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Characterization of Two [^{3}H]Ketanserin Recognition Sites in Rat Striatum

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Abstract: Two [^{3}H]ketanserin recognition sites are present in the rat striatum. The high-affinity site (K_D, 0.39 n*M*) is similar to the 5-hydroxytryptamine$_2$ (5-HT$_2$) site previously characterized by various investigators. The low-affinity site (K_D, 21.8 n*M*) has a unique pharmacologic specificity and is preferentially localized to rat striatum and septum. Conventional 5-HT$_2$ antagonists as well as 5-HT and 5-HT uptake inhibitors are ineffective at inhibiting [^{3}H]-ketanserin binding to this low-affinity site. Also, chronic treatment with *p*-chlorophenylalanine, which depletes brain 5-HT, upregulates only the high-affinity site. Thus, in the striatum and septum, [^{3}H]ketanserin labels a unique recognition site. This site has recently been shown to be associated with dopaminergic nerve endings and may regulate biogenic amine release. **Key Words:** Serotonin receptors—5-Hydroxytryptamine$_2$ receptors—Ketanserin—Recognition site—**Roth B. L. et al.** Characterization of two [^{3}H]ketanserin recognition sites in rat striatum. *J. Neurochem.* **49**, 1833–1838 (1987).

Serotonin (5-hydroxytryptamine; 5-HT) receptors are subdivided into two major classes, 5-HT$_1$ and 5-HT$_2$, on the basis of radioligand binding studies (Peroutka and Snyder, 1979) and coupling to biochemical processes that mediate signal transduction. The 5-HT$_1$ sites are proposed to activate adenylate cyclase (Barbaccia et al., 1983) and phospholipase C (Janowsky et al., 1984) in rat hippocampus. The 5-HT$_2$ sites, on the basis of studies with 5-HT$_2$ selective antagonists such as ketanserin, are suggested to be specifically associated with a phosphoinositide-specific phospholipase C in rat aorta (Roth et al., 1984, 1986*a*) and platelets (DeChaffoy de Courcelles et al., 1985). Conn and Sanders-Bush (1984, 1985) and Kendall and Nahorski (1985) proposed a similar association in rat frontal cortex. For a recent review see Roth and Chuang (1987).

Since its introduction as a selective radioligand for 5-HT$_2$ recognition sites, [^{3}H]ketanserin has been used extensively to study the tissue distribution (Leysen et al., 1982), cellular and subcellular distribution (Luabeya et al., 1986), and brain distribution of 5-HT$_2$ sites (Schotte et al., 1985). [^{3}H]Ketanserin sites have been shown by autoradiography to be localized to layer IV of the cortex and striatum (Pazos et al., 1985). Also, ^{125}I-lysergic acid diethylamide (LSD) binding to 5-HT$_2$ receptors was reported to be highest in rat cortex and striatum (Kadan et al., 1984). Intense serotonergic innervation of both the striatum (Steinbush, 1981) and cortex (Lidov et al., 1980) has been demonstrated, so it is not surprising that [^{3}H]-ketanserin binding is high in these regions. It has been assumed, though, that the sites labelled by radioactive 5-HT$_2$ antagonists in the cortex and extracortical regions are pharmacologically identical, although this assumption has not been completely tested.

Pazos et al. (1985) recently confirmed the finding of Slater and Patel (1982) that [^{3}H]ketanserin sites are

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The opinions and assertions contained herein are the private ones of the authors and are not to be construed as reflecting the views of the U.S. Navy, the naval service at large, or the Department of Defense.

The experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Resources, National Research Council, DHEW, publication No. (NIH) 85-23.

Abbreviations used: DOPAC, dihydroxyphenylacetic acid; Gpp(NH)p, 5′-guanylylimidodiphosphate; 5-HT, 5-hydroxytryptamine; PCPA, *p*-chlorophenylalanine.

enriched in striatum and cortex. Pazos et al. (1985) noted, though, that the sites in striatum were incompletely displaced by 5-HT or spiperone. Leysen et al. (1984) presented preliminary evidence as well that the striatal site might be unique, and recently reported that this site is associated with dopaminergic nerve terminals and regulates dihydroxyphenylacetic acid (DOPAC) release in vitro (Leysen et al., 1987). In this article we report a detailed characterization of this pharmacologically unique, anatomically distinct, nonserotonergic site in rat striatum. A preliminary account of these findings was previously published in abstract form (Roth et al., 1986*b*).

MATERIALS AND METHODS

Materials

[^{3}H]Ketanserin (70 Ci/mmol) and Aquassure were from New England Nuclear (Boston, MA, U.S.A.). The following compounds were obtained from pharmaceutical companies: mianserin, ketanserin, spiroperidol, and haloperidol from Janssen Pharmaceuticals (Piscataway, NJ, U.S.A.); amitryptyline from Merck, Sharp and Dohme Research Laboratories (West Point, PA, U.S.A.); prazosin from Pfizer Chemical (Ridgefield, NJ, U.S.A.); pizotifen from Sandoz (Basel, Switzerland); and LY53857 from Eli Lilly (Indianapolis, IN, U.S.A.). Other biochemicals were from Sigma (St. Louis, MO, U.S.A.).

Binding assays

Male Sprague–Dawley rats (150–200 g) supplied from Zivic Miller were given intraperitoneal injections of the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (PCPA) or saline for 18 days as previously described by Brunello et al. (1982). The animals were killed, their brains removed, and the corpus striatum and frontal cortex dissected and frozen on dry ice and stored at −70°C or immediately homogenized with a Polytron in binding buffer (50 m*M* Tris-Cl, pH 7.40 at 25°C) at 4°C. A crude membrane preparation was made by centrifugation at 20,000 *g* for 20 min. Aliquots of this crude membrane preparation were incubated in a total volume of 1.0 ml of binding buffer with 0.5–1.0 n*M* [^{3}H]ketanserin and various concentrations of unlabelled ligands. Following a 90-min incubation at 25°C, membranes were harvested with a Brandel SM-24 cell harvester onto Whatman GF/B glass fiber filters and washed with 3 × 3 ml rinses of ice-cold binding buffer. Samples were counted in an LKB Rack Beta Counter at 35% efficiency after soaking overnight in Aquassure. The concentration of membranes used during the binding assays was 200 μg protein/ml.

Data analysis

The computerized, nonlinear, least-squares regression algorithm developed by Munson and Rodbard (1980) was used as previously described by Roth and Coscia (1984) using the National Institutes of Health DEC/10 computer. This weighted curve-fitting program assumes binding according to the law of mass action to independent classes of binding sites. An *F* test incorporated into the program distinguishes among various binding models (one, two, or more sites) while a runs test ascertains systematic deviations from the model under study. A $p < 0.05$ was used to distinguish among binding models. Nonspecific binding is a fitted function when this program is utilized and the *N* value was between 0.04 and 0.05. For this analysis, nonspecific binding is a shared parameter that is fitted to all curves simultaneously.

Autoradiography studies

Male rats (200–300 g) were decapitated, their brains rapidly removed, placed into isopentane precooled to −30°C, and then frozen in dry ice and stored (−35°C) until cut. Frozen brains were mounted onto cryostat chucks with mounting medium and 20-μm thick horizontal sections were cut, thaw-mounted onto gelatin-coated slides, placed

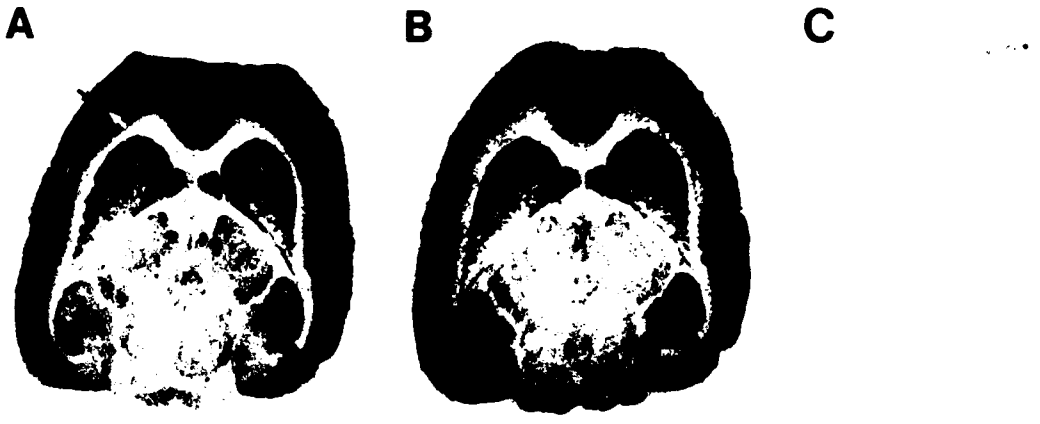


FIG. 1. Twenty-micron-thick horizontal sections of fresh rat brain were cryostat-cut, thaw-mounted onto gelatin-coated slides and stored according to the method of Herkenham and Pert (1982). Incubation of the slides was performed using binding conditions identical to those for membrane studies. **A:** [^{3}H]Ketanserin densely labels the caudate and septum and layers IV and V of the cortex. Moderate labeling is present in layers I and V of the prelimbic (PL) cortex. **B:** Addition of 100 n*M* mianserin displaces [^{3}H]ketanserin to all cortical areas, but has no effect on binding to striatum or septum. **C:** Adjacent sections incubated with 1 μ*M* ketanserin showed minimal binding in cortex, striatum, or septum.

into slide boxes kept on ice, and then placed in a desiccator and stored under vacuum for 24 h at 4°C (Herkenham and Pert, 1982). The sections were then stored at −70°C until use.

The slides were then removed from the freezer, allowed to come to room temperature, and incubated with 1 n*M* [^{3}H]ketanserin for 90 min at 25°C. Following incubation the slides were rinsed sequentially (4 × 1 min) in ice-cold buffer and rapidly dried under a stream of cold air.

The slides were placed into a cassette (Wolf), overlaid with tritium-sensitive Ultrofilm (LKB), exposed for 2 months, and developed with D-19 (Kodak).

RESULTS

Demonstration of anatomically distinct [^{3}H]ketanserin binding sites

The striatum and layer V of the cortex are selectively enriched in [^{3}H]ketanserin recognition sites. Moderate densities of binding sites are present in the septum and layers I and IV of the frontal cortex (Fig. 1A). Incubation of adjacent sections with 100 n*M* mianserin displaces [^{3}H]ketanserin from cortical binding sites, but does not affect binding in the septum and striatum (Fig. 1B). Incubation of adjacent sections with 1,000 n*M* ketanserin abolishes binding to the cortex, striatum, and septum (Fig. 1C).

Characterization of striatal [^{3}H]ketanserin sites

Because this mianserin-resistant ketanserin site is enriched in corpus striatum, we performed detailed binding studies using striatal tissue. For comparison with conventional ketanserin sites (5-HT$_2$) we also examined the frontal cortex. We evaluated eight separate compounds over a concentration range of 0.1–10,000 n*M*. We previously evaluated this series of compounds for the inhibition of 5-HT$_2$-stimulated phosphoinositide hydrolysis in rat aorta (Roth et al., 1986*a*). As is seen in Figs. 2–4, the competition binding isotherms for each 5-HT$_2$ antagonist are complex in rat striatum. With the exception of ketanserin (Fig. 2) and haloperidol (not shown), a distinct plateau is seen with each drug. This suggests that there might be two distinct ketanserin sites in rat striatum. Prazosin, an α_1-adrenergic antagonist, is relatively ineffective at inhibiting ketanserin binding to rat striatum and cortex (Table 1). Also, the 5-HT uptake inhibitors imipramine (Fig. 3) and amitryptyline (Table 1) had low affinities for the low-affinity ketanserin sites in rat striatum, and high potency for the higher affinity site. Also, the selective 5-HT$_2$ antagonist LY53857 is ineffective at the low-affinity ketanserin site (Table 1). Finally, mianserin, which is proposed to label a distinct class of 5-HT$_2$ receptors (Gandolfi et al., 1985),

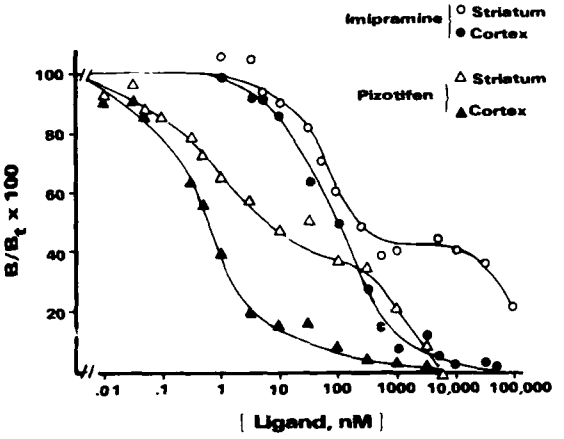


FIG. 3. Inhibition of [^{3}H]ketanserin binding by imipramine and pizotifen in striatum and cortex. Membranes were prepared and data analyzed as in Fig. 2 using imipramine and pizotifen as unlabeled ligands.

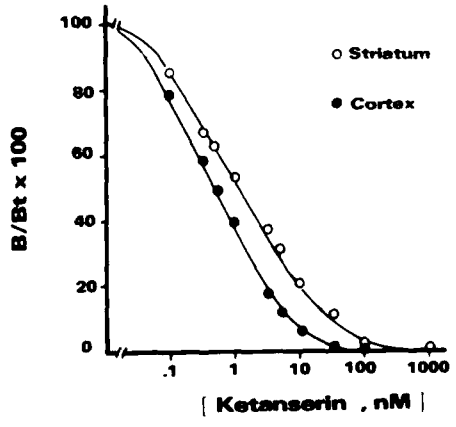


FIG. 2. Inhibition binding isotherm for ketanserin in cortex and striatum. Membranes were prepared (see Materials and Methods) and ketanserin versus [^{3}H]ketanserin isotherms prepared. Data represent the ratio of specifically bound [^{3}H]ketanserin at each dose of unlabelled drug. Nonspecific binding is a fitted parameter (see Munson and Rodbard, 1980).

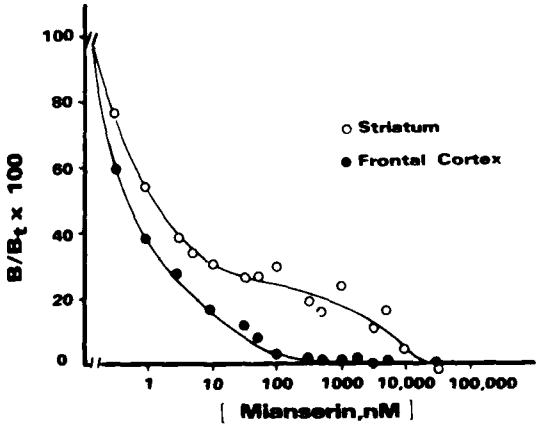


FIG. 4. Inhibition of [^{3}H]ketanserin binding by mianserin in striatum and cortex. Membranes were prepared and data analyzed as in Fig. 2 using mianserin as unlabeled ligand.

TABLE 1. *Binding constants for various 5-HT$_2$ antagonists for displacement of [^{3}H]ketanserin binding in rat cortex and striatum*

Drug	Striatum			Cortex
	K_{D1}	K_{D2}	K_{D1}/K_{D2}	K_D
Ketanserin	0.39 ± 0.1	21.8 ± 7.2	55.9	0.89 ± 0.3
Mianserin	0.69 ± 0.44	2,907 ± 639	4,213	0.55 ± 0.2
Haloperidol	14.6 ± 4.6	5,107 ± 639	349	30.6 ± 10
Amitryptyline	2.77 ± 0.12	12,310 ± 3,000	4,444	1.9 ± 0.7
Imipramine	23.9 ± 11.0	206,500 ± 90,000	8,640	—
Pizotifen	0.21 ± 0.05	1,286 ± 180	6,123	0.29 ± 0.05
Spiroperidol	0.103 ± 0.05	1,956 ± 300	18,990	0.12 ± 0.04
LY53857	1.158 ± 0.34	6,610 ± 1,800	5,708	2.27 ± 0.8
5-HT	42.4 ± 22	200,000 ± 43,000	4,716	—
5-HT + 100 μM Gpp(NH)p	20.4 ± 10	209,400 ± 36,000	10,245	—

Data represent means ± SD of computer-derived estimates for two to five separate experiments. K_D values are expressed in terms of nanomolar affinity. The percent high affinity (% R_H) decreased from 4.9 ± 0.5% to 2.6 ± 0.4% ($p < 0.05$) in the presence of 100 μM Gpp(NH)p.

easily inhibits only the high-affinity ketanserin binding site (Fig. 4).

Analysis of the competition isotherms by weighted, nonlinear least-squares regression analysis (Munson and Rodbard, 1980) discloses high- and low-affinity ketanserin sites in rat striatum (Table 1). The high-affinity site has a subnanomolar affinity for ketanserin and a B_{max} of 104 fmol/mg protein whereas the low-affinity site has nanomolar affinity for ketanserin and a B_{max} of 2,993 fmol/mg protein (Table 2). The various 5-HT$_2$ receptor antagonists compete potently for the high-affinity ketanserin binding sites, but weakly for the low-affinity sites (Table 1). Spiroperidol has the greatest selectivity ratio (K_{D1}/K_{D2}) of 18,990, although it has moderate affinity for the ketanserin site (K_D, 2 μM). In contrast, imipramine (Fig. 3) has extremely low affinity for the second ketanserin site (K_D, 206,000 nM), whereas 5-HT does not inhibit binding to the low-affinity site (K_D > 100 μM).

In general, the capacity of the 5-HT$_2$ receptor antagonists to inhibit the high-affinity site correlates with their capacity to inhibit [^{3}H]ketanserin binding in frontal cortex (Fig. 5) and 5-HT-stimulated phosphoinositide metabolism in rat aorta (see Roth et al., 1986*a*). In contrast, all the agents tested were relatively ineffective (K_D values > 1,000 nM) at inhibiting the low-affinity ketanserin site.

Alteration of striatal [^{3}H]ketanserin binding with PCPA treatment

To determine whether the striatal sites were physiologically related to 5-HT levels, we treated rats chronically with PCPA as previously described by Brunello et al. (1982). The low-affinity sites were recently shown to be altered by 6-hydroxydopamine (6-OHDA) which lesions dopaminergic terminals (Leysen et al., 1987). Chronic PCPA treatment increases the number of high-affinity ketanserin sites and caused a statistically insignificant increase in the number of low-affinity sites (Table 2). Thus, the high-affinity sites may be selectively altered by PCPA whereas the low-affinity sites are significantly altered only by lesioning dopaminergic terminals.

Guanine nucleotide interactions with agonist (5-HT) binding in striatum

[^{3}H]Ketanserin binding to the high-affinity site is displaced by various concentrations of 5-HT in the

TABLE 2. *Alteration of striatal ketanserin sites by chronic treatment with PCPA*

	K_{D1} (nM)	K_{D2} (nM)	B_{max1} (fmol/mg)	B_{max2} (fmol/mg)
Control				
Ketanserin	0.21 ± 0.18	19.1 ± 4.2	104 ± 18	2,993 ± 391
Mianserin	0.69 ± 0.44	2,907 ± 639		
PCPA-treated				
Ketanserin	0.45 ± 0.27	25.3 ± 5.3	175 ± 13[a]	3,934 ± 532
Mianserin	1.26 ± 0.55	2,343 ± 150		

For this experiment binding isotherms of ketanserin versus [^{3}H]ketanserin (0.5 nM) and mianserin versus [^{3}H]ketanserin (1.0 nM) were performed and analyzed simultaneously (n = 3 separate experiments). K_D values are expressed in nanomolar affinity and B_{max} values are in terms of fmol/mg protein.
[a] $p < 0.05$ versus control.

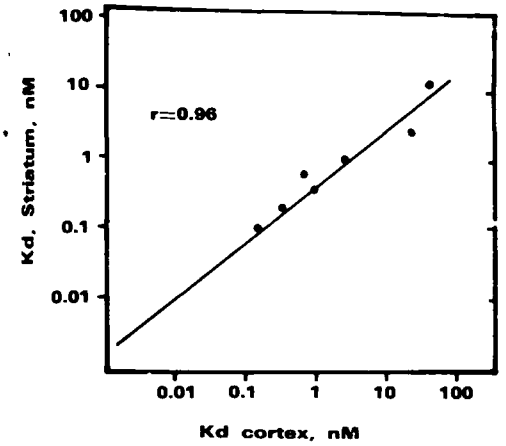


FIG. 5. Correlation of high-affinity ketanserin binding with cortical 5-HT_2 receptors. Shown is the correlation between the high-affinity K_D values (K_{D1}) for various antagonists for striatal ketanserin binding sites and K_D values for cortical 5-HT_2 sites. $r = 0.96$ ($p < 0.01$). Antagonists used were (from left to right in figure) spiroperidol, pizotifen, ketanserin, mianserin, LY53857, amitryptyline, and haloperidol.

presence and absence of 5'-guanylylimidodiphosphate [Gpp(NH)p] (100 μM) (Table 1). A twofold decrease in the number of high-affinity sites was obtained when 100 μM Gpp(NH)p was included. In contrast, ketanserin binding to the low-affinity site is not affected by either Gpp(NH)p or 5-HT (not shown). The efficacy of guanine nucleotides to alter the capacity of 5-HT to inhibit the high-affinity ketanserin site agrees with the findings of Kendall and Nahorski (1983).

DISCUSSION

In this article we characterize the presence of two [^{3}H]ketanserin binding sites in rat striatum. One site represents binding to a classic 5-HT_2 receptor based on antagonist binding profiles as well as 5-HT displacement studies. The second site represents a pharmacologically unique binding site. This second site is unaffected by altering brain 5-HT levels with PCPA but was recently shown to be abolished by 6-OHDA lesioning of dopaminergic terminals (Leysen et al., 1987).

The autoradiographic studies suggest that this low-affinity site is anatomically distinct (Fig. 1). Analysis of horizontal brain sections labeled with [^{3}H]-ketanserin reveals these sites to be enriched in corpus striatum and septum. In contrast, the 5-HT_2 sites are enriched in layers I, IV, and V of the cortex, in agreement with previous studies (Altar et al., 1985; Blue et al., 1986).

Because of the unique specificity and localization of this low-affinity site, the possibility is raised that it represents a "drug recognition site." These types of recognition molecules have been previously described for spirodecadones (Palacios et al., 1981) and benzomorphans (Tam, 1983) and have shown striking regional specificity (Howlett et al., 1979). Recent studies by Leysen et al. (1987) cast doubt on this idea and support the hypothesis that the low-affinity site represents a functional binding site for ketanserin and certain congeners.

These authors noted (Leysen et al., 1987) that ketanserin and a related compound, ritanserin (Leysen et al., 1985), provoked the release of DOPAC from striatal slices. Further, the affinity profiles of several "ketanserin-like" compounds for evoking the release of DOPAC and for binding to the low-affinity site were highly correlated ($r = 0.996$) (Leysen et al., 1987). Thus, although our findings show that the low-affinity site is unrelated to 5-HT uptake mechanisms it apparently is related to a tetrabenazine-sensitive dopamine uptake mechanism (Leysen et al., 1987). The affinity for ketanserin reported in our study (K_D, 19.1 ± 4.2) is quite similar to that reported by Leysen et al. (K_D, 14.7 ± 1.4 nM) in their preliminary study.

In conclusion, we have characterized two [^{3}H]-ketanserin recognition sites in rat striatum and septum. The high-affinity site represents the 5-HT_2 receptor whereas the low-affinity site, although unrelated to serotonergic functioning, may regulate DOPAC release on dopaminergic terminals.

REFERENCES

Altar C. A., O'Neil S., Walter R. J., and Marshall J. F. (1985) Brain dopamine and serotonin receptor sites revealed by digital subtraction autoradiographic studies. *Science* **228**, 597–600.

Barbaccia M. L., Brunello N., Chuang D.-M., and Costa E. (1983) Serotonin-elicited amplification of adenylate cyclase in hippocampal membranes from adult rats. *J. Neurochem.* **40**, 1671–1679.

Blue M. E., Yagaloff K. A., Mamounas L. A., Hartig P. R., and Molliver M. E. (1986) Correspondence of 5HT_2 receptor distribution with serotonin innervation in rat cortex. *Soc. Neurosci. Abstr.* **12**, 145.

Brunello N., Chuang D.-M., and Costa E. (1982) Different synaptic location of mianserin and imipramine binding sites. *Science* **215**, 1112–1115.

Conn P. J. and Sanders-Bush E. (1984) Selective 5HT_2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. *Neuropharmacol.* **23**, 993–996.

Conn P. J. and Sanders-Bush E. (1985) Serotonin-stimulated phosphoinositide turnover: mediation by the S_2 binding site in rat cerebral cortex but not in subcortical regions. *J. Pharmacol. Exp. Ther.* **234**, 195–203.

DeChaffoy de Courcelles D., Leysen J. E., DeClerck F., VanBelle H., and Janssen P. A. J. (1985) Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S_2 receptor sites. *J. Biol. Chem.* **260**, 7603–7608.

Gandolfi O., Barbaccia M. L., and Costa E. (1985) Different effects of serotonin antagonists on ^{3}H-mianserin and ^{3}H-ketanserin recognition sites. *Life Sci.* **36**, 713–721.

Herkenham M. and Pert C. B. (1982) Light microscopic localization of brain opiate receptors: a general autoradiographic method which preserves tissue quality. *J. Neurosci.* **2**, 1129–1149.

Howlett D. R., Morris H., and Nahorski S. R. (1979) Anomolous properties of [^{3}H]-spiperone binding sites in various areas of the rat limbic system. *Mol. Pharmacol.* **15**, 506–514.

Janowsky A., Labarca R., and Paul S. A. (1984) Characterization of neurotransmitter receptor mediated phosphatidylinositol hydrolysis in the rat hippocampus. *Life Sci.* **35**, 1953–1961.

Kadan M. J., Krohn A. M., Evans M. J., Waltz R. L., and Hartig P. R. (1984) Characterization of [^{125}I]-lysergic acid diethylamide binding to serotonin receptors in rat frontal cortex. *J. Neurochem.* **43**, 601–606.

Kendall D. A. and Nahorski S. R. (1983) Temperature-dependent 5-hydroxytryptamine (5HT)-sensitive [^{3}H]-spiperone binding to rat cortical membranes: regulation by guanine nucleotide and antidepressant treatment. *J. Pharmacol. Exp. Ther.* **227**, 427–434.

Kendall D. A. and Nahorski S. R. (1985) 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterization and effects of antidepressants. *J. Pharmacol. Exp. Ther.* **233**, 473–479.

Leysen J. E., Niemegeer C. J. E., Van Neuten J. M., and Laduron P. M. (1982) [^{3}H]Ketanserin (R 41 468), as selective ^{3}H-ligand for serotonin$_2$ receptor binding sites: binding properties, brain distribution and functional role. *Mol. Pharmacol.* **21**, 304–314.

Leysen J. E., De Chaffoy de Courcelles D., DeClerck F., Niemegeers C. J. E., and Van Neuten J. M. (1984) Serotonin-S_2 receptor binding sites and functional correlates. *Neuropharmacology* **23**, 1493–1498.

Leysen J. E., Gommeren W., Van Gompel P., Wynants J., Janssen P. A. J., and Laduron P. M. (1985) Receptor binding properties in vitro and in vivo of ritanserin, a very potent and long acting serotonin-S_2 antagonist. *Mol. Pharmacol.* **27**, 600–609.

Leysen J. E., Eens A., Gommeren W., Van Gompel P., Wynants J., and Janssen P. A. J. (1987) Non-serotonergic [^{3}H]-ketanserin binding sites in striatal membranes are associated with a dopac release system on dopaminergic nerve endings. *Eur. J Pharmacol.* **134**, 373–375.

Lidov H. G. W., Grzanna R., and Molliver M. E. (1980) The serotonin innervation of the cerebral cortex in the rat: an immunohistochemical analysis. *Neuroscience* **5**, 207–227.

Luabeya M. K., Maloteaux J. M., DeRoe C., Trouet A., and Laduron P. M. (1986) Different subcellular localization of muscarinic and serotonin (S_2) receptors in human, dog and rat brain. *J. Neurochem.* **46**, 405–412.

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

Palacios J. M., Niehoff D. L., and Kuhar M. J. (1981) ^{3}H-Spiperone binding sites in brain. Autoradiographic localization of receptors. *Brain Res.* **213**, 277–289.

Pazos A., Cortex A., and Palacios J. M. (1985) Quantitative autoradiographic mapping of serotonin receptors in rat brain. II serotonin-2 receptors. *Brain Res.* **346**, 231–249.

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

Roth B. L. and Chuang D.-M. (1987) Minireview: Multiple mechanisms of serotonergic signal transduction. *Life Sci.* **41**, 1051–1064.

Roth B. L. and Coscia C. J. (1984) Microsomal opiate receptors: characterization of smooth microsomal and synaptic membrane opiate receptors. *J. Neurochem.* **42**, 1677–1684.

Roth B. L., Nakaki T., Chuang D.-M., and Costa E. (1984) Aortic recognition sites for serotonin (5HT) are coupled to phospholipase C and modulate phosphatidylinositol turnover. *Neuropharmacology* **23**, 1234–1237.

Roth B. L., Nakaki T., Chuang D.-M. and Costa E. (1986*a*) Characterization of $5HT_2$ receptors coupled to phospholipase C in rat aorta: modulation of phosphoinositide metabolism by phorbol ester. *J. Pharmacol. Exp. Ther.* **238**, 480–485.

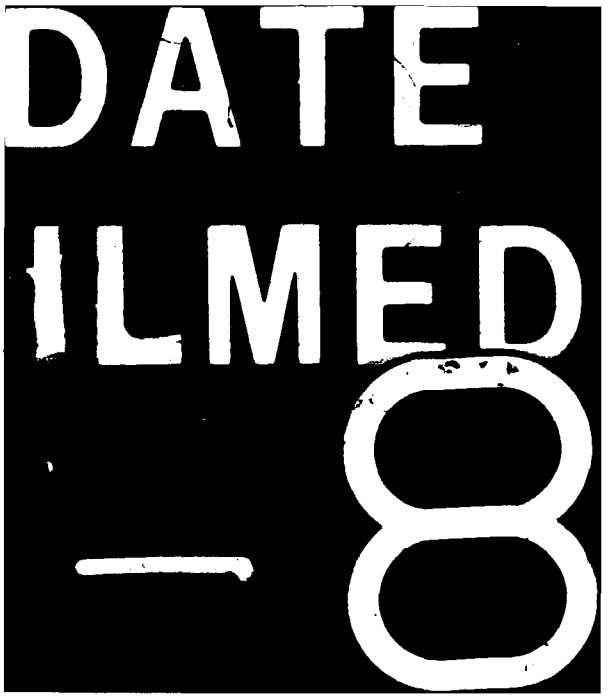
Roth B. L., Zhu X.-Z., Chuang D.-M., and McLean S. (1986*b*) Characterization of a unique [^{3}H]-ketanserin recognition site in rat striatum. *Soc. Neurosci. Abstr.* **12**, 423.

Schotte A., Maloteaux J. M., and Laduron P. M. (1985) Characterization and regional distribution of serotonin S_2 receptors in human brain. *Brain Res.* **276**, 231–235.

Slater P. and Patel S. (1982) Autoradiographic distribution of serotonin$_2$ receptors in rat brain. *Eur. J. Pharmacol.* **92**, 297–298.

Steinbush H. W. M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat. Cell bodies and terminals. *Neuroscience* **6**, 557–618.

Tam S. W. (1983) Naloxone-inaccessible receptor in rat central nervous system. *Proc. Natl. Acad. Sci. USA* **80**, 6703–6707.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