. Pharmacol. *14:* 50–59.

Fozard, J. (1984) Neuronal 5-HT receptors in the periphery. Neuropharmacology *23:* 1473–1486.

Gozlan, H., S. El Mestikawy, L. Pichat, J. Glowinski, and M. Hamon (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature *305:* 140–142.

Hall, M. D., S. El Mestikawy, M. B. Emerit, L. Pichat, M. Hamon, and H. Gozlan (1985) [^{3}H]8-Hydroxy-2-(di-*n*-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. J. Neurochem. *44:* 1685–1696.

Heuring, R. A., J. R. Schlegel, and S. J. Peroutka (1986) Species variations in RU 24969 interactions with non-5-HT$_{1A}$ binding sites. Eur. J. Pharmacol. *122:* 279–282.

Hoyer, D., G. Engel, and H. O. Kalkman (1985a) Characterization of the 5-HT$_{1B}$ recognition site in rat brain: Binding studies with (−)[^{125}I]iodocyanopindolol. Eur. J. Pharmacol. *118:* 1–12.

Hoyer, D., G. Engel, and H. O. Kalkman (1985b) Molecular pharmacology of 5-HT$_1$ and 5-HT$_2$ recognition sites in rat and pig brain membranes: Radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)[^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. Eur. J. Pharmacol. *118:* 13–23.

Leysen, J. E., and J. P. Tollenaere (1982) Biochemical models for serotonin receptors. Annu. Rep. Med. Chem. *17:* 1–10.

Leysen, J. E., C. J. E. Niemegeers, J. P. Tollenaere, and P. M. Laduron (1978) Serotonergic component of neuroleptic receptors. Nature *272:* 168–171.

Leysen, J. E., D. De Chaffoy De Courcelles, F. De Clerck, C. J. E. Niemegeers, and J. M. Van Nueten (1984) Serotonin-S$_2$ receptor binding sites and functional correlates. Neuropharmacology *23:* 1493–1501.

Mallat, M., and M. Hamon (1982) Ca^{2+}-guanine nucleotide interactions in brain membranes. I. Modulation of central 5-hydroxytryptamine receptors in the rat. J. Neurochem. *38:* 151–161.

McPherson, G. A. (1983) A practical computer-based approach to the analysis of radioligand binding experiments. Comput. Programs Biomed. *17:* 107–114.

Middlemiss, D. N. (1986) Functional correlates of the subtypes of the 5-HT$_1$ recognition site. Trends Pharmacol. Sci. *7:* 52–53.

Middlemiss, D. N., and J. R. Fozard (1983) 8-Hydroxy-2-(DI-*n*-propylamino)-tetralin discriminates between subtypes of the 5-HT$_1$ recognition site. Eur. J. Pharmacol. *90:* 151–153.

Munson, P. J., and D. Rodbard (1980) LIGAND: A versatile computerized approach for characterization of ligand binding systems. Anal. Biochem. *107:* 220–239.

Norman, A. B., G. Battaglia, and I. Creese (1985) [^{3}H]WB4101 labels the 5-HT$_{1A}$ serotonin receptor subtype in rat brain. Mol. Pharmacol. *28:* 487–494.

Pazos, A., and J. M. Palacios (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res. *346:* 205–230.

Pazos, A., D. Hoyer, and J. M. Palacios (1984) The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. Eur. J. Pharmacol. *106:* 539–546.

Pedigo, N. W., H. I. Yamamura, and D. L. Nelson (1981) Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem. *36:* 220–226.

Peroutka, S. J. (1984) 5-HT$_1$ receptor sites and functional correlates. Neuropharmacology *23:* 1487–1492.

Peroutka, S. J. (1985) Selective labeling of 5-HT$_{1A}$ and 5-HT$_{1B}$ binding sites in bovine brain. Brain Res. *344:* 167–171.

Peroutka, S. J. (1986) Pharmacological differentiation and characterization of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites in rat frontal cortex. J. Neurochem. *47:* 529–540.

Peroutka, S. J., and S. H. Snyder (1979) Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. Mol. Pharmacol. *16:* 687–699.

Peroutka, S. J., and S. H. Snyder (1981) Two distinct serotonin receptors: Regional variations in receptor binding in mammalian brain. Brain Res. *208:* 339–347.

Peroutka, S. J., R. M. Lebovitz, and S. H. Snyder (1979) Serotonin receptor binding sites affected differentially by guanine nucleotides. Mol. Pharmacol. *16:* 700–708.

Peroutka, S. J., R. E. Heuring, M. D. Mauk, and J. D. Kocsis (1986a) Serotonin receptor subtypes: Biochemical, physiological, behavioral, and clinical implications. Psychopharmacol. Bull. *22:* 813–817.

Peroutka, S. J., S. Huang, and G. S. Allen (1986b) Canine basilar artery contractions mediated by 5-hydroxytryptamine$_{1A}$ receptors. J. Pharmacol. Exp. Ther. *237:* 901–906.

Peroutka, S. J., P. J. Ison, D. U. Liu, and R. W. Barrett (1986c) Artifactual high-affinity and saturable binding of ^{3}H-5-hydroxytryptamine induced by radioligand oxidation. J. Neurochem. *47:* 38–45.

Peroutka, S. J., M. D. Mauk, and J. D. Kocsis (1987) Modulation of hippocampal neuronal activity by 5-hydroxytryptamine and 5-hydroxytryptamine$_{1A}$ selective drugs. Neuropharmacology (in press).

Raiteri, M., G. Maura, G. Bonanno, and A. Pittaluga (1986) Differential pharmacology and function of two 5-HT$_1$ receptors modulating transmitter release in rat cerebellum. J. Pharmacol. Exp. Ther. *237:* 644–648.

Ransom, R. W., K. B. Asarch, and J. C. Shih (1986) [^{3}H]1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)piperazine: A selective radioligand for 5-HT$_{1A}$ receptors in rat brain. J. Neurochem. *46:* 68–75.

Robaut, C., M. P. Fillion, S. Dufois, C. Fayolle-Bauguen, J. C. Rousselle, G. Gillet, S. Benkirane, and G. Fillion (1986) Multiple high affinity binding sites for 5-hydroxytryptamine: A new class of sites distinct from 5-HT$_1$ and S$_2$. Brain Res. *346:* 250–262.

Schlegel, J. R., and S. J. Peroutka (1986) Nucleotide interactions with 5-HT$_{1A}$ binding sites directly labeled by ^{3}H-8-OH-DPAT. Biochem. Pharmacol. (in press).

Schnellman, R. G., S. J. Waters, and D. L. Nelson (1984) [^{3}H]5-Hydroxytryptamine binding sites: Species and tissue variation. J. Neurochem. *42:* 65–70.

Sills, M. A., B. B. Wolfe, and A. Frazer (1984) Determination of selective and nonselective compounds for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor subtypes in rat frontal cortex. J. Pharmacol. Exp. Ther. *231:* 480–487.

Smith, L. M., and S. J. Peroutka (1986) Differential effects of 5-hydroxytryptamine$_{1A}$ selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. *24:* 1513–1519.

Sprouse, J. S., and G. K. Aghajanian (1985) Serotonergic dorsal raphe neurons: Electrophysiological responses in rats to 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor subtype ligands. Soc. Neurosci. Abstr. *11:* 47.

Taylor, E. W., S. P. Duckles, and D. L. Nelson (1986) Dissociation constants of serotonin agonists in the canine basilar artery correlate to K_i values at the 5-HT$_{1A}$ binding site. J. Pharmacol. Exp. Ther. *236:* 118–125.

Tricklebank, M. D. (1985) The behavioural response to 5-HT receptor agonists and subtypes of the central 5-HT receptor. Trends Pharmacol. Sci. *6:* 403–407.

Trulson, M. E., and K. Arasteh (1985) Buspirone decreases the activity of 5-hydroxytryptamine-containing dorsal raphe neurons in vitro. J. Pharm. Pharmacol. *38:* 380–382.

VanderMaelen, C. P., and R. C. Wilderman (1984) Buspirone, a non-benzodiazepine anxiolytic drug, causes inhibition of serotonergic dorsal raphe neurons in the rat brain slice. Soc. Neurosci. Abs. *10:* 259.

Vane, J. R. (1959) The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. Br. J. Pharmacol. *14:* 87–98.

Whitaker, P. M., and P. Seeman (1978) High-affinity ^{3}H-serotonin binding to caudate: Inhibition by hallucinogens and serotonergic drugs. Psychopharmacology *59:* 1–5.

Yagaloff, K. A., and P. R. Hartig (1985) ^{125}I-lysergic acid diethylamide binds to a novel serotonergic site on rat choroid plexus epithelial cells. J. Neurosci. *5:* 3178–3183.