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[^{3}H]WB4101 Labels the 5-HT$_{1A}$ Serotonin Receptor Subtype in Rat Brain

Guanine Nucleotide and Divalent Cation Sensitivity

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SUMMARY

In the presence of a 30 nM prazosin mask, [^{3}H]-2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane ([^{3}H]WB4101) can selectively label 5-HT$_1$ serotonin receptors. Serotonin exhibits high affinity (K_i = 2.5 nM) and monophasic competition for [^{3}H]WB4101 binding in cerebral cortex. Furthermore, we have found a significant correlation (r = 0.96) between the affinities of a number of serotonergic and nonserotonergic compounds at [^{3}H]WB4101-binding sites in the presence of 30 nM prazosin and [^{3}H]lysergic acid diethylamide ([^{3}H]LSD)-labeled 5-HT$_1$ serotonin receptors in homogenates of rat cerebral cortex. Despite similar pharmacological profiles, distribution studies indicate that, in the presence of 5 mM $MgSO_4$, the B_{max} of [^{3}H]WB4101 is significantly lower than the B_{max} of [^{3}H]LSD in various brain regions. WB4101 competition for [^{3}H]LSD-labeled 5-HT$_1$ receptors fits best to a computer-derived model assuming two binding sites, with the K_H for WB4101 being similar to the K_D of [^{3}H]WB4101 binding derived from saturation experiments. This suggests that [^{3}H]WB4101 labels only one of the subtypes of the 5-HT$_1$ serotonin receptors labeled by [^{3}H]LSD. Interestingly, the selective 5-HT$_{1A}$ serotonin receptor antagonist, spiperone, and the selective 5-HT$_{1A}$ agonist, 8-hydroxy-2-(di-*n*- propylamino) tetraline, exhibit high affinity and monophasic competition for [^{3}H]WB4101 but compete for multiple [^{3}H]LSD 5-HT$_1$ binding sites. These data indicate that [^{3}H]WB4101 selectively labels the 5-HT$_{1A}$ serotonin receptor, whereas [^{3}H]LSD appears to label both the 5-HT$_{1A}$ and the 5-HT$_{1B}$ serotonin receptor subtypes. The divalent cations, Mn^{2+}, Mg^{2+}, and Ca^{2+} were found to markedly increase the affinity and B_{max} of [^{3}H]WB4101 binding in cerebral cortex. Conversely, the guanine nucleotides guanylylimidodiphosphate and GTP, but not the adenosine nucleotide ATP, markedly reduce the B_{max} of [^{3}H]WB4101 binding. These characteristics are typical of agonists interacting with receptors which modulate cellular function via a guanine nucleotide-regulatory subunit.

INTRODUCTION

Two distinct putative receptor populations, believed to be specific for serotonergic systems, have been biochemically characterized in radioligand binding experiments in the past 10 years (1, 2). One receptor population has high nanomolar affinity for various serotonergic agonists and can be labeled by [^{3}H]5-HT[3] and [^{3}H]LSD. Additionally, these receptors have been reported to exhibit lower affinity for serotonergic antagonists and have been designated the 5-HT$_1$ serotonin receptor subtype (1, 2). The second population is distinguished by its relatively lower affinity for serotonergic agonists but high nanomolar affinity for various serotonergic antagonists. This site has been designated the 5-HT$_2$, or S$_2$, serotonin receptor subtype and has been labeled by [^{3}H]LSD and [^{3}H]spiperone (1, 2) and, more recently, by [^{3}H]ketanserin (3, 4).

Detailed characterization of 5-HT$_1$ serotonin receptors has been hampered by the lack of specific ligands. While [^{3}H]5-HT and [^{3}H]LSD have been the most widely used ligands for the labeling of the 5-HT$_1$ serotonin receptor, it has recently been reported that [^{3}H]5-HT, in fact, labels a heterogeneous population of sites. The antagonist spiperone competes for [^{3}H]5-HT binding in a shallow biphasic manner which has been resolved into distinct high and low affinity components. These com-

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[3] The abbreviations used are: 5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide; WB4101, 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane; Gpp(NH)p, guanylylimidodiphosphate; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetraline; EDTA, ethylenediaminetetraacetate; TFMPP, trifluoromethylphenyl piperazine.

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ponents of [^{3}H]5-HT binding defined by the biphasic spiperone displacement have been putatively designated as distinct receptor subtypes based on their differential pharmacology and regional distribution (5–8). The [^{3}H]5-HT-binding site having higher affinity (K_D ~30–120 nM) for spiperone has been designated the 5-HT_{1A} serotonin receptor, while the site having lower affinity (K_D ~1–15 μM) for spiperone has been designated the 5-HT_{1B} serotonin receptor (5).

[^{3}H]WB4101 classically has been identified as an α-adrenergic receptor antagonist ligand in the central nervous system (9), although its selectivity has been questioned but not defined (10–12). This laboratory has recently found that [^{3}H]WB4101 labels multiple sites in rat hippocampus discriminated by high nanomolar affinity (K_D ~0.1 nM) or low micromolar affinity (K_D ~6 μM) for prazosin (13). The sites having high affinity for prazosin proved to correspond to the classical α_1-adrenergic receptor (13). Interestingly, we have found that serotonergic compounds have high affinity for the non-α_1-adrenergic receptor component of [^{3}H]WB4101 binding in rat cerebral cortex (14). We herein extend these latter observations and demonstrate that [^{3}H]WB4101 can selectively label the 5-HT_{1A} subtype of serotonin receptors in rat brain.

EXPERIMENTAL PROCEDURES

Materials. [^{3}H]WB4101 (19.8 Ci/mmol) and [^{3}H]LSD (46.0 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). 5-Hydroxytryptamine creatine sulfate, 5-methoxy-*N*,*N*-dimethyltryptamine, 5-methoxytryptamine, dopamine, (−)-epinephrine, (−)-norepinephrine, Gpp(NH)p, GTP, and ATP were purchased from Sigma Chemical Co. (St. Louis, MO). WB4101 was purchased from Amersham Corp. (Arlington Heights, IL). 8-OH-DPAT and TFMPP were purchased from Research Biochemicals, Inc. (Wayland, MA). LSD was purchased from Alltech/Applied Science (Deerfield, IL). Haloperidol, ketanserin, and spiperone were donated by Janssen Pharmaceutica N.V. (Beerse, Belgium). Prazosin hydrochloride was donated by Pfizer (Brooklyn, NY). Phentolamine hydrochloride was donated by Ciba-Geigy (Summit, NJ). Lisuride hydrogen maleate was donated by Schering A.G. (Berlin, Germany). Dihydroergocryptine was donated by Sandoz (E. Hanover, NJ). Metergoline was donated by Farmitalia (Milan, Italy) and Dr. David Nelson (University of Arizona, Tucson, AZ). Chlorpromazine was donated by Smith, Kline and French (Philadelphia, PA). (−)-Sulpiride was donated by Ravizza (Milan, Italy).

Procedures. Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA; 180–220 g) were used for all experiments. Rats were decapitated and the brains immediately removed into ice-cold saline. Brain regions were rapidly dissected, placed into plastic vials, frozen in liquid nitrogen, and stored at −70° until use. Tissue was homogenized in 50 volumes of ice-cold 50 mM Tris buffer (pH 7.7 at 25°) using a Tekmar Tissuemizer (setting 7, 10 sec) and centrifuged twice (48,000 × *g* for 10 min, 4°) with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer (pH 7.4 at 37°) except for [^{3}H]WB4101 saturation experiments investigating the effect of ions, where the $MgSO_4$ was omitted from the resuspension buffer. Competition experiments with dopamine, (−)-epinephrine, and (−)-norephinephrine also contained 0.02% ascorbic acid. This concentration of ascorbic acid had no effect on serotonin competition for [^{3}H]WB4101. [^{3}H]WB4101 and [^{3}H]LSD competition curves were generated using three intermediate concentrations of inhibitor per log unit and a concentration of 3.0 nM [^{3}H]WB4101 or 1.0 nM [^{3}H]LSD. Saturation experiments employed a concentration range of 0.5–8 nM for both radioligands. Tissue (7.5 mg/tube and 5 mg/tube for [^{3}H]WB4101 and [^{3}H]LSD binding, respectively) was added to a total assay volume of 2.5 ml for both competition and saturation experiments in cortex. [^{3}H]WB4101 binding assays were conducted in the presence of a 30 nM prazosin mask unless stated otherwise. All [^{3}H]LSD binding experiments were conducted in the presence of a 40 nM ketanserin mask to block 5-HT_2 serotonin receptor binding of the radiogland. WB4101 competition curves for [^{3}H]LSD binding in the striatum also were generated in the presence of 200 nM (−)-sulpiride and 100 mM NaCl to selectively preclude binding of [^{3}H]LSD to D_2 dopamine receptors (the presence of 100 mM NaCl and 200 nM (−)-sulpiride reduced the affinity of [^{3}H]LSD for 5-HT_1 serotonin receptors in the striatum less than 2-fold). Nonspecific binding was defined using 0.5 or 1 μM serotonin unless stated otherwise. [^{3}H]LSD binding assays were covered with aluminum foil to prevent photodecomposition of the radioligand. The glass test tubes were incubated at 37° for 40 min and then filtered under vacuum through Whatman GF/C filters and washed three times with 5 ml of ice-cold Tris buffer using a modified Brandel cell harvester. Filters were placed in plastic mini-scintillation vials and 4 ml of Cytoscint (Westchem) were added. Radioactivity trapped on the filters was counted using a Beckman LS7500 scintillation spectrometer at an efficiency of 52%.

Data analysis. The computer analyses employed the weighted, nonlinear least squares curve fitting program, LIGAND (15, 16), using a general model for complex ligand-receptor binding systems (17). The exact treatment of experimental data has previously been described in detail (18). Briefly, competiton curves were first analyzed according to a four-parameter logistic equation, and the slope factor (pseudo-Hill coefficient, designated n_H) was derived. The curves were then analyzed according to a model for the binding of the radioligand and competing drug to one, two, or more binding sites. Deviation of the observed points from the predicted values were weighted according to the reciprocal of the predicted variance (19, 20). Testing for statistical difference between models was obtained by comparing their residual variances of fits to the data by an F test (15), and $p < 0.05$ was taken as indicating a significant difference. A model for two binding sites was retained only when it fitted the data significantly better than a model for a single binding site. Saturation curves were analyzed by Scatchard analysis (21). In some experiments, statistical significance was determined using a two-tailed Student's *t* test for comparison of means.

RESULTS

This laboratory has previously shown that [^{3}H]WB4101 labels multiple sites in rat hippocampus having differential affinity for [^{3}H]WB4101 (13). LIGAND analysis of [^{3}H]WB4101 saturation data using 10 μM phentolamine to define nonspecific binding demonstrated the presence of two distinct binding sites with K_D values of approximately 0.35 nM and 1.8 nM for [^{3}H]WB4101.

As seen in Fig. 1, prazosin displacement of 3.0 nM [^{3}H]WB4101 binding is biphasic. Thus, for computer-assisted two-site analysis of prazosin competition for [^{3}H]WB4101 binding (Fig. 1), using a K_D value of 0.35 nM for [^{3}H]WB4101 at the site having high affinity for prazosin and a K_D value of 1.8 nM for the site having low affinity for prazosin yielded respective K_i values for the two components of prazosin competition of approximately 0.2 nM and 5.3 μM. The high affinity α_1-adrenergic receptor component of [^{3}H]WB4101 binding, which likewise exhibits high affinity for prazosin, has been shown to have a pharmacological profile consistent with the α_1-adrenergic receptor (13). As prazosin displays a greater than 1000-fold difference in its affinity for the two components of [^{3}H]WB4101 binding, a concentration of prazosin (30 nM) in the plateau region of its competition curve (see Fig. 1) will selectively block binding to all of the high affinity component of [^{3}H]WB4101 binding

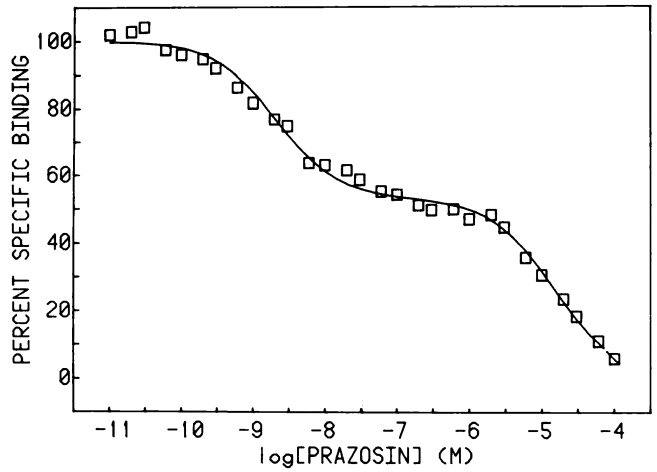

FIG. 1. *Prazosin competition for 3.0 nM [^{3}H]WB4101 binding in the rat cerebral cortex*

Data shown represent the mean of 4 separate experiments with duplicate determinatons at each concentration of inhibitor. Competition curves were generated in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer (pH 7.4 at 37°). Competition curves were analyzed by the computer program LIGAND. Data fit best to a model assuming two binding sites, $K_H = 0.2 \pm 0.03$ nM, $\%R_H = 39\% \pm 2$; $K_L = 5.3 \pm 0.7$ μM. For data analysis the affinities of [^{3}H]WB4101 for its high and low affinity binding sites were assigned values of 0.35 nM and 1.8 nM, respectively. Nonspecific binding was defined by 10 μM phentolamine.

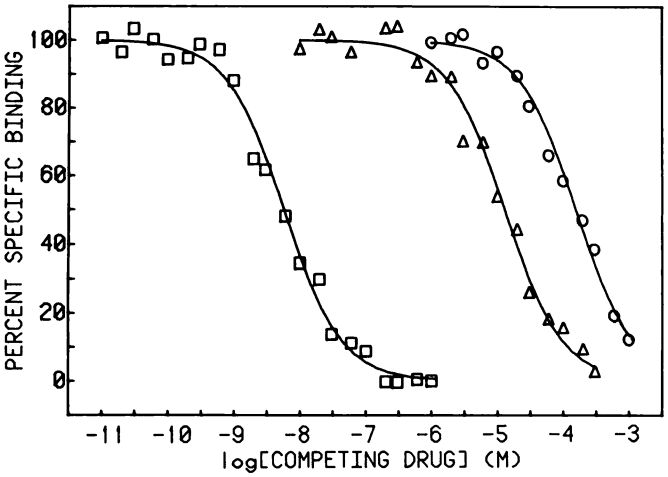

FIG. 2. *Competition of serotonin (□), dopamine (△) and (−)-norepinephrine (○) for 3.0 nM [^{3}H]WB4101 binding in rat cerebral cortex*

The assays were conducted in 50 mM Tris, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer containing 0.02% ascorbic acid (pH 7.4 at 37°). All assays were conducted in the presence of 30 nM prazosin. Nonspecific binding was defined by 500 nM serotonin. All competition data fit to a computer-derived model assuming a single binding site, and the fit was not significantly improved by a model assuming two binding sites. Serotonin: $K_i = 2.5 \pm 0.3$ nM, pseudo-Hill coefficient (n_H) = 1.08; dopamine: $K_i = 2.6 \pm 0.8$ μM, $n_H = 0.98$; (−)-norepinephrine: $K_i = 40.5 \pm 12$ μM, $n_H = 1.03$.

while having no effect on binding to the low affinity component.

In order to characterize the non-adrenergic component of [^{3}H]WB4101 binding, all subsequent assays were thus carried out in the presence of a 30 nM prazosin mask. As seen in Fig. 2, the neurotransmitter serotonin had a very high nanomolar affinity, (K_i ~2.5 nM), while the neurotransmitters dopamine and (−)-norepinephrine exhibited markedly lower affinity for [^{3}H]WB4101 binding sites ($K_i = 2.6$ μM and 40.5 μM, respectively). Since the 5-HT$_1$ serotonin receptor is the only receptor for which serotonin has been shown to exhibit high nanomolar affinity, these data suggest that the site labeled by [^{3}H]WB4101 under these assay conditions may represent the 5-HT$_1$ serotonergic receptor. Competition by serotonin, (−)-norepinephrine, and dopamine for [^{3}H]WB4101 binding were best described by a computer-derived model assuming a single population of binding sites with pseudo-Hill coefficients not significantly different from 1 (Fig. 2). Since the maximal inhibition by serotonin of [^{3}H]WB4101 binding was obtained at a concentration of 500 nM and this was comparable to the inhibition by 10 μM phentolamine, this concentration of serotonin was used to define nonspecific binding in all further experiments. In the presence of 30 nM prazosin, Scatchard analysis of [^{3}H]WB4101 saturation data using a 500 nM serotonin baseline demonstrated the presence of only a *single* saturable binding site in hippocampus, cerebral cortex, and striatum having B_{max} values of approximately 25.8, 8.9, and 1.9 pmol/g of tissue, respectively, and a K_D of approximately 1.4–2.1 nM (Table 1, Fig. 3). Under the conditions employed in competition assays the mean total and nonspecific binding of 2.8–3.2 nM [^{3}H]WB4101 (19.8 Ci/mmol) was 1998 and 948 cpm, respectively, in cerebral cortex (7.5 mg of tissue) and 2885 and 939 cpm in hippocampus (5 mg of tissue). Thus, specific binding constitutes approximately 53% and 68% of total binding in cortex and hippocampus, respectively. At these concentrations of [^{3}H]WB4101, the majority of nonspecific binding is to the GF/C filters rather than the tissue.

As shown in Fig. 4, the affinities (i.e., IC_{50} values) of various serotonergic and nonserotonergic compounds for [^{3}H]WB4101 binding in cerebral cortex indicate that the pharmacological profile of this site is clearly serotonergic. Compounds such as 5-methoxytryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine, LSD, and lisuride exhibit high nanomolar affinities for this binding site. Interestingly, the selective 5-HT$_2$ serotonin receptor antagonist ketanserin had low affinity for the [^{3}H]WB4101 binding site (Fig. 4), precluding the possibility that this binding site may represent the 5-HT$_2$ serotonin receptor. Additionally, a significant correlation ($r = 0.96$, $p < 0.001$) was found between the affinities (IC_{50} values) of numerous serotonergic and nonserotonergic compounds for the site labeled by [^{3}H]WB4101 and 5-HT$_1$ serotonin receptor-specific [^{3}H]LSD binding (defined as the binding of [^{3}H]LSD in the presence of a 40 nM ketanserin mask to preclude [^{3}H]LSD binding to the 5-HT$_2$ serotonin receptor). These data demonstrate that, in the presence of 30 nM prazosin, the binding of [^{3}H]WB4101 has a pharmacological profile consistent with its labeling 5-HT$_1$ serotonin receptors.

Although the majority of compounds listed in Fig. 4 displayed monophasic competition and inhibited [^{3}H]WB1401 binding to a similar degree as serotonin, some compounds (WB4101, chlorpromazine, and clozapine) inhibited below the serotonin baseline at higher concentrations. This could indicate the presence of an addi-

TABLE 1

Regional distribution of [^{3}H]WB4101 and [^{3}H]LSD binding to 5-HT$_1$ serotonin receptors in rat brain

[^{3}H]WB4101 and [^{3}H]LSD saturation assays were conducted in the presence of 30 nM prazosin or 40 nM ketanserin, respectively, in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer (pH 7.4 at 37°). Nonspecific binding was defined by 500 nM serotonin in all experiments. For [^{3}H]WB4101 and [^{3}H]LSD binding, 7.5 mg and 5 mg of tissue per replicate were used, respectively, and a concentration range of 0.5 to 7 nM was used for both radioligands. B_{max} and K_D values were determined from Scatchard analysis of saturation data. Values represent the mean ± SE of the number of separate experiments shown in parentheses.

	[^{3}H]WB4101			[^{3}H]LSD		
		B_{max}	K_D		B_{max}	K_D
		pmol/g tissue	*nM*		*pmol/g tissue*	*nM*
Hippocampus	(n = 11)	25.8 ± 1.3	1.4 ± 0.2	(n = 3)	35.5 ± 1.0	1.8 ± 0.3
Cortex	(n = 9)	8.9 ± 0.3	1.8 ± 0.1	(n = 7)	24.0 ± 1.1	2.9 ± 0.3
Striatum	(n = 3)	1.9 ± 0.5	2.1 ± 0.4	(n = 3)	15.4 ± 1.7	3.6 ± 0.8

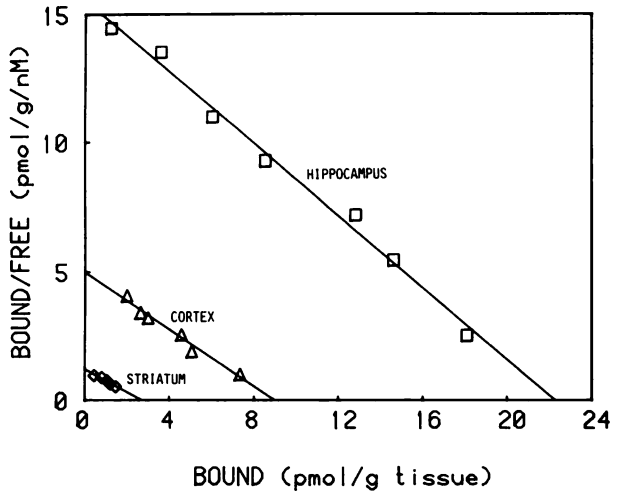

FIG. 3. *Scatchard analysis of [^{3}H]WB4101 binding to membranes of rat hippocampus, cerebral cortex, and striatum*

Assays were conducted in 50 mM Tris, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer (pH 7.4 at 37°) in the presence of 30 nM prazosin. Nonspecific binding was defined by 500 nM serotonin. The amounts of tissue used were 7.5 mg of tissue per replicate for cortex and striatum and 5 mg of tissue per replicate for hippocampus. The concentration range of [^{3}H]WB4101 was 0.5–8 nM. B_{max} and K_D values of these representative experiments were: hippocampus (□), 22.3 pmol/g of tissue and 1.4 nM; cortex (△), 8.9 pmol/g of tissue and 1.8 nM; striatum (◇), 2.7 pmol/g of tissue and 2.2 nM.

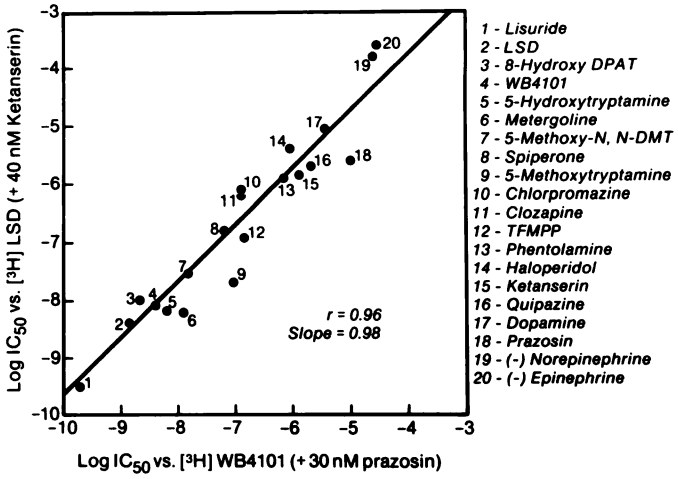

FIG. 4. *Correlation of the potencies of various serotonergic and non-serotonergic compounds for 3.0 nM [^{3}H]WB4101 and 1.0 nM [^{3}H]LSD binding in rat cerebral cortex*

All [^{3}H]WB4101 and [^{3}H]LSD competition assays were conducted in the presence of 30 nM prazosin and 40 nM ketanserin, respectively. All assays were conducted in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer (pH 7.4 at 37°). Nonspecific binding was defined by 500 nM serotonin. *Points* represent the mean of IC_{50} values from 2–8 separate experiments.

tional component of [^{3}H]WB4101 binding not corresponding to either α_1-adrenergic receptors or 5-HT$_1$ serotonin receptors. It was found, however, that this was the result of a small degree of inhibition of nonspecific [^{3}H]WB4101 binding from GF/C filters which could be demonstrated in the absence of tissue.

Heterogeneity of [^{3}H]5-HT-labeled 5-HT$_1$ serotonin receptors based on the biphasic competition of spiperone has been reported. The [^{3}H]5-HT-binding site having highest affinity (~30–120 nM) for spiperone has been designated the 5-HT$_{1A}$ receptor, while the site having lower affinity (~1–15 μM) for spiperone has been designated the 5-HT$_{1B}$ receptor (5). As shown in Fig. 5A, the antagonist spiperone reveals a similar heterogeneity in competition for [^{3}H]LSD-labeled 5-HT$_1$ receptors. Competition for [^{3}H]LSD binding by the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (22) is likewise biphasic and the high affinity component of spiperone and 8-OH-DPAT for [^{3}H]LSD binding is similar to their reported affinities for the 5-HT$_{1A}$ serotonin receptor subtype. This suggests that [^{3}H]LSD, like [^{3}H]5-HT, labels both subtypes of 5-HT$_1$ serotonin receptors. Additionally, we found that computer assisted analysis of the shallow WB4101 competition curve for [^{3}H]LSD binding to 5-HT$_1$ receptors was best described by a computer-derived model assuming two binding sites which have differential affinity for WB4101 (Fig. 5B, Table 2). Scatchard analysis of saturation data, from experiments using the same tissue preparation, demonstrated that B_{max} values for [^{3}H]WB4101 binding were significantly less than the B_{max} value of [^{3}H]LSD binding to 5-HT$_1$ serotonin receptors in all brain regions examined (Table 1). These data support the contention that [^{3}H]WB4101 labels a subpopulation of the 5-HT$_1$ receptors labeled by [^{3}H]LSD. The computer analysis of WB4101 competition for [^{3}H]LSD binding to 5-HT$_1$ receptors in the cortex (Fig. 5B) demonstrated that the high affinity component of WB4101 competition had similar affinity to the K_D of [^{3}H]WB4101 binding as determined in the saturation studies (Table 1). Furthermore, the percentage of [^{3}H]

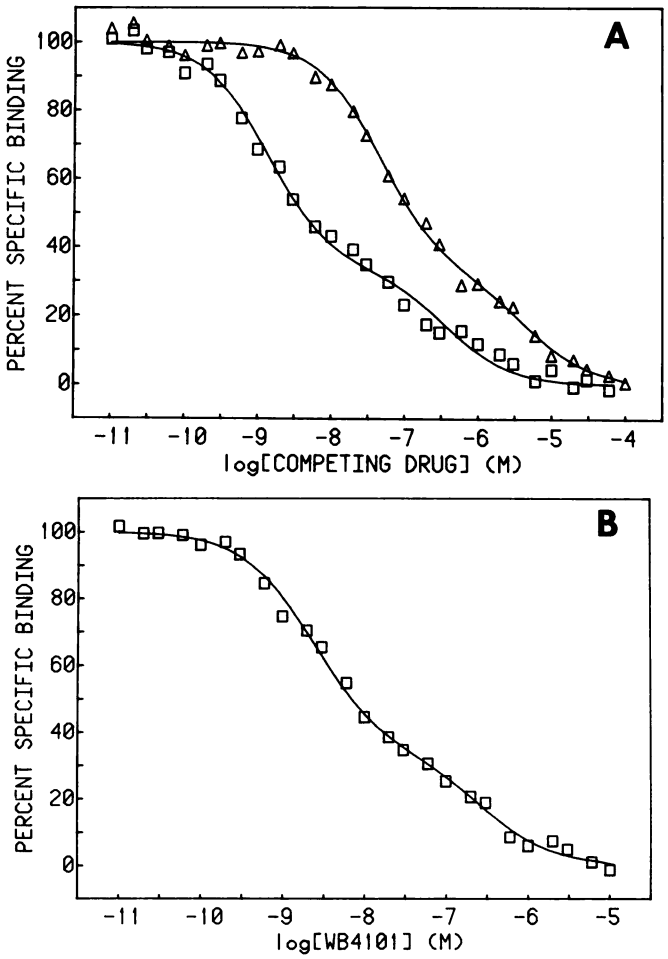

FIG. 5. *Competition of 8-OH-DPAT and spiperone (A) or WB4101 (B) for 1.0 nM [^{3}H]LSD binding in rat cerebral cortex*

All assays were conducted in the presence of 40 nM ketanserin in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer (pH 7.4 at 37°). Nonspecific binding was defined by 500 nM serotonin. The *curves* represent the mean of 4 (8-OH-DPAT) and 6 (spiperone and WB4101) separate experiments with duplicate determinations at each concentration of inhibitor. Competition data were analyzed by the computer program LIGAND. All competition curves were best described by a model assuming two binding sites. *A*, 8-OH-DPAT (□): K_H = 1.1 ± 0.1 nM, K_L= 281.5 ± 48.5 nM, $\%R_H$ = 67 ± 3; spiperone (△): K_H = 36.1 ± 4.1 nM, K_L = 5901.0 ± 2082, $\%R_H$ = 68 ± 3. *B*, WB4101: K_H = 2.1 ± 0.4 nM, K_L = 241.0 ± 21.5 nM, $\%R_H$ = 62 ± 2.

TABLE 2

Computer-derived binding parameters of WB4101 competition for [^{3}H] LSD-labeled 5-HT$_1$ serotonin receptors in various brain regions

Competition data were analyzed using the computer program LIGAND. All competition curves for 1.0 nM [^{3}H]LSD were generated as described in "Experimental Procedures." Nonspecific binding was defined by 0.5 μM serotonin. Values represent the mean ± SE of the number of separate experiments shown in parentheses.

		K_H	R_H	K_L
		nM	%	nM
Hippocampus	(n = 3)	2.0 ± 0.7	76 ± 1	243.1 ± 81.6
Cortex	(n = 6)	2.1 ± 0.4	62 ± 2	241.0 ± 21.5
Striatum	(n = 3)	3.3 ± 0.9	13 ± 6	310.1 ± 75.5

LSD-binding sites having high affinity ($\%R_H$) for WB4101 in the cortex (Fig. 5B) was similar to the $\%R_H$ of spiperone or 8-OH-DPAT for their respective competition for [^{3}H]LSD binding (Fig. 5A). However, in contrast to the biphasic competitions of spiperone and 8-OH-DPAT for [^{3}H]LSD binding (Fig. 5A), the competition for [^{3}H]WB4101 binding by both the 5-HT$_{1A}$-selective antagonist spiperone and the selective agonist 8-OH-DPAT, as well as serotonin (Fig. 2), was adequately described by a computer-derived model assuming a single binding site (Fig. 6). That these compounds exhibit nanomolar affinity for a *homogeneous* population of 5-HT$_1$ serotonergic sites labeled by [^{3}H]WB4101 indicates that [^{3}H]WB4101 can selectively label the 5-HT$_{1A}$ serotonin receptor subtype.

In support of the contention that [^{3}H]WB4101 selectively labels the putative 5-HT$_{1A}$ serotonin receptor subtype, the regional distribution of [^{3}H]WB4101 binding (Table 2) in brain was found to parallel the regional distribution of the 5-HT$_{1A}$ serotonin receptor previously reported (6, 7, 23). Additionally, the overall IC_{50} of WB4101 competition for [^{3}H]LSD-labeled 5-HT$_1$ serotonin receptors was lowest in the hippocampus and highest in the striatum, and LIGAND analysis of these competition curves demonstrated that a low affinity WB4101 binding site was predominant in the striatum while a high affinity binding site was predominant in the hippocampus (Table 2). This corresponds with the higher B_{max} of [^{3}H]WB4101 binding in the hippocampus compared to the markedly lower B_{max} of [^{3}H]WB4101 binding in the striatum (Table 1).

As shown in Table 3, the binding of [^{3}H]WB4101 to the putative 5-HT$_{1A}$ serotonin receptor in rat cortex in 50 mM Tris, 0.5 mM EDTA buffer had a K_D of approxi-

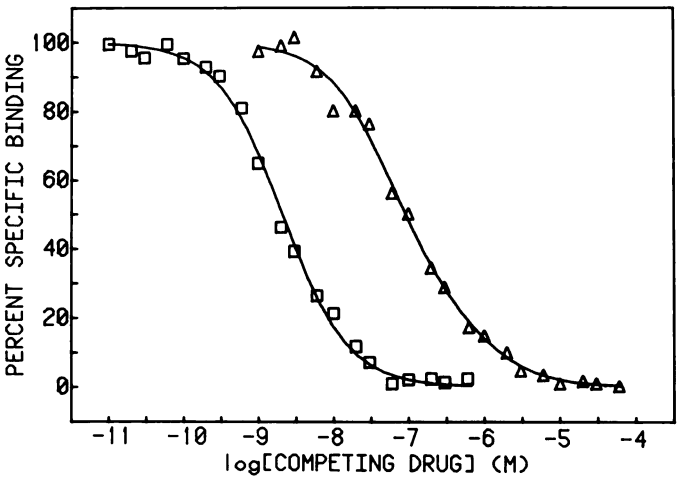

FIG. 6. *Competiton of 8-OH-DPAT (□) and spiperone (△) for 3.0 nM [^{3}H]WB4101 binding in rat cerebral cortex*

Both *curves* represent the mean of 3 separate experiments with duplicate determinations at each concentration of inhibitor. All assays were conducted in the presence of 30 nM prazosin in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer. Nonspecific binding was defined by 500 nM serotonin. Competition data were analyzed by the computer program LIGAND. Both *curves* were adequately described by a model assuming a single binding site, and the fit was not significantly improved by a model assuming two binding sites. 8-OH-DPAT: K_i = 0.8 ± 0.1 nM, n_H = 1.02; spiperone: K_i = 29.9 ± 6 nM, n_H = 0.90.

TABLE 3

Effect of monovalent and divalent cations on the binding of [^{3}H]WB4101 to 5-HT$_{1A}$ serotonin receptors in rat cerebral cortex

All saturation assays were conducted in the presence of 30 nM prazosin in 50 mM Tris, 0.5 mM EDTA buffer in the presence or absence of appropriate ions. Nonspecific binding was defined by 500 nM serotonin. B_{max} and K_D values represent the mean ± SE of the number of separate experiments shown in parentheses and were determined from Scatchard analysis of saturation data using a concentration range of 0.5–7 nM [^{3}H]WB4101.

	B_{max}	K_D
	pmol/g tissue	*nM*
Control (n = 7)	6.9 ± 0.4	5.9 ± 0.8
Monovalent cations		
100 mM NaCl (n = 3)	6.2 ± 0.3	4.9 ± 0.4
100 mM KCl (n = 3)	6.9 ± 0.3	5.0 ± 1.4
5 mM LiCl (n = 3)	6.7 ± 0.4	6.1 ± 1.0
Divalent cations		
5 mM $MnCl_2$ (n = 3)	12 ± 0.3[a]	1.9 ± 0.1[a]
5 mM $MgSO_4$ (n = 9)	8.9 ± 0.3[b,c]	1.8 ± 0.1[a]
5 mM $CaCl_2$ (n = 4)	7.9 ± 0.4[b,c]	2.0 ± 0.1[a]

[a] Significantly different from control values, $p < 0.005$, two-tailed t test.

[b] Significantly different from control values, $p < 0.01$, two-tailed t test.

[c] Significantly different from the B_{max} in the presence of 5 mM $MnCl_2$, $p < 0.005$, two-tailed t test.

TABLE 4

Effects of guanine nucleotides and adenosine nucleotides on the binding of [^{3}H]WB4101 to 5-HT$_{1A}$ serotonin receptors in rat cerebral cortex

All saturation assays were conducted in the presence of 30 nM prazosin in 50 mM Tris, 5 mM $MgSO_4$, 0.5 mM EDTA buffer (pH 7.4 at 37°). Nonspecific binding was defined by 0.5 μM serotonin. B_{max} and K_D values represent the mean ± SE of the number of separate experiments shown in parentheses and were determined from Scatchard analysis of saturation data using a concentration range of 0.5–7 nM [^{3}H]WB4101.

	B_{max}	K_D
	pmol/g tissue	*nM*
Control (n = 9)	8.9 ± 0.3	1.8 ± 0.1
100 μM ATP (n = 3)	8.5 ± 0.5	2.4 ± 0.5
100 μM GTP (n = 4)	4.3 ± 0.7[a]	2.6 ± 0.4[a]
100 μM Gpp(NH)p (n = 5)	3.8 ± 0.8[a]	3.3 ± 0.5[a]

[a] Significantly different from control value ($p < 0.01$, two-tailed t test).

mately 6 nM. The monovalent cations Na^+, K^+, and Li^+ were found to have no significant effect on the B_{max} or K_D of [^{3}H]WB4101 in the cortex. Mn^{2+}, Mg^{2+}, and Ca^{2+}, at a concentration of 5 mM, increased the affinity of [^{3}H]WB4101 binding to a similar extent (Table 3). However, significant differences in the B_{max} of [^{3}H]WB4101 binding were observed in the presence of these divalent cations. The B_{max} of [^{3}H]WB4101 binding was significantly greater in the presence of 5 mM Mn^{2+} than in the presence of 5 mM Mg^{2+} or 5 mM Ca^{2+} ($p < 0.005$, two-tailed t-test, Table 3). This difference in B_{max} in the presence of Mn^{2+} and Mg^{2+} was not due to submaximal concentrations of Mg^{2+} ions being employed, as 5 mM Mg^{2+} was found to be a maximally effective concentration (data not shown). As seen in Table 4, the guanine nucleotides GTP and Gpp(NH)p at a concentration of 100 μM were found to markedly reduce the B_{max} of [^{3}H]WB4101 binding. A small (less than 2-fold) but significant ($p < 0.01$, two-tailed t-test) decrease in the affinity of [^{3}H]WB4101 binding was also observed in the presence of these guanine nucleotides. That these effects were specific for guanine nucleotides is demonstrated by the finding that equimolar concentrations of ATP were found to have no significant effect on either the B_{max} or the K_D of [^{3}H]WB4101 binding (Table 4).

DISCUSSION

We have demonstrated that prazosin can discriminate two [^{3}H]WB4101-binding sites in homogenates of rat cerebral cortex (Fig. 1). The site having high nanomolar affinity for prazosin has previously been shown to represent binding to α_1-adrenergic receptors in rat hippocampus (13). We have recently reported that the second [^{3}H]WB4101-binding site has a pharmacology similar to that of the 5-HT$_1$ serotonin receptor (14). We report here that the detailed pharmacology of [^{3}H]WB4101-labeled 5-HT$_1$ serotonin receptors demonstrates: 1) that the site with lower affinity for both [^{3}H]WB4101 (K_D = 1.8 nM) and prazosin (K_i = 5.6 μM) exhibits a pharmacology consistent with the putative 5-HT$_{1A}$ serotonin receptor subtype, and 2) that this site exhibits divalent cation and guanine nucleotide sensitivity.

Although the selectivity of [^{3}H]WB4101 for α_1-adrenergic receptors has been questioned in previous studies (10–12), we are now in a position to clarify the binding characteristics of this radioligand. In saturation studies employing 30 nM prazosin to define nonspecific binding, [^{3}H]WB4101 will selectively identify α_1-adrenergic receptors. In typical competition experiments using concentrations of [^{3}H]WB4101 at the K_D of this ligand for the α_1-adrenergic receptor (~0.3 nM), which is approximately 7-fold lower than its K_D at the putative 5-HT$_{1A}$ receptor, [^{3}H]WB4101 will label less than 10% of the 5-HT$_{1A}$ receptor population. Thus, only in tissues which have a large density of 5-HT$_{1A}$ receptor-binding sites will this component become significant. If the binding of [^{3}H]WB4101 is conducted in the absence of divalent cations, the binding of [^{3}H]WB4101 to the 5-HT$_{1A}$ receptor will be markedly reduced and the ligand will be even more selective at low concentrations for the α_1-adrenergic receptor since the binding of [^{3}H]WB4101 to the α_1-adrenergic receptor is not affected by 5 mM $MgSO_4$ (data not shown).

In brain, serotonin receptors have been subdivided into two major subtypes designated 5-HT$_1$ and 5-HT$_2$ serotonin receptors (1, 2). 5-HT$_1$ receptors exhibit high nanomolar affinity for serotonin derivatives and lower affinity for antagonists. 5-HT$_2$ receptors exhibit lower affinity for serotonin and serotonergic agonists and high affinity for antagonists such as ketanserin, which has nanomolar affinity for this receptor. We have found that, in the presence of 30 nM prazosin, the site labeled by [^{3}H]WB4101 exhibits nanomolar affinity for serotonin and serotonergic agonists. That the selective 5-HT$_2$ serotonin receptor antagonist ketanserin was found to have very low affinity for [^{3}H]WB4101 binding precludes the possibility that [^{3}H]WB4101 could be labeling 5-HT$_2$

serotonin receptors. The significant correlation found between the affinities (IC_{50} values) of various compounds for [^{3}H]LSD-labeled 5-HT$_1$ receptors and this [^{3}H]WB4101-binding site indicated that the overall pharmacology of this site was consistent with that of the 5-HT$_1$ serotonin receptor.

5-HT$_1$ serotonin receptors have more recently been subdivided into putative 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor subtypes on the basis of shallow spiperone competition curves for [^{3}H]5-HT binding in various regions of the rat brain (6, 7). In the present study, competition of spiperone for [^{3}H]WB4101 binding was monophasic and was described by a computer-derived model assuming a single binding site with a K_i of approximately 30 nM (Fig. 6). This is consistent with the affinity of spiperone for the 5-HT$_{1A}$ site labeled by [^{3}H]5-HT (6–8). These data suggested that [^{3}H]WB4101 was selectively labeling a single homogeneous population of binding sites corresponding to the 5-HT$_{1A}$ serotonin receptor subtype. To further test the selectivity of [^{3}H]WB4101 as a label for the 5-HT$_{1A}$ site, we investigated in detail the competition of a number of selective and nonselective serotonergic agonists for [^{3}H]WB4101 binding. We found that the selective 5-HT$_{1A}$ receptor agonists, 8-OH-DPAT (22) and 5-methoxy-*N*,*N*-dimethyltryptamine (8), exhibited nanomolar affinities and monophasic competition for [^{3}H]WB4101 binding. It was of significant interest that all of these compounds, as well as serotonin itself and various antagonists, were best described by a computer-derived model assuming a single homogeneous population of binding sites. In contrast, the sites labeled by [^{3}H]LSD, in the presence of a 40 nM ketanserin mask to preclude 5-HT$_2$ serotonin receptor binding, appear to be heterogeneous with respect to the selective 5-HT$_{1A}$ antagonist spiperone and agonist 8-OH-DPAT (Fig. 5A). That serotonin competition for [^{3}H]LSD binding is of high affinity and not heterogeneous (data not shown) suggests that [^{3}H]LSD labels the same 5-HT$_1$ serotonin receptor subtypes labeled by [^{3}H]5-HT. Consistent with this hypothesis, we found that WB4101 can discriminate between the two subtypes of 5-HT$_1$ receptors labeled by [^{3}H]LSD: WB4101 competition for [^{3}H]LSD binding was shallow, and computer-assisted analysis demonstrated that the high affinity component of WB4101 competition had an affinity similar to the K_D of [^{3}H]WB4101 for the putative 5-HT$_{1A}$ serotonin receptor. Therefore, these data demonstrate that [^{3}H]LSD-labeled 5-HT$_1$ serotonin receptors (in the presence of 40 nM ketanserin mask to preclude the possibility of labeling 5-HT$_2$ serotonin receptors) represents a heterogeneous population of binding sites, one of which corresponds to the novel [^{3}H]WB4101 binding site. That the overall pharmacology of the sites labeled by 3.0 nM [^{3}H]WB4101 and 1.0 nM [^{3}H]LSD correlate so closely (Fig. 4) despite the subtype selectivity of some compounds (e.g., 8-OH-DPAT, spiperone, WB4101) can be explained by our finding that, at 1.0 nM [^{3}H]LSD in the cortex, 60–70% of the binding is to the 5-HT$_{1A}$ serotonin receptor subtype (Fig. 5 A and B). Furthermore, most of the compounds used in this study have similar affinities for the putative 5-HT$_1$ serotonin receptor subtypes.

5-HT$_{1A}$- and 5-HT$_{1B}$-binding sites have been reported to display marked regional variations in density (6, 7, 23). There is much more high affinity spiperone competition for [^{3}H]5-HT binding in the frontal cortex than in the striatum, where spiperone displacement is essentially all low affinity. This indicates that the striatum contains largely 5-HT$_{1B}$-binding sites, whereas the cortex contains a significantly greater proportion of 5-HT$_{1A}$-binding sites. Likewise, we have found the regional distribution of [^{3}H]WB4101 binding to display a marked regional variation which parallels the distribution of the putative 5-HT$_{1A}$ serotonin receptors.

5-HT$_1$ serotonin receptors have been hypothesized to mediate the effects of serotonin on the stimulation of adenylate cyclase in the rat brain (24, 25). Adenylate cyclase-coupled receptors generally display very characteristic binding properties. For ^{3}H-antagonist-labeled receptors the population appears homogeneous with respect to antagonists, whereas agonists display biphasic competition with high and low affinity binding components. These components represent the high and low affinity agonist-binding states of these receptors which have been shown to be interconvertible by divalent cations and guanine nucleotides (15, 26). Consistent with this, ^{3}H-agonists label only the high affinity agonist component of the receptor population, and, therefore, competition for ^{3}H-agonist binding is homogeneous with respect to both agonists and antagonists as demonstrated by pseudo-Hill coefficients which are not significantly different from 1. That serotonin and 8-OH-DPAT, two agonists, competed for [^{3}H]WB4101 binding with pseudo-Hill coefficients not significantly different from 1 suggested that [^{3}H]WB4101 could not be labeling these binding sites in a manner characteristic of typical antagonists at adenylate cyclase-coupled receptors. Alternatively, the 5-HT$_{1A}$ serotonin receptors labeled by [^{3}H]WB4101 may not represent the subtype of 5-HT$_1$ serotonin receptor mediating stimulation of adenylate cyclase by serotonergic agonists. We therefore investigated the regulation by cations and guanine nucleotides of [^{3}H]WB4101 binding to determine the sensitivity of the putative 5-HT$_{1A}$ subtype to these modulators. That significant increases in the B_{max} of [^{3}H]WB4101 binding were observed in the presence of Ca^{2+}, Mg^{2+}, and Mn^{2+} suggests that these cations induce formation of the high affinity state of this receptor. Additionally, we have found that the increase in the B_{max} of [^{3}H]WB4101 binding by 5 mM Mn^{2+} was significantly greater than the maximal increase elicited by Mg^{2+}, indicating that if the 5-HT$_{1A}$ serotonin receptor indeed represents a two-state receptor, the formation of high affinity [^{3}H]WB4101-labeled sites is differentially regulated by these divalent cations. Furthermore, in the presence of 5 mM $MgSO_4$ there is a selectivity for the guanine nucleotides, GTP and Gpp(NH)p, to significantly reduce the B_{max} of [^{3}H]WB4101 binding, whereas ATP at equimolar concentrations was ineffective (Table 4). Thus, although WB4101 is an antagonist at α_1-adrenergic receptors (9), our data indicate that [^{3}H]WB4101 has binding characteristics consistent with it acting as an agonist at the putative 5-HT$_{1A}$ serotonin receptor subtype.

A recent report by Hall *et al.* (27) demonstrates that the binding of [^{3}H]8-OH-DPAT has a pharmacological profile similar to that demonstrated for [^{3}H]WB4101 binding. However, it is interesting to note that competition of various compounds for [^{3}H]8-OH-DPAT binding in the cerebral cortex demonstrated pseudo-Hill coefficients significantly less than 1, indicating the labeling of multiple sites by [^{3}H]8-OH-DPAT. Furthermore, the B_{max} of [^{3}H]8-OH-DPAT binding in the striatum (which contains mainly the putative 5-HT_{1B}- rather than the 5-HT_{1A}-binding site (7, 23)) was 64% of that in the cerebral cortex, while in the present study the B_{max} of [^{3}H]-WB4101 binding in the striatum was only approximately 21% of that found in the cerebral cortex. It is therefore possible that [^{3}H]WB4101, under the conditions employed in the present study, is a more selective radioligand for the 5-HT_{1A} receptor subtype than was [^{3}H]8-OH-DPAT.

In conclusion, these data clearly indicate that [^{3}H] WB4101, in the presence of 30 nM prazosin, is a selective radioligand for the putative 5-HT_{1A} receptor-binding site. Thus, [^{3}H]WB4101 may be useful in the classification of multiple 5-HT_1 serotonin receptor subtypes, especially in light of recent reports (28) of multiple 5-HT_1 serotonin receptors distinct from the 5-HT_{1A} and 5-HT_{1B} serotonin receptors identified by Nelson and coworkers (5–7, 23). The 5-HT_{1A} serotonin receptor subtype identified in this study would appear to possess multiple affinity states which may be discriminated by WB4101 and differentially regulated by divalent cations and guanine nucleotides. Thus, the 5-HT_{1A} serotonin receptor appears to have binding characteristics consistent with a receptor which modulates cellular function via a guanine nucleotide-regulatory subunit. This could reflect coupling of the receptor to either adenylate cyclase or phosphoinositide metabolism. Indeed, both of these effectors have been proposed to be coupled to 5-HT_1 serotonin receptors (24, 25, 29). Furthermore, the 5-HT_{1A} serotonin receptor subtype has recently been shown to be linked to the stimulation of adenylate cyclase (30). Whether the 5-HT_{1A} receptor subtype is also associated with the stimulation of phosphoinositide metabolism remains to be elucidated.

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