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Binding Characteristics of a Radiolabeled Agonist and Antagonist at Central Nervous System *Alpha* Noradrenergic Receptors

DAVID C. U'PRICHARD,[1] DAVID A. GREENBERG, AND SOLOMON H. SNYDER[2]

Departments of Pharmacology and Experimental Therapeutics and of Psychiatry and the Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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SUMMARY

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Binding of the *alpha* noradrenergic agonist [^{3}H]clonidine and the *alpha* antagonist [^{3}H]WB-4101 (2-([2',6'-dimethoxy]phenoxyethylamino)methylbenzodioxan) to rat brain membranes exhibits characteristics expected of *alpha* receptors for norepinephrine. Binding of both [^{3}H]ligands is saturable, with K_D values of 5.8 nM and 0.48 nM for [^{3}H]clonidine and [^{3}H]WB-4101, respectively. A series of catecholamines inhibits the binding of both ligands with the potency order epinephrine > norepinephrine >> isoproterenol, corresponding to the relative activities of these agents at *alpha* receptors in the periphery. Competition for binding is stereoselective, with (−) isomers of phenylethanolamines many times more potent than the corresponding (+) isomers. Classical *alpha* antagonists inhibit binding of both ligands at low concentrations, but *beta* antagonists are much weaker. *Alpha* agonists are more potent in displacing [^{3}H]clonidine than [^{3}H]WB-4101 binding, while *alpha* antagonists compete more avidly for [^{3}H]WB-4101 sites. Partial agonist ergot alkaloids display similar affinities for the binding sites of both [^{3}H]ligands. These findings may be explained by the existence of discrete agonist and antagonist states of the *alpha* receptor, which preferentially bind [^{3}H]clonidine and [^{3}H]WB-4101, respectively. Regional variations in the binding of both [^{3}H]ligands in the brain are not pronounced, although levels tend to be highest in hypothalamus and cerebral cortex and lowest in cerebellum. Treatment with 6-hydroxydopamine fails to decrease the binding of either [^{3}H]ligand, suggesting that binding occurs to postsynaptic sites.

INTRODUCTION

Pharmacological, behavioral, and neurophysiological studies indicate that central actions of norepinephrine involve both *alpha* and *beta* receptors (1, 2). *Beta* adrenergic receptors in brain tissue have been labeled by the binding of [^{3}H]dihydroalprenolol (3, 4), [^{3}H]propranolol (5), and [^{125}I]hydroxybenzylpindolol (6). Direct binding of [^{3}H]dihydroergocryptine to *alpha* receptor sites in rabbit uterine membranes (7) and of [^{3}H]clonidine and [^{3}H]WB-4101 (2-([2',6'-dimethoxy]phenoxyethylamino)methylbenzodioxan) to *alpha* receptors in mammalian brain membranes (8) has recently been reported. While the binding characteristics of both clonidine and WB-4101 indicate that these ligands label *alpha* noradrenergic recep-

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tors, the relative potencies of various drugs in competing for binding suggest that WB-4101 selectively labels an "antagonist state" of the receptor while the binding of clonidine is associated with an "agonist state." In the present study we have explored in detail the characteristics of [^{3}H]WB-4101 and [^{3}H]clonidine binding to rat brain membranes.

METHODS

Methods were the same as described previously (8). Fresh whole brains (for [^{3}H]WB-4101 experiments) or whole brains minus cerebella (for [^{3}H]clonidine experiments) from adult male Sprague-Dawley rats were homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°) with a Brinkmann Polytron PT-10. The homogenates were centrifuged twice at 50,000 × g for 10 min, with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was homogenized in 50 volumes of cold 50 mM Tris-HCl buffer (pH 7.7 at 25° for [^{3}H]WB-4101 experiments; pH 7.5 at 25° for [^{3}H]clonidine experiments).

Preliminary experiments showed that elimination of the cerebellum from the tissue preparation enhanced [^{3}H]clonidine binding by increasing the maximum number of sites without altering the K_D for [^{3}H]clonidine. [^{3}H]WB-4101 binding was not affected by inclusion of the cerebellum. With a series of Tris-HCl buffers, [^{3}H]-clonidine binding was maximum at pH 7.5, and [^{3}H]WB-4101 binding was maximum at pH 7.7. However, over the pH range 7.0–8.0, binding of both ligands was close to maximum values.

Binding assays. [^{3}H]Clonidine hydrochloride, 1.6 Ci/mmole, was generously donated by Boehringer Ingelheim. Radiochemical purity was greater than 95% as determined by thin-layer chromatography on Eastman Chromogram sheet 6060 silica gel G plates in two solvent systems: chloroform–methanol–acetone–ammonium hydroxide (50:20:10:1), R_F 0.9; 1-butanol–acetic acid–water (4:1:5) (upper phase), R_F 0.7. WB-4101 (Ward-Blenkinsop), supplied by Dr. D. R. Mottram, was tritiated at New England Nuclear Corporation. Fifteen milligrams of precursor were dissolved in 0.25 ml of trifluoroacetic acid, to which were added 25 mg of platinum black catalyst and 10 Ci of tritiated water. The reaction mixture was stirred overnight at 80°. Labile tritium was removed under vacuum, using ethanol as the solvent. After filtration from the catalyst, the compound was again taken to dryness under vacuum and taken up in 10 ml of ethanol. [^{3}H]WB-4101 migrated with a single peak of radioactivity corresponding to authentic WB-4101 when chromatographed on Eastman plates as above, in three solvent systems: 1-butanol–acetic acid–water (4:1:1), R_F 0.8; 1-butanol–acetone–diethylamine–water (10:10:2:5), R_F 0.9; chloroform–benzene–ethanol (4:2:1), R_F 0.6. Specific activity was determined by ultraviolet absorption at 274 nm to be 7 Ci/mmole.

Triplicate incubation tubes each received 15–30 μl of either [^{3}H]ligand to give a final concentration of 4 nM for [^{3}H]clonidine or 0.22 nM for [^{3}H]WB-4101, 20–200 μl of various concentrations of unlabeled drugs, 0.8–1.0 ml of 50 mM Tris-HCl buffer (pH 7.7 at 25° for [^{3}H]WB-4101 experiments; pH 7.5 at 25° for [^{3}H]clonidine experiments), and 1.0 ml of tissue suspension (20 mg of the original wet weight of the tissue). The total incubation volume was 2 ml.

Tubes were incubated at 25° for 15 min ([^{3}H]WB-4101) or 30 min ([^{3}H]clonidine), and the contents were rapidly filtered under vacuum through Whatman GF/B filters with 10-ml ([^{3}H]WB-4101) or 15-ml ([^{3}H]clonidine) rinses of ice-cold 50 mM Tris buffer (pH 7.7 at 25°). Filters were counted by liquid scintillation spectrometry in 10 ml of Hydromix (Yorktown Research) at efficiencies of 36–40%.

Specific binding of [^{3}H]clonidine was defined as the excess over blanks taken in the presence of 10 μM (−)-norepinephrine or 1 μM clonidine. Blanks for [^{3}H]WB-4101 binding were taken in the presence of 100 μM (−)-norepinephrine or 0.1 μM WB-4101. Bound or dissociated radioactivity from incubation with either ligand migrated with the authentic substances on thin-layer chromatograms (8).

6-Hydroxydopamine experiments. Twelve adult male Sprague-Dawley rats

were injected intraventricularly with 250 μg of 6-hydroxydopamine in 20 μl of 0.9% NaCl containing 0.1% ascorbic acid on two occasions 24 hr apart. Control animals received equivalent injections of vehicle. The animals were killed 10–14 days after injections. Some control and 6-hydroxydopamine-treated brains were homogenized in 0.32 M sucrose, and aliquots of the homogenate were taken for assay of endogenous norepinephrine (9) or [^{3}H]norepinephrine uptake (10). Twenty volumes (original weight) of Tris-HCl buffer were added to the remainder of the homogenates, which were centrifuged at 50,000 × *g* for 20 min. Thereafter the pellets were washed and resuspended for binding assays as described above. Initial homogenization in sucrose did not affect the specific binding of either ligand.

Drugs. Catecholamine and phenylephrine isomers were donated by Sterling-Winthrop; ergot alkaloids, by Sandoz; α-methylnorepinephrine isomers, by Dr. P. N. Patil; tramazoline and St-600, by Boehringer Ingelheim; minoxidil, by Upjohn; and indoramin, by Wyeth. Other drugs were obtained from the pharamceutical company of origin or commercial sources.

RESULTS

Saturation of [^{3}H]WB-4101 and [^{3}H]-clonidine binding. The binding of both [^{3}H]clonidine and [^{3}H]WB-4101 to rat brain membranes is saturable. The ratio of total [^{3}H]clonidine binding to nonspecific binding measured in the presence of 10 μM (−)-norepinephrine is about 2 at a [^{3}H]-clonidine concentration of 4 nM, which was used for routine binding assays. Nonspecific binding increases linearly up to 80 nM [^{3}H]clonidine, the highest concentration examined (Fig. 1). Specific [^{3}H]clonidine binding, the difference between total and nonspecific binding, begins to form a plateau at 20–30 nM [^{3}H]clonidine, and half-maximal binding is apparent at about 6 nM [^{3}H]clonidine. Scatchard analysis of these data indicates a single population of binding sites, with a dissociation constant (K_D) of 5.8 nM. The calculated maximal number of binding sites is 14 pmoles/g of whole rat brain minus cerebellum. A Hill plot of these data is linear, with a Hill coefficient of 0.98, indicating the apparent absence of positively or negatively cooperative interactions in [^{3}H]clonidine binding.

The nonspecific binding of [^{3}H]WB-4101, measured in the presence of 100 μM (−)-norepinephrine, increases linearly up to 2 nM [^{3}H]WB-4101, the highest concentration examined (Fig. 2). The ratio of total to nonspecific binding is 3 at a [^{3}H]WB-4101 concentration of 0.22 nM, which was employed for routine assays. Specific [^{3}H]WB-4101 binding, the difference between total and nonspecific binding, forms a plateau between 1 and 2 nM [^{3}H]WB-4101. Half-maximal binding is apparent at about 0.4 nM [^{3}H]WB-4101. Scatchard analysis indicates the presence of a single population of binding sites, with a K_D value of about 0.48 nM. The maximal number of binding sites is 11 pmoles/g of whole rat brain, similar to the calculated receptor density for [^{3}H]clonidine binding. A Hill plot of these data is linear, with a Hill coefficient of 1.04, indicating the absence of positive or negative cooperativity.

Kinetics of [^{3}H]clonidine and [^{3}H]WB-4101 binding. The rate of association of [^{3}H]clonidine binding at 25° is rapid. Binding appears to reach equilibrium by 3 min (Fig. 3). Half-maximal binding is attained at about 20 sec. The mean bimolecular rate constant for association was determined from the first two points on the curve to be 0.18 nM^{-1} min^{-1}, using the equation $k_1 = [1/t(F\text{-}R)] \ln [R(F - B)/F(R - B)]$, where F is the initial concentration of [^{3}H]clonidine (4.0 nM), R is the receptor concentration (0.14 nM; see above), and B is the concentration of specifically bound [^{3}H]-clonidine at time t. The association is accelerated at higher temperatures, so that at 37° half-maximal binding is attained in 10 sec. At lower temperatures the rate of association is greatly slowed, so that at 4° 10 min are required for half-maximal binding.

The rate of dissociation of [^{3}H]clonidine was examined by incubating membranes to equilibrium at 25° and then adding 10 μM (−)-norepinephrine to prevent rebinding of dissociated [^{3}H]clonidine (Fig. 3).

The [^{3}H]clonidine remaining bound was then assessed at three temperatures after various time intervals. In the experiments conducted at 25°, nonradioactive clonidine (1 μM) was also employed as a displacing agent. At 37° dissociation is extremely rapid, with a $t_{1/2}$ of about 10 sec, while dissociation at 4° is much slower, so that 50% dissociation requires about 4 min. At 25°, using either (−)-norepinephrine or clonidine as displacer, the $t_{1/2}$ for dissociation is 40 sec. At all three temperatures the dissociation rate is linear when plotted on a semilogarithmic scale. At 25° the rate constant for dissociation (k_{-1}) is 1.05 min^{-1}. The dissociation constant, calculated from the ratio of k_{-1} to k_1, is 5.8 nM, similar to the value obtained in saturation experiments.

[^{3}H]WB-4101 associates more slowly than [^{3}H]clonidine (Fig. 4). At 25° equilibrium is apparent at about 5 min, while half-maximal binding occurs at about 2 min. The mean bimolecular rate constant for association, calculated by the above formula from the first three points of these data, is 0.35 nM^{-1} min^{-1}. At 4° half-maximal binding occurs at 10 min, while at 37° half-maximal binding is observed at 30 sec.

The dissociation of bound [^{3}H]WB-4101 was evaluated by incubating membranes to equilibrium at 25°, whereupon 100 μM (−)-norepinephrine was added and [^{3}H]WB-4101 remaining bound was measured at different time intervals (Fig. 4). At 4°, 25°, and 37° the dissociation of [^{3}H]WB-4101 is slower than that of [^{3}H]clonidine at the corresponding temperatures. When plotted on a semilogarithmic scale, the dissociation at these temperatures is linear, indicating a first-order process. At 37° the $t_{1/2}$ for dissociation is about 1 min, while at 4° the $t_{1/2}$ is about 50 min. At 25°, 50% dissociation is apparent at about 8 min. The rate constant for dissociation at 25° is calculated to be 0.09 min^{-1}. The dissociation constant, calculated from the ratio of k_{-1} to k_1, is 0.26 nM, similar to the dissociation constant determined in saturation experiments.

Mutual competition of clonidine and WB-4101 binding. Displacement curves for reduction of [^{3}H]clonidine binding by WB-4101 and by clonidine are parallel (Fig. 5). Displacement of [^{3}H]clonidine binding by both clonidine and WB-4101 reaches a plateau at about the same level of binding, corresponding to nonspecific [^{3}H]clonidine binding in the presence of 10 μM (−)-norepinephrine. The conclusion that WB-4101 and clonidine bind to the same sites is supported by experiments demonstrating the absence of additivity in the displacement of [^{3}H]clonidine by WB-4101 and clonidine. Thus no greater displacement of [^{3}H]clonidine binding occurs with a mixture of 10 μM WB-4101 and 1 μM clonidine than with either drug alone. Moreover, the displacement by respective mixtures of 10 μM (−)-norepinephrine and 1 μM clonidine or of 10 μM (−)-norepinephrine and 10 μM WB-4101 is also the same as with any of these drugs alone.

Half-maximal reduction of [^{3}H]clonidine binding by nonradioactive clonidine occurs at about 9 nM, similar to the half-maximal value for binding of progressively increasing concentrations of [^{3}H]clonidine. WB-4101 is much weaker in competing for [^{3}H]clonidine binding, producing half-maximal displacement at about 400 nM WB-4101.

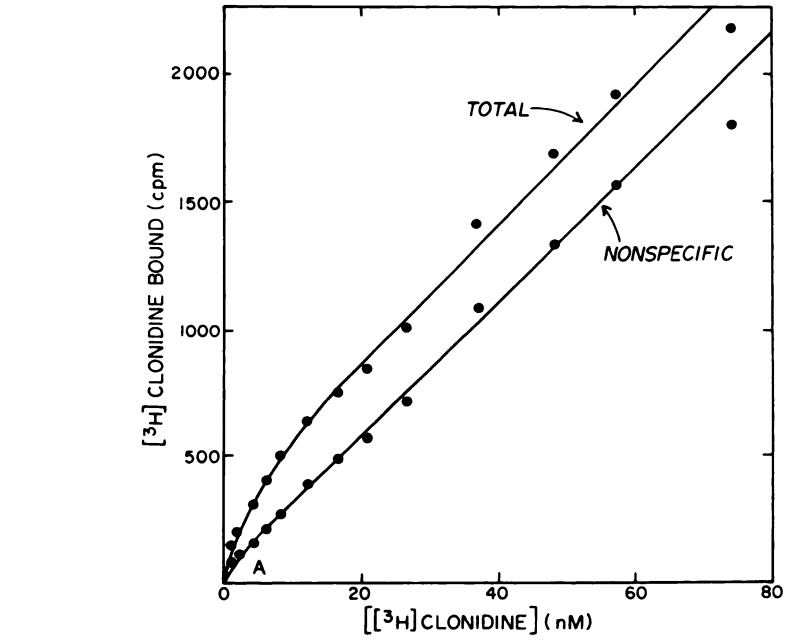
The displacement curves for competition by clonidine and WB-4101 for [^{3}H]WB-4101 binding are also parallel. Moreover, maximal displacements by clonidine and WB-4101 are similar and correspond to maximal displacement in the presence of 100 μM (−)-norepinephrine (Fig. 5). No greater displacement of binding is obtained using various combinations of 100 μM (−)-norepinephrine, 1 μM WB-4101, and 100 μM clonidine than with any of these drugs alone. The absence of additivity in displacement further supports the conclusion that norepinephrine, clonidine, and WB-4101 compete for the same sites labeled by [^{3}H]WB-4101.

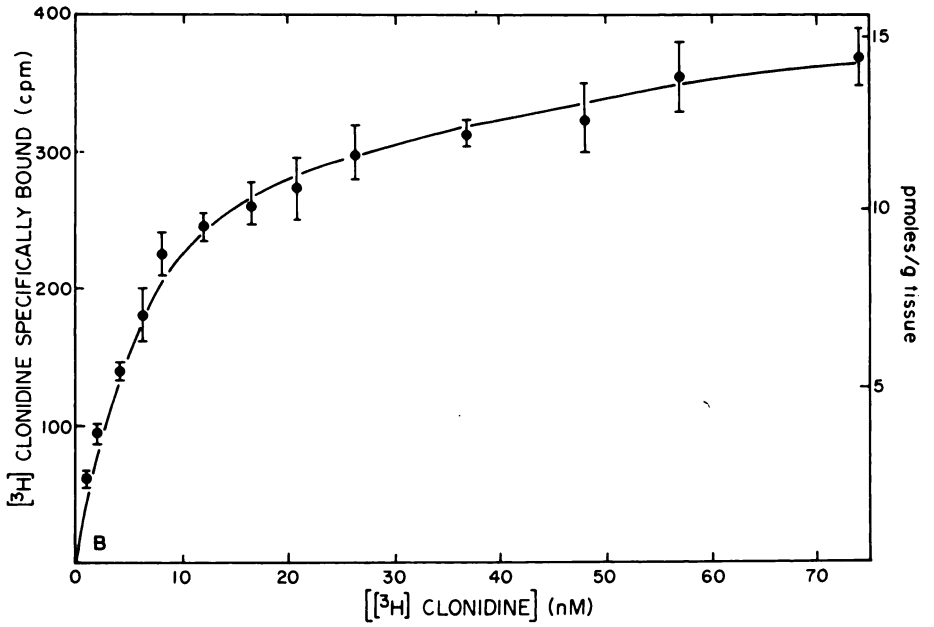
Although [^{3}H]clonidine and [^{3}H]WB-4101 appear to compete for the same or similar sites, there are striking differences between the affinities of the unlabeled drugs for [^{3}H]WB-4101 and [^{3}H]clonidine binding sites. [^{3}H]WB-4101 binding is reduced 50% by 0.9 nM WB-4101, while over

400-fold higher concentrations of WB-4101 are required to reduce [^{3}H]clonidine binding 50%. Similarly, the clonidine concentration required to reduce [^{3}H]WB-4101 binding 50% is about 1 μM, over 100 times the IC_{50} for reduction of [^{3}H]clonidine binding by clonidine.

Hill plots of the displacement by clonidine and WB-4101 of either [^{3}H]ligand give slopes of about 1.0.

Effect of phenylethylamines on [^{3}H]WB-4101 and [^{3}H]clonidine binding. A variety of phenylethylamines compete for both [^{3}H]clonidine and [^{3}H]WB-4101 binding, providing parallel displacement curves with Hill slopes of about 1.0, and similar





FIGURES 1A and 1B

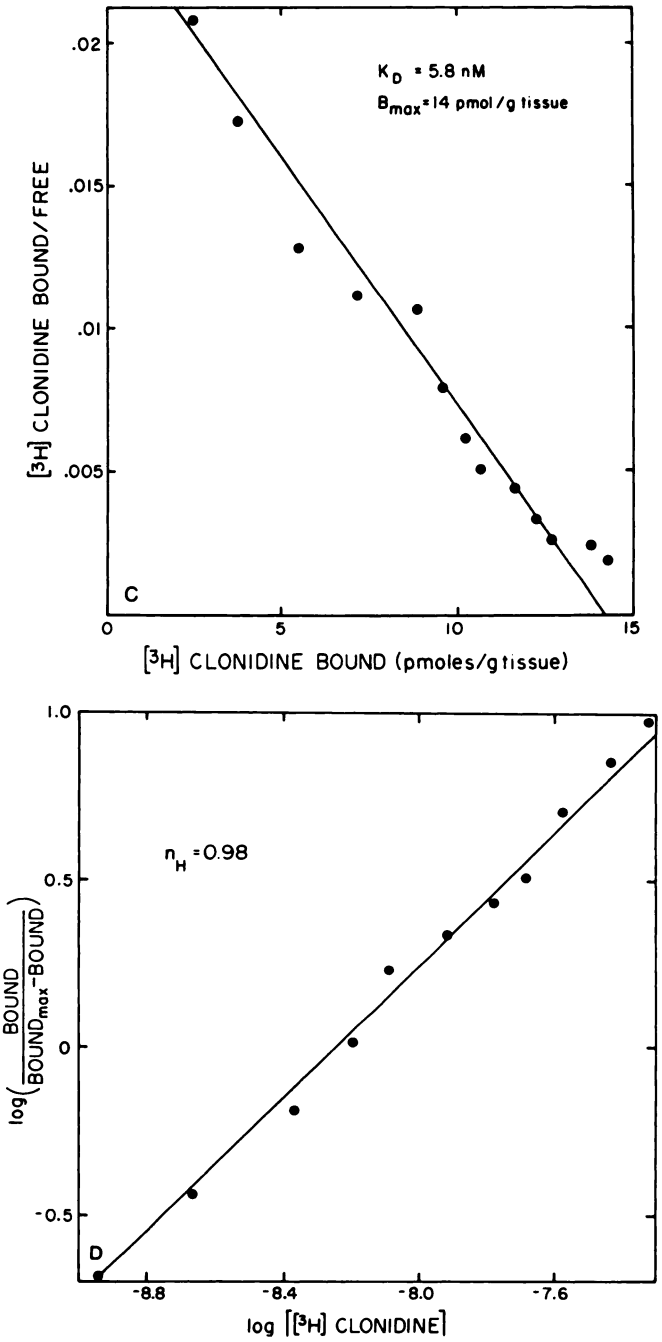
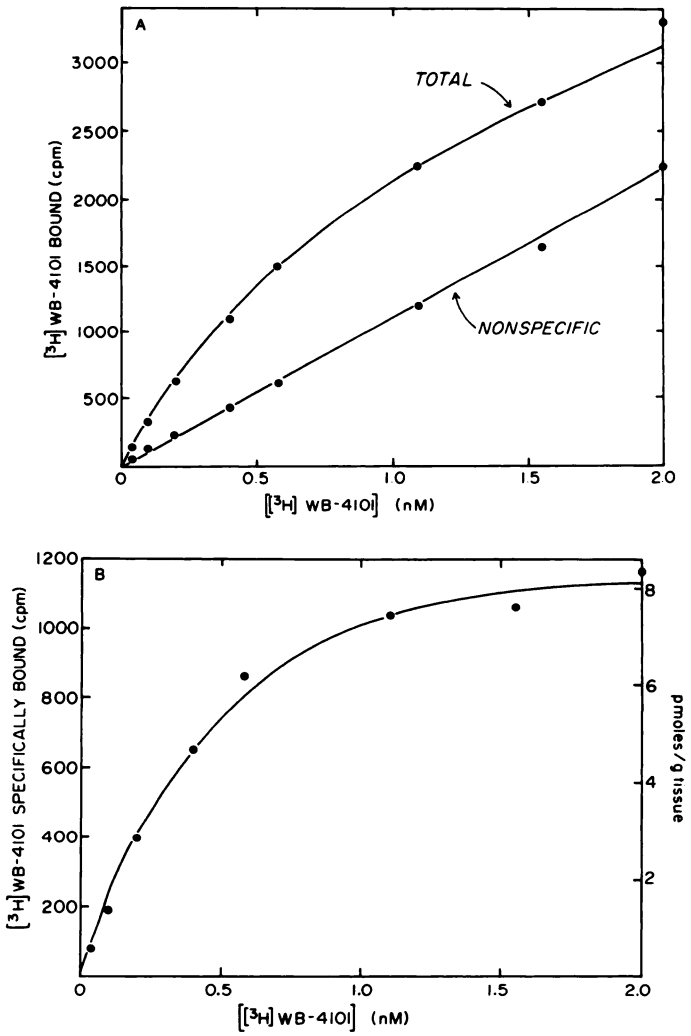


FIG. 1. *[³H]Clonidine binding as a function of increasing concentrations of [³H]clonidine*

Homogenates of rat brain minus cerebellum (20 mg of the original wet weight of the tissue) were incubated for 30 min at 25°, as described in METHODS, with various concentrations of [³H]clonidine. Nonspecific binding was that occurring in the presence of 10 μM (−)-norepinephrine. Each point represents the mean of four experiments, each conducted in triplicate, with standard errors for specific binding determinations indicated by bars. A. Total and nonspecific binding. B. Specific binding, defined as the difference between total and nonspecific. C. Scatchard plot, showing a K_D value of 5.8 nM and 14 pmoles of binding sites per gram of tissue. D. Hill plot, with n_H = 0.98.



FIGURES 2A and 2B

maximal levels of displacement for both [^{3}H]ligands (Fig. 6 and Table 1). The most potent is (−)-epinephrine, whose apparent K_i value for [^{3}H]clonidine binding is 6 nM. (−)-Epinephrine is about 100 times more potent in competing for [^{3}H]clonidine than for [^{3}H]WB-4101 binding. The effects of epinephrine are stereoselective at both [^{3}H]clonidine and [^{3}H]WB-4101 binding sites, the (−) isomer being 10 and 50 times more potent than the (+) isomer in displacing [^{3}H]clonidine and [^{3}H]WB-4101, respectively. The relative affinities of various catecholamines are characteristic of *alpha* noradrenergic receptors (11), with (−)-epinephrine 2–3 times more potent than (−)-norepinephrine, while (−)-isoproterenol is less than 1% as active as (−)-epinephrine in competing for both [^{3}H]clonidine and [^{3}H]WB-4101 binding. Although dopamine is only about 2% as potent an agonist as (−)-epinephrine, it is still 22 times more potent than (−)-isoproterenol. Stereoselectivity is apparent for the isomers of phenylephrine, norepinephrine, and α-methylnorepinephrine, where the β-carbon is asymmetrical. The extent of stereoselectivity appears greater for isomers of α-methylnorepinephrine than for any of the other catecholamines. Little stereoselectivity is apparent with the amphetamine isomers, where only the α-carbon is asymmetrical.

For other neurotransmitter receptors

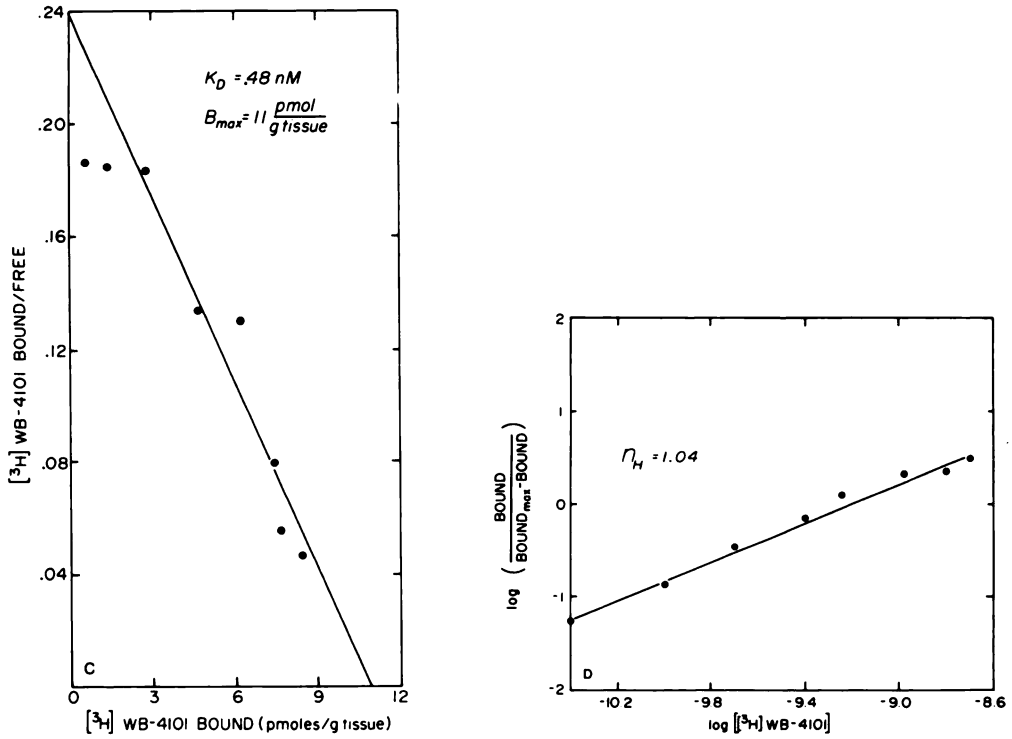


FIG. 2. *[3H]WB-4101 binding as a function of increasing concentrations of [3H]WB-4101*

Whole rat brain homogenates (20 mg of the original wet weight of the tissue) were incubated for 15 min at 25°, as described in METHODS, in the presence of various concentrations of [3H]WB-4101. Nonspecific binding was determined by the addition of 100 μM (−)-norepinephrine. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated three times. A. Total and nonspecific binding. B. Specific binding, defined as the difference between total and nonspecific. C. Scatchard plot, showing a K_D value of 0.48 nM and a receptor density of 11 pmoles/g of tissue. D. Hill plot, with n_H = 1.04.

which have been labeled with both agonist and antagonist ligands, the known relative potencies of structural analogues of the endogenous transmitter are reflected most accurately in their abilities to displace [3H]agonists (12, 13). This appears to be the case for the interaction of phenylethylamines with the *alpha* noradrenergic receptor as well. A variety of substitutions in the basic phenylethylamine structure known to confer enhanced *alpha* agonist activity (2, 14) also result in increased affinity for [3H]clonidine binding sites. Both hydroxyl groups in the catechol moiety are important for activity, as evidenced by the greater potency of dopamine and norepinephrine than of tyramine and octopamine. Potency is diminished more profoundly by loss of the *m*- than the *p*-hydroxyl substituent, since more activity is retained by phenylephrine than by tyramine, octopamine, and hydroxyamphetamine.

β-Hydroxylation of phenylethylamine to phenylethanolamine, tyramine to octopamine, and dopamine to norepinephrine results in more potent *alpha* agonists. Likewise, α-methylation of norepinephrine, phenylethylamine, and tyramine increases their ability to displace [3H]clonidine. *N*-Methylation of norepinephrine to epinephrine or of amphetamine to methamphetamine also enhances affinity for the binding sites of both [3H]ligands. Finally, the presence of a more bulky *N*-substituent in isoproterenol results in much weaker competition for [3H]clonidine and [3H]WB-4101 sites.

All phenylethylamines examined are more potent in reducing [3H]clonidine

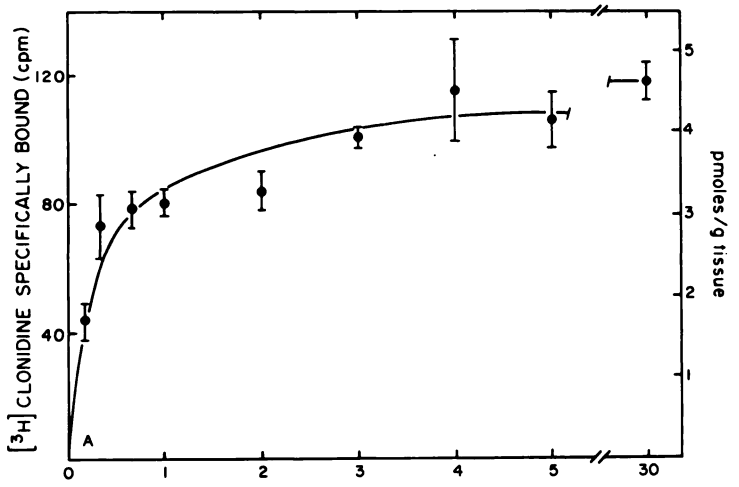


FIGURE 3A

than [³H]WB-4101 binding. The difference in potency is most pronounced with catecholamines, especially *N*- or α-methylated derivatives.

Effects of non-phenylethylamine alpha noradrenergic agonist drugs on [³H]clonidine and [³H]WB-4101 binding. Several imidazoline derivatives related to clonidine have considerable potency in competing for [³H]clonidine and [³H]WB-4101 binding (Table 2). Oxymetazoline is the most potent, with about 3 and 18 times greater affinity than clonidine for [³H]clonidine and [³H]WB-4101 binding, respectively. Oxymetazoline, xylometazoline, naphazoline, tetrahydrozoline, tramazoline, and St-600, like clonidine, are substantially more potent in competing for [³H]clonidine than for [³H]WB-4101 binding. Within this group, relative effects on the [³H]ligands vary. Tetrahydrozoline and the 2-aminoimidazolines (tramazoline, clonidine, and St-600), drugs with central hypotensive effects thought to be mediated by central *alpha* receptor activation (15), exhibit the greatest preference for [³H]clonidine binding sites.

A variety of sympathomimetic amines employed clinically as anorectics, pressor agents, and nasal decongestants, including mephentermine, methoxamine, phentermine, cyclopentamine, and tuaminoheptane, are substantially weaker than the imidazolines in competing for binding sites. The most potent of these agents, mephentermine, has only about 1% of the affinity of clonidine for [³H]clonidine binding sites. These agents are between 10 and 23 times more potent in reducing [³H]-clonidine than [³H]WB-4101 binding.

Influence of alpha noradrenergic antagonist drugs on [³H]clonidine and [³H]WB-4101 binding. The classical *alpha* noradrenergic antagonists phentolamine and phenoxybenzamine are highly potent competitors for [³H]WB-4101 binding, with respective apparent K_i values of 3.6 nM and 4.0 nM (Table 3). Whereas *alpha* agonists such as epinephrine have much greater affinity for clonidine than for WB-4101 binding sites, the reverse holds true for these antagonists; the potency ratios of [³H]clonidine displacement to [³H]WB-4101 displacement are 0.16 and 0.07, respectively, for phentolamine and phenoxybenzamine. However, as with the *alpha* agonists, the Hill slopes of the displacement of either [³H]ligand by antagonists are approximately 1.0. The most potent antagonist is WB-4101 itself, with an apparent K_i value of 0.6 nM for reducing [³H]WB-4101 binding. This correlates well with the finding that WB-4101 is a very potent *alpha* antagonist in the rat vas deferens, having a pA_2 value there of 9.8 (16). The *alpha* antagonists indoramin and WB-4101 display the most pronounced discrepancies between affinities for WB-4101 and clonidine binding sites, respectively possessing 1000 and 300 times greater affinity for WB-4101

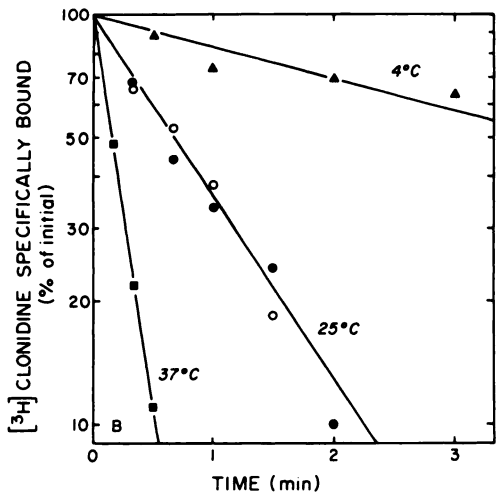


FIG. 3. *Time course of association and dissociation of [3H]clonidine binding*

A. Association of [3H]clonidine binding to rat brain homogenates (20 mg of the original tissue weight) at 25° was measured at various time intervals following the addition of 4 nM [3H]clonidine. Specific binding was defined as the difference between binding obtained in the absence and presence of 10 μM (−)-norepinephrine. Incubation conditions were the same as described in METHODS. Each point represents the mean of four experiments, each conducted in triplicate, with standard errors indicated by bars. B. Dissociation of bound [3H]clonidine was measured at 4°, 25°, and 37° following incubation with [3H]clonidine under standard assay conditions and subsequent equilibration for 5 min to the indicated temperatures. At zero time 10 μM (−)-norepinephrine (●) or 1 μM clonidine (○) was added to the incubation mixtures, and the reactions were terminated by filtration at various time intervals. Points shown are the means of triplicate values from two to four experiments, which varied less than 20%.

than for clonidine binding. The strong *alpha* adrenolytic properties of the benzodioxan drug dibozane (17) are reflected in its high [3H]WB-4101 displacement potency. The haloalkylamine derivative dibenamine is pharmacologically much less potent than its congener phenoxybenzamine (18), and also possesses less affinity for [3H]WB-4101 binding sites. Piperoxan, an *alpha* antagonist with some agonist properties (19), has only about 0.3% of the potency of WB-4101 in competing for [3H]WB-4101 binding, and actually displays greater affinity for [3H]clonidine than for [3H]WB-4101 binding.

Effects of ergot alkaloids and related drugs and partial agonists on [3H]clonidine and [3H]WB-4101 binding. The ergot alkaloids are well characterized as mixed agonist-antagonist drugs at *alpha* noradrenergic receptors in smooth muscle (20). Most of the ergots are quite potent competitors for [3H]clonidine and [3H]WB-4101 binding. Absence of the peptide side chain in such ergots as ergonovine, methysergide, and *d*-LSD[3] greatly reduces potency at *alpha* adrenergic receptors in smooth muscle (18) and is associated with a pronounced lessening of affinity for [3H]clonidine and [3H]WB-4101 binding sites. Thus, while the apparent K_i values of the peptide-containing ergots for both [3H]clonidine and [3H]WB-4101 binding are between 2 and 18 nM, the corresponding values for *d*-LSD, ergonovine, and methysergide are in the range of 220–23,000 nM.

If the binding of [3H]clonidine represents a fairly selective interaction with a form of the *alpha* receptor specific for agonists and that of [3H]WB-4101 labels an "antagonist" form of the receptor, one would expect mixed agonist-antagonists to have similar affinities for clonidine and WB-4101 binding sites. Thus it is of interest that the ergots have similar potencies in competing for [3H]clonidine and [3H]-WB-4101 binding. Dihydrogenation of ergots tends to increase their antagonist properties (18). Of a series of four ergots and their dihydrogenated derivatives which we examined, dihydrogenation leads in three cases to an increase in relative affinity for [3H]WB-4101 binding compared with [3H]clonidine binding. The only exception is dihydroergotamine, which possesses significant pressor properties compared with the other dihydroergots (21). Dihydrogenation also increases the absolute potencies of ergots at both [3H]clonidine and [3H]WB-4101 binding sites.

Tolazoline displays varying proportions of agonist and antagonist activity, with agonist activity predominating in some systems (22). Tolazoline is about 12 times

[3] The abbreviation used is: *d*-LSD, *d*-lysergic acid diethylamide.

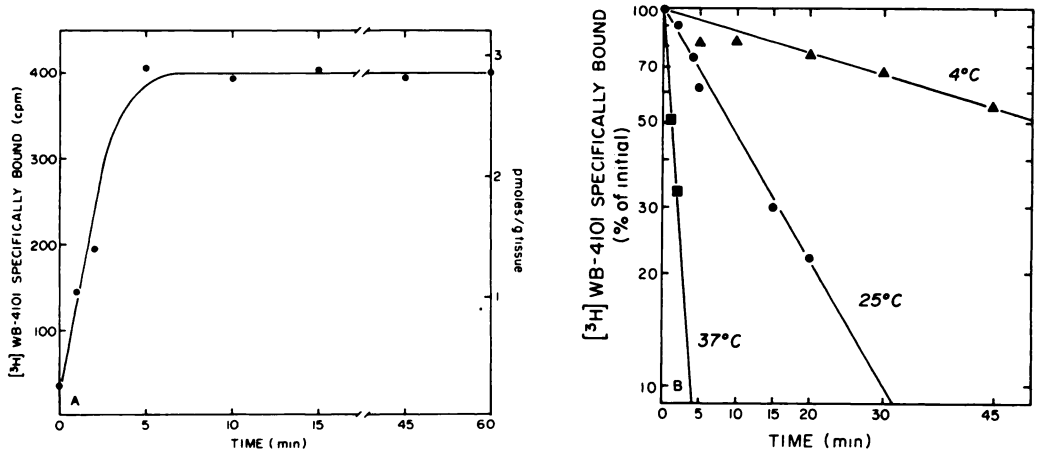


FIG. 4. *Time course of association and dissociation of [³H]WB-4101 binding*

A. Association of [³H]WB-4101 binding to rat brain homogenates (20 mg of the original tissue weight) at 25° was measured at various time intervals following the addition of 0.22 nM [³H]WB-4101. Specific binding was defined as the difference between binding obtained in the absence and presence of 100 μM (−)-norepinephrine. Incubation conditions were the same as described in METHODS. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated three times. B. Dissociation of bound [³H]WB-4101 was measured at 4°, 25°, and 37° following incubation with [³H]WB-4101 under standard assay conditions and subsequent equilibration for 5 min to the indicated temperatures. At zero time 100 μM (−)-norepinephrine was added to the incubation mixtures, and the reactions were terminated by filtration at various time intervals. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated twice.

more potent in competing for [³H]clonidine than for [³H]WB-4101 binding, and has considerably less affinity for both ligands than its pharmacologically more potent congener, phentolamine. Yohimbine also possesses both agonist and antagonist properties at *alpha* noradrenergic receptors (19) and displays 3 times more affinity for [³H]clonidine than for [³H]WB-4101 sites.

Effects of miscellaneous drugs on [³H]clonidine and [³H]WB-4101 binding. To ascertain the specificity of clonidine and WB-4101 binding, we examined a number of drugs of different classes (Table 4). The *beta* receptor antagonists alprenolol and propranolol are much less potent than *alpha* antagonists in competing for binding. With the exception of tranylcypromine, monoamine oxidase inhibitors are extremely weak displacers of both [³H]ligands. The stereoselectivity of the tranylcypromine isomers is similar to, but less marked than, catecholamine stereoselectivity. The tricyclic antidepressants amitriptyline and imipramine display substantial potency in competing for [³H]WB-4101 binding, with apparent K_i values of 20–50 nM and with 25–55 times more affinity for WB-4101 than for clonidine binding sites. The *alpha* blocking activities of tricyclic antidepressants have been well characterized in smooth muscle systems (23, 24). Among the muscarinic anticholinergics, atropine and trihexyphenidyl are weak in competing for binding, while benztropine has substantial affinity for WB-4101 binding sites. The antihistaminic phenothiazine promethazine has a potency similar to that of benztropine in competing for [³H]WB-4101 binding, while the H_2 antihistamine burimamide and H_1 antihistamine diphenhydramine are much weaker.

In some peripheral systems, antipsychotic drugs appear to be as potent as classical *alpha* antagonists in their adrenergic blocking abilities (25). Among the antipsychotics, the phenothiazines thioridazine and chlorpromazine have potencies resembling some of the most powerful classical *alpha* adrenergic antagonists,

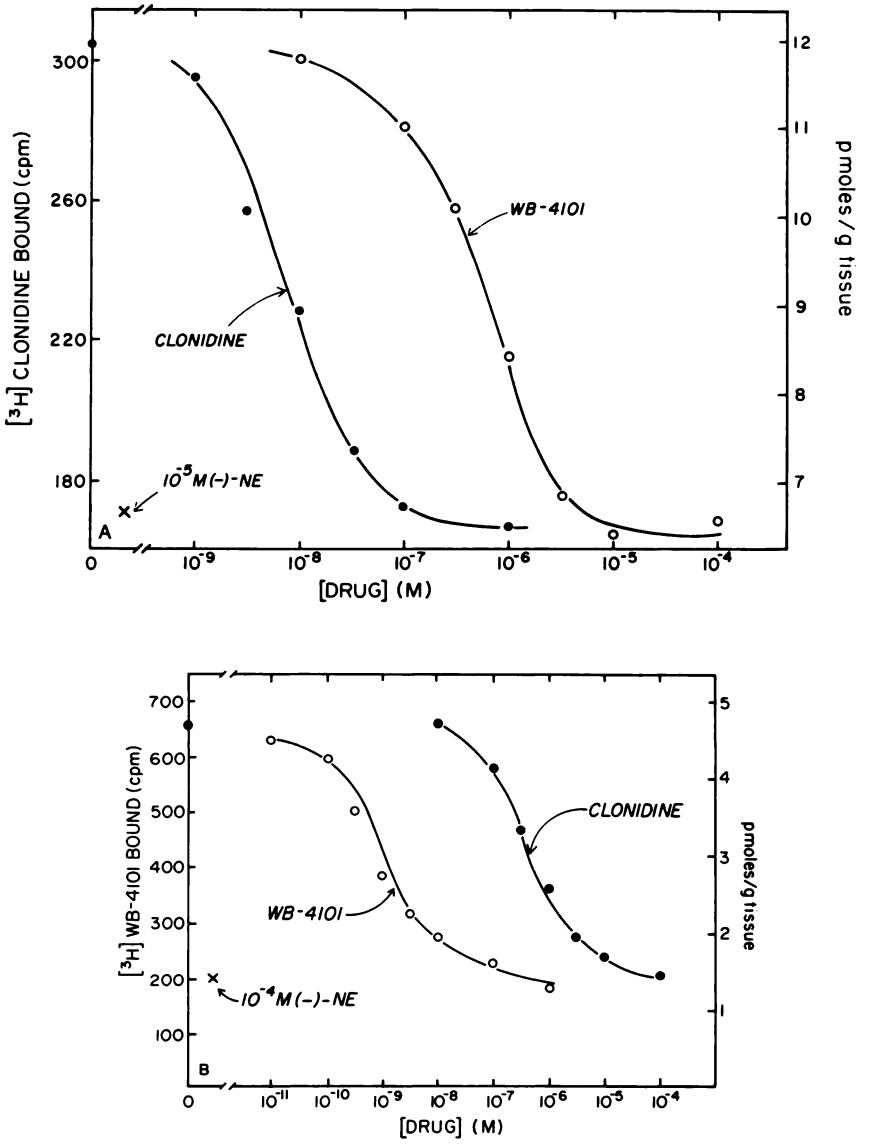


FIG. 5. *Inhibition of [³H]clonidine and [³H]WB-4101 binding by nonradioactive clonidine and WB-4101*

A. Inhibition of [³H]clonidine binding was determined by incubating 4 nM [³H]clonidine with various concentrations of clonidine and WB-4101 under standard assay conditions. Points shown are the means of triplicate values from two experiments, which varied less than 10%. IC_{50} values were determined as the concentration of unlabeled drug which displaced 50% of [³H]clonidine specific binding [10 μM (−)-norepinephrine ((−)-NE) shown (×)]. Total [³H]clonidine binding in the absence of any displacing agent is indicated on the *y* axis (●). B. Inhibition of [³H]WB-4101 binding was determined by incubating 0.22 nM [³H]WB-4101 with various concentrations of clonidine and WB-4101 under standard assay conditions. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated three times. IC_{50} values were determined as the concentration of unlabeled drug which displaced 50% of [³H]WB-4101 specific binding [100 μM (−)-norepinephrine blank shown (×)]. Total [³H]WB-4101 binding in the absence of any displacing agent is indicated on the *y* axis (●).

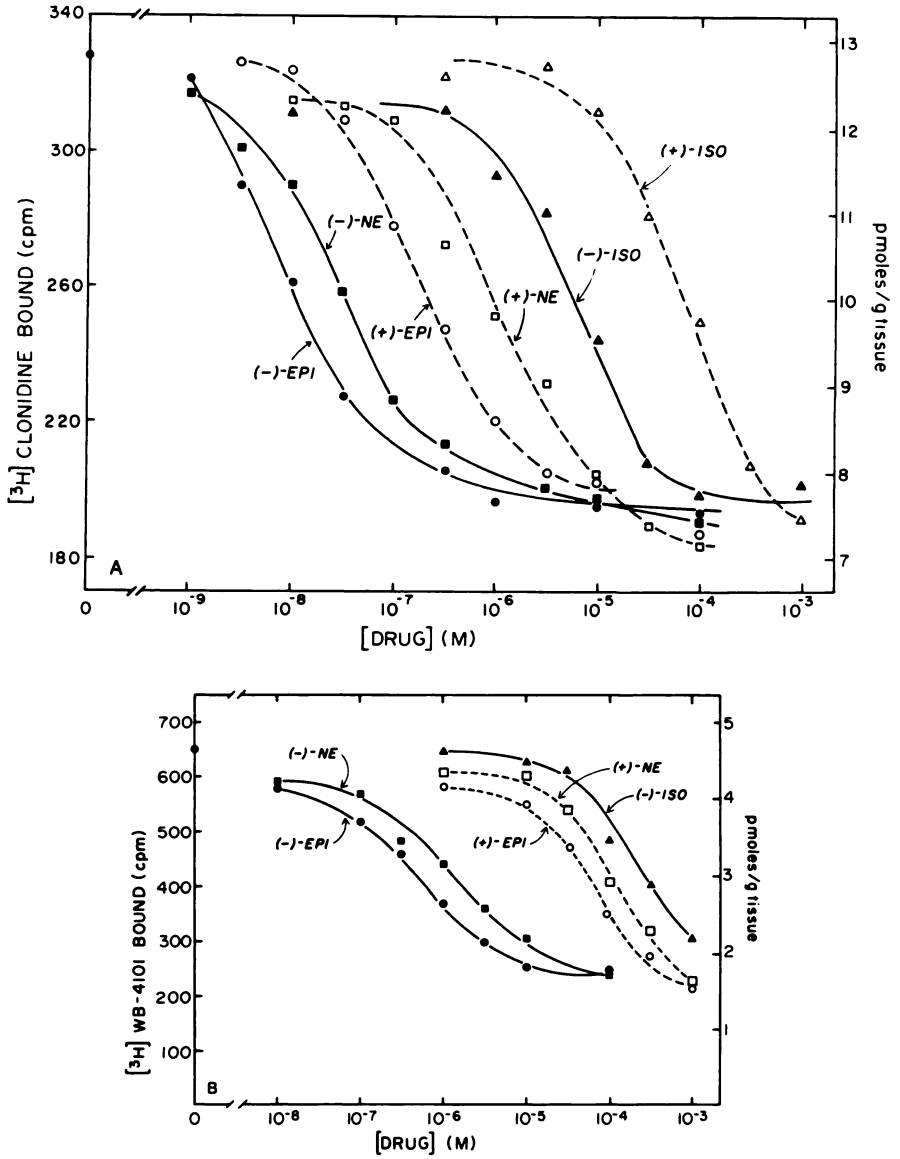


FIG. 6. *Inhibition of [³H]clonidine and [³H]WB-4101 binding by catecholamine isomers*

Inhibition of binding of both [³H]ligands by stereoisomers of epinephrine (EPI), norepinephrine (NE), and isoproterenol (ISO) was determined as described in Fig. 5. A. Inhibition of [³H]clonidine binding by catecholamine isomers. B. Inhibition of [³H]WB-4101 binding by catecholamine isomers.

while trifluoperazine is 7–8 times weaker. The butyrophenone haloperidol is also quite potent, reducing [³H]WB-4101 binding with an apparent K_i of 11 nM. All antipsychotics examined are about 100 times more potent in competing for [³H]WB-4101 than for [³H]clonidine binding, corresponding to their known actions as *alpha* antagonists (17).

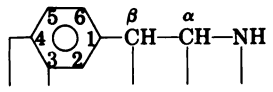
Hydralazine and minoxidil, antihypertensive agents which act directly on smooth muscle, are quite weak in competing for the binding of both [³H]drugs.

Regional variations in [³H]clonidine and [³H]WB-4101 binding. There are marked regional variations in endogenous norepinephrine concentrations, with the highest concentrations in the hypothala-

TABLE 1

Inhibition of [³H]clonidine and [³H]WB-4101 binding by phenylethylamine derivatives

Drug displacement studies were conducted as described in Fig. 5, under standard assay conditions (see METHODS). IC_{50} values were determined by log probit analysis, and apparent K_i values were calculated from the equation $K_i = IC_{50}/(1 + [[^3H]\text{ligand}]/K_D)$ for each ligand. Values are means ± standard errors of three to nine separate experiments, each conducted in triplicate.

Drug						K_i for [³H]clonidine binding (A)	K_i for [³H]WB-4101 binding (B)	B:A
						nM	*nM*	
Phenylethylamine	H	H	H	H	H	2,100 ± 600	12,000 ± 400	5.7
(±)-Phenylethanolamine	H	H	OH	H	H	1,100 ± 600	23,000 ± 6,000	21
(+)-Amphetamine	H	H	H	CH_3	H	1,600 ± 700	10,000 ± 400	6.3
(−)-Amphetamine	H	H	H	CH_3	H	2,200 ± 700	12,000 ± 1,000	5.5
(+)-Methamphetamine	H	H	H	CH_3	CH_3	600 ± 100	6,700 ± 900	11
(±)-Ephedrine	H	H	OH	CH_3	CH_3	700 ± 60	24,000 ± 5,000	34
Tyramine	OH	H	H	H	H	5,900 ± 600	23,000 ± 7,000	3.9
(±)-Octopamine	OH	H	OH	H	H	1,560 ± 30	11,000 ± 400	7.1
(±)-Hydroxyamphetamine	OH	H	H	CH_3	H	5,000 ± 300	39,000 ± 1,700	7.8
(−)-Phenylephrine	H	OH	OH	H	CH_3	270 ± 110	2,600 ± 900	9.6
(±)-Phenylephrine	H	OH	OH	H	CH_3	450 ± 150	5,000 ± 900	11
(+)-Phenylephrine	H	OH	OH	H	CH_3	12,000 ± 600	>70,000	
Dopamine	OH	OH	H	H	H	250 ± 30	44,000 ± 11,000	180
(−)-Norepinephrine	OH	OH	OH	H	H	17 ± 3	1,000 ± 190	59
(+)-Norepinephrine	OH	OH	OH	H	H	600 ± 140	67,000 ± 2,400	110
(±)-α-Methyl-(−)-norepinephrine	OH	OH	OH	CH_3	H	7.7 ± 0.6	2,800 ± 240	360
(±)-α-Methyl-(±)-norepinephrine	OH	OH	OH	CH_3	H	16 ± 2	6,800 ± 170	430
(±)-α-Methyl-(+)-norepinephrine	OH	OH	OH	CH_3	H	9,500 ± 2,900	>70,000	
(−)-Epinephrine	OH	OH	OH	H	CH_3	5.9 ± 0.8	590 ± 170	100
(+)-Epinephrine	OH	OH	OH	H	CH_3	65 ± 20	28,000 ± 3,500	430
(−)-Isoproterenol	OH	OH	OH	H	$CH(CH_3)_2$	5,600 ± 1,800	>70,000	
(+)-Isoproterenol	OH	OH	OH	H	$CH(CH_3)_2$	38,000 ± 5,000	>70,000	

mus and the lowest levels in the cerebellum. Variations in [³H]clonidine binding in brain regions are much less, but are still significant and, except for the cerebral cortex, show a rough parallelism with norepinephrine levels (Table 5). The cerebral cortex displays the highest level of binding, followed by the hypothalamus, whereas the cerebellum exhibits the lowest levels of [³H]clonidine binding, less than half the values of hypothalamus and cerebral cortex. There are no significant regional differences in [³H]WB-4101 binding, although, as with [³H]clonidine binding, the cerebral cortex has the highest, and the cerebellum the lowest, levels (Table 5). The regional binding levels obtained at the single subsaturating concentration of each [³H]ligand are proportional to the receptor density, as recent displacement studies indicate no regional differences in the affinities of either [³H]clonidine or [³H]WB-4101.[4]

Effects of 6-hydroxydopamine on [³H]-clonidine and [³H]WB-4101 binding. For both peripheral and central nervous systems there is evidence for the existence

[4] D. A. Greenberg, D. C. U'Prichard, and S. H. Snyder, manuscript in preparation.

TABLE 2

Inhibition of [³H]clonidine and [³H]WB-4101 binding by alpha agonists

Apparent K_i values were determined as described in Table 1.

Drug	K_i for [³H]clonidine binding (A)	K_i for [³H]WB-4101 binding (B)	B:A
	nM	*nM*	
Imidazoline derivatives			
Oxymetazoline	1.9 ± 0.3	24 ± 6	13
Xylometazoline	4.8 ± 1.4	39 ± 5	8.1
Naphazoline	5.7 ± 2.1	110 ± 32	19
Tetrahydrozoline	11 ± 4	300 ± 26	27
Tramazoline	4.2 ± 1.5	110 ± 13	26
Clonidine	5.7 ± 0.8	430 ± 64	75
St-600	7.6 ± 2.4	620 ± 110	82
Other drugs			
Mephentermine	650 ± 60	15,000 ± 2,000	23
Methoxamine	940 ± 160	11,000 ± 4,000	12
Phentermine	1,400 ± 100	24,000 ± 3,100	17
Cyclopentamine	2,500 ± 500	32,000 ± 2,900	13
Tuaminoheptane	7,100 ± 1,000	68,000 ± 16,000	9.6

TABLE 3

Inhibition of [³H]clonidine and [³H]WB-4101 binding by alpha antagonists and partial agonists

Apparent K_i values were determined as described in Table 1.

Drug	K_i for [³H]clonidine binding (A)	K_i for [³H]WB-4101 binding (B)	B:A
	nM	*nM*	
Ergot alkaloids and related drugs			
α-Ergocryptine	8 ± 0.4	9 ± 2.2	1.1
Ergocornine	11 ± 3	10 ± 3	0.9
Ergotamine	12 ± 4	12 ± 2.5	1.0
2-Bromo-α-ergocryptine	16 ± 2	9 ± 1.5	0.6
Ergocristine	18 ± 3	6.3 ± 0.3	0.4
Dihydroergotamine	2.4 ± 0.6	3.5 ± 0.2	1.5
Dihydro-α-ergocryptine	7 ± 1	2.4 ± 0.4	0.3
Dihydroergocornine	9 ± 4	3.7 ± 1.1	0.4
Dihydroergocristine	10 ± 5	2.4 ± 0.2	0.2
d-LSD	220 ± 80	220 ± 40	1.0
Ergonovine	1,400 ± 200	1,800 ± 100	1.3
Methysergide	4,700 ± 1,200	1,900 ± 50	0.4
Serotonin	18,000 ± 1,800	23,000 ± 2,400	1.3
Other partial agonists			
Yohimbine	150 ± 40	480 ± 10	3.2
Tolazoline	180 ± 60	2,100 ± 600	12
Antagonists			
Phentolamine	22 ± 4	3.6 ± 0.3	0.16
Phenoxybenzamine	60 ± 3	4.0 ± 0.5	0.07
Dibenamine	270 ± 50	83 ± 21	0.31
Piperoxan	95 ± 24	180 ± 5	1.9
Dibozane	100 ± 20	19 ± 5	0.19
WB-4101	200 ± 60	0.6 ± 0.1	0.003
Indoramin	6,500 ± 600	5.9 ± 1.2	0.0009

of presynaptic "autoreceptors" on norepinephrine-containing neurons, which behave pharmacologically much like *alpha* receptors (27-29). To determine whether [³H]clonidine and [³H]WB-4101 binding sites are situated on norepinephrine-containing neurons, we treated animals with intraventricular doses of 6-hydroxydopa-

TABLE 4

Inhibition of [³H]clonidine and [³H]WB-4101 binding by miscellaneous drugs

Apparent K_i values were determined as described in Table 1. The following drugs failed to displace binding of either [³H]ligand by 50% at 100 μM: cocaine, γ-aminobutyric acid, L-glutamate, glycine, iproniazid, isocarboxazid, morphine, naloxone, nialamide, and oxotremorine.

Drug	K_i for [³H]clonidine binding (A)	K_i for [³H]WB-4101 binding (B)	B:A
	nM	*nM*	
Beta-antagonists			
(−)-Alprenolol	4,700 ± 1,200	380 ± 100	0.08
(±)-Propranolol	5,900 ± 1,200	6,900 ± 100	1.2
Antidepressants			
Amitriptyline	530 ± 120	21 ± 5	0.04
Imipramine	2,900 ± 700	51 ± 8	0.018
(−)-Tranylcypromine	140 ± 40	2,800 ± 300	20
(+)-Tranylcypromine	940 ± 240	7,000 ± 700	7.4
Pargyline	14,000 ± 3,500	>70,000	
Anticholinergics			
Benztropine	420 ± 90	47 ± 1	0.11
Trihexyphenidyl	5,000 ± 1,500	18,000 ± 400	3.6
Atropine	>60,000	660 ± 40	
d-Tubocurarine	>60,000	6,900 ± 700	
Antihistaminics			
Promethazine	1,200 ± 100	55 ± 6	0.046
Diphenhydramine	3,500 ± 900	3,500 ± 100	1.0
Burimamide	1,030 ± 30	1,900 ± 140	1.9
Antipsychotics			
Thioridazine	520 ± 20	5.1 ± 1	0.010
Chlorpromazine	590 ± 60	6.2 ± 0.2	0.011
Haloperidol	1,200 ± 60	11 ± 3	0.009
Trifluoperazine	4,400 ± 1,000	44 ± 17	0.010
Antihypertensives			
Hydralazine	7,700 ± 2,900	52,000 ± 10,000	6.8
Minoxidil	>60,000	29,000 ± 10,000	
Other drugs			
Apomorphine	950 ± 350	2,300 ± 900	2.4
Strychnine	>60,000	4,500 ± 300	

mine sufficient to produce permanent destruction of most noradrenergic cells (30). Between 10 and 14 days after a series of two injections of 6-hydroxydopamine, whole brain levels of norepinephrine are reduced 80% and [³H]norepinephrine uptake into synaptosomal preparations is reduced 67% (Table 6). By contrast, at this time small but significant enhancement of both [³H]clonidine and [³H]WB-4101 binding is detected, amounting to about a 23% increase for clonidine and 8% for WB-4101 binding.

The failure of treatment with 6-hydroxydopamine to reduce [³H]clonidine and [³H]WB-4101 binding, under conditions which cause destruction of norepinephrine neurons, suggests that the binding sites are not presynaptic. However, it is conceivable that part of the binding could be presynaptic and part postsynaptic, and that a substantial reduction in presynaptic binding might be offset by a corresponding enhancement of postsynaptic binding. There appear to be differences in the relative potencies of *alpha* agonists (31) and *alpha* antagonists (32) at presynaptic and postsynaptic norepinephrine receptors in several sympathetically innervated tissues. In the rabbit pulmonary artery, oxymetazoline and clonidine appear to be 6–7 times more potent at pre- than at postsynaptic norepinephrine receptors, while (−)-norepinephrine and phenylephrine are respectively 2 and 30 times more potent at postsynaptic sites (31). If 6-hydroxydopamine treatment altered the ratio of presynaptic to postsynaptic receptors, one

might expect a change in the affinities of these drugs. Accordingly, we examined the relative potencies of these agents in competing for [^{3}H]clonidine and [^{3}H]WB-4101 binding 2 weeks after treatment with 6-hydroxydopamine (Table 7). In the few brains examined, no obvious change in the affinities of any of these drugs for [^{3}H]clonidine or [^{3}H]WB-4101 binding is apparent as a function of 6-hydroxydopamine treatment.

TABLE 5

Regional distribution of [^{3}H]clonidine and [^{3}H]WB-4101 specific binding in rat brain

Standard binding assays were conducted with membrane suspensions from each region, as described in METHODS. Values are given as mean amounts of [^{3}H]ligand bound per gram of the original wet weight of the tissue, ± standard errors, with the number of determinations indicated in parentheses. Each determination was performed on a pool of four rat brains. One-way analysis of variance showed no significant regional variation of [^{3}H]WB-4101 binding ($F = 2.37, p > 0.05$), but highly significant [^{3}H]clonidine regional differences ($F = 13.25, p < 0.001$). Subsequent *a posteriori* comparisons of means were performed using the Student-Newman-Keuls test (26).

Region	[^{3}H]Clonidine	[^{3}H]WB-4101
	pmoles/g	*pmoles/g*
Cerebral cortex	4.2 ± 0.4[a] (5)	2.7 ± 0.4 (5)
Hypothalamus	3.4 ± 0.2[b] (4)	2.2 ± 0.2 (5)
Thalamus-midbrain	3.0 ± 0.1 (5)	1.8 ± 0.1 (5)
Medulla-pons	2.7 ± 0.1 (4)	1.8 ± 0.2 (5)
Hippocampus	2.5 ± 0.2 (5)	2.1 ± 0.3 (5)
Corpus striatum	2.3 ± 0.2 (5)	1.8 ± 0.2 (5)
Cerebellum	1.4 ± 0.2[a] (4)	1.7 ± 0.2 (5)

[a] Significantly different from all other groups ($p < 0.05$).

[b] Significantly different from corpus striatum ($p < 0.05$).

DISCUSSION

The major finding of this study is that the binding of [^{3}H]clonidine and [^{3}H]WB-4101 to rat brain membranes has properties indicating an association with postsynaptic *alpha* noradrenergic receptor sites, as indicated in preliminary findings (8). WB-4101 is a potent antagonist, and clonidine a potent agonist, at peripheral *alpha* adrenoceptors (16, 28). In the central nervous system clonidine appears to act as an *alpha* agonist (29, 33, 34), although it antagonizes methoxamine-induced accumulation of cyclic 3',5'-AMP in rat cortical slices (35). The relative potencies of various catecholamines in displacing the binding of these [^{3}H]ligands reflect their relative effects at *alpha* receptor sites. Potent *alpha* antagonists also have substantial affinity for the binding sites. The relative potencies of both agonists and antagonists resemble those reported for binding of [^{3}H]dihydroergocryptine to *alpha* receptor sites in rabbit uterine membranes (7).

The much greater affinity of agonists than of antagonists for [^{3}H]clonidine binding sites and the greater affinity of antagonists than of agonists for [^{3}H]WB-4101

TABLE 6

Effect of intraventricular 6-hydroxydopamine on endogenous norepinephrine levels, [^{3}H]norepinephrine uptake, and specific [^{3}H]clonidine and [^{3}H]WB-4101 binding in rat brain

Animals received two intraventricular injections of 250 μg of 6-hydroxydopamine and were killed 10–14 days later for assay of endogenous norepinephrine, [^{3}H]norepinephrine uptake, and [^{3}H]clonidine and [^{3}H]WB-4101 binding as described in METHODS. Values are the means ± standard errors of the number of determinations in parentheses. Values of p were determined by Student's t-test.

Treatment	Endogenous norepinephrine	[^{3}H]Norepinephrine uptake	Specific binding	
			[^{3}H]Clonidine	[^{3}H]WB-4101
	ng/g tissue	*nmole/g/5 min*	*cpm*	*cpm*
Control	217 ± 20 (3)	0.52 ± 0.19 (3)	91 ± 3 (11)	440 ± 13 (7)
6-Hydroxydopamine	46 ± 3[a] (3)	0.17 ± 0.01[a] (3)	112 ± 6[b] (12)	473 ± 8[c] (8)
Change	−79%	−67%	+23%	+8%

[a] Significantly different from control ($p < 0.001$).

[b] Significantly different from control ($p < 0.01$).

[c] Significantly different form control ($p < 0.05$).

TABLE 7

Drug effects on [³H]clonidine and [³H]WB-4101 binding in control and 6-hydropamine-treated rat brain homogenates

Treatments, tissue preparation, and binding assays were performed as described in METHODS. Values given are means from one or two determinations, with individual values indicated in parentheses.

Drug	IC_{50} for [³H]clonidine binding		IC_{50} for [³H]WB-4101 binding	
	Control	6-Hydroxydopamine	Control	6-Hydroxydopamine
	nM	*nM*	*nM*	*nM*
(−)-Norepinephrine	29 (41, 17)	26 (34, 18)	800	950 (1200, 700)
Oxymetazoline	3.9 (4.0, 3.8)	2.5 (4, 1)	27 (36, 18)	21 (25, 17)
Clonidine	10 (13, 7)	8 (9, 7)	410 (430, 390)	390
(±)-Phenylephrine	350	270 (290, 250)	5400 (5700, 5100)	5400 (5800, 5000)

sites suggest that [³H]clonidine and [³H]WB-4101 bind respectively to agonist and antagonist states of the receptor. The finding that mixed agonist-antagonist drugs have similar affinities for clonidine and WB-4101 sites supports this hypothesis. Similar evidence for the existence of discrete agonist and antagonist states of neurotransmitter receptors in the brain has been provided for the dopamine receptor (13), the postsynaptic serotonin receptor (12), and the opiate receptor (36, 37). There are several possible explanations for the differences in binding properties of agonists and antagonists to neurotransmitter receptors. One model postulates the existence of "agonist" and "antagonist" states of the receptor, which may interconvert freely or under restraint. A freely interconverting system (38, 39) would necessarily imply that a drug would have equal affinity in displacing an agonist or antagonist [³H]ligand. Such is not the case in these studies. The findings for the dopamine and serotonin receptors of differential affinities of drugs in displacing agonist and antagonist ligands, and Hill coefficients of much less than 1.0 for agonists displacing [³H]antagonists and vice versa, have been explained by a two-state receptor model with restricted interconvertibility (12, 13). The present findings of Hill coefficients of 1.0 seen with any combination of *alpha* noradrenergic ligand and displacing drug does not favor a two-state model with interconverting states, except in the case of extremely slow interconversion. Another model, which has been suggested for the muscarinic and opiate receptors (40, 41), postulates the existence of two independent sites with high and low affinities for agonists, respectively, but with identical affinities for antagonists. The simplest model to explain our findings involves discrete "agonist"- and "antagonist"-favoring sites which do not interconvert. Consistent with this last model is the finding that a concentration of unlabeled clonidine which saturates [³H]clonidine binding sites fails to displace [³H]WB-4101 binding. Moreover, at concentrations which should saturate [³H]WB-4101 binding, unlabeled WB-4101 does not displace [³H]clonidine binding (Figs. 1, 2, and 5).

Regional variations in [³H]clonidine and [³H]WB-4101 binding do not correlate well with regional levels of endogenous norepinephrine, as has also been found with *beta* noradrenergic receptor binding (4). The relative activity of norepinephrine-sensitive cyclic AMP-accumulating systems associated with *alpha* norepinephrine receptor sites also does not show regional variations that parallel those of endogenous norepinephrine (42). Interestingly, [³H]-clonidine and [³H]WB-4101 binding levels do not correlate regionally with each other, [³H]WB-4101 binding having almost no regional variation. Conceivably, various brain regions have differing proportions of presumed agonist and antagonist forms of the *alpha* receptor. There appear to be differing ratios of *alpha* and *beta* receptor binding in some brain regions; thus *beta* receptor binding is low in the hypothalamus and high in the cerebellum (4), while the converse is true for *alpha* receptor binding. The exception is the cer-

ebral cortex, which has little endogenous norepinephrine but exhibits substantial *alpha* and *beta* receptor binding. In several other cases, regional variations in neurotransmitter receptor binding fail to parallel variations in endogenous content of neurotransmitter (3, 6, 12).

The high potency of antipsychotic agents in displacing [^{3}H]WB-4101 might suggest that this ligand labels receptors for dopamine in addition to *alpha* receptors. However, several findings in the present study argue against such an interpretation. Binding sites for [^{3}H]WB-4101 are distributed with relative uniformity in the seven rat brain regions examined. By contrast, binding of [^{3}H]dopamine and [^{3}H]haloperidol is markedly higher in striatal tissues than in any other region of calf brain (13). In addition, marked differences are observed in the affinities of classical dopaminergic and *alpha* adrenergic agents for [^{3}H]WB-4101 and [^{3}H]haloperidol sites. Dopamine and the dopamine agonist apomorphine are respectively 65 and 45 times more potent displacers of [^{3}H]haloperidol than of [^{3}H]WB-4101, while the classical *alpha* antagonists phenoxybenzamine and phentolamine display 35 and 550 times greater affinity for [^{3}H]-WB-4101 than for [^{3}H]haloperidol sites. Although antipsychotic agents are potent displacers of both [^{3}H]haloperidol and [^{3}H]WB-4101, their relative orders of potency at these sites are different. Of the four antipsychotics examined here, thioridazine is most potent in displacing [^{3}H]WB-4101, but least potent in displacing [^{3}H]haloperidol (13). Trifluoperazine, which is 5 times more potent than chlorpromazine in displacing [^{3}H]haloperidol, has 7 times less affinity than chlorpromazine for [^{3}H]WB-4101 sites. Studies of [^{3}H]WB-4101 displacement by an extensive series of antipsychotic agents (43) indicate that the potent dopamine antagonist (+)-butaclamol has 44 times less affinity for [^{3}H]WB-4101 than for [^{3}H]haloperidol sites, and reveal a specific correlation of [^{3}H]WB-4101 displacement potency with alpha adrenergic antagonism *in vivo*.

6-Hydroxydopamine treatment produces small but significant enhancement of [^{3}H]clonidine and [^{3}H]WB-4101 binding in rat brain membranes. Further [^{3}H]clonidine saturation studies have shown that this increased binding reflects an augmentation of receptor sites,[4] which may be associated with "denervation supersensitivity." Increased *beta* noradrenergic receptor binding in the brain has also been observed following 6-hydroxydopamine treatment (44). Moreover, after 6-hydroxydopamine lesions of the nigrostriatal dopamine pathway, substantial enhancement of dopamine receptor binding can be demonstrated in membranes of the corpus striatum, associated with an increased number of binding sites with no change in their affinity for dopamine or dopamine antagonists (45).

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