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Pharmacological Properties of [^{3}H]Dihydroergokryptine Binding Sites Associated with *Alpha* Noradrenergic Receptors in Rat Brain Membranes

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SUMMARY

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[^{3}H]Dihydroergokryptine, a mixed agonist-antagonist at *alpha* noradrenergic receptors, binds in saturable fashion and with high affinity to membranes of rat cerebral cortex. Saturation studies reveal a dissociation constant (K_D) of 1.6 nM and a receptor density of 21 pmoles/g, similar to the sum of the maximal number of binding sites observed for the [^{3}H]*alpha* agonist clonidine and the [^{3}H]*alpha* antagonist WB-4101 alone. The relative affinities of a wide range of drugs indicate that binding to cerebral cortical membranes selectively involves *alpha* noradrenergic receptors. Dopamine and serotonin inhibit binding only weakly, indicating that labeling of receptors for these neurotransmitters is negligible. The regional distribution of [^{3}H]dihydroergokryptine binding in rat brain coincides with that observed for *alpha* receptor binding of [^{3}H]clonidine and [^{3}H]WB-4101, except for disproportionately high levels in corpus striatum. This suggests that in striatal membranes [^{3}H]dihydroergokryptine is able to bind to dopamine as well as *alpha* receptors. The slopes of logit-log plots for the inhibition of [^{3}H]dihydroergokryptine binding by mixed agonist-antagonists are about 1.0, while pure agonists or antagonists display shallower inhibition curves. These data are consistent with a model of the *alpha* noradrenergic receptor in which agonists and antagonists bind selectively to discrete, noninterconverting sites, while mixed agonist-antagonists can bind to either site.

INTRODUCTION

Alpha noradrenergic receptor sites in mammalian brain membranes can be labeled with the agonists [^{3}H]clonidine, [^{3}H]epinephrine, and [^{3}H]norepinephrine (1-4), and with the antagonist [^{3}H]WB-4101 (2-[(2', 6'-dimethoxy)phenoxyethylamino]methylbenzodioxan) (1, 2). *Alpha* noradrenergic agonists have substantially greater potency in competing for the binding of the [^{3}H]agonists clonidine, epinephrine, and norepinehprine than for the binding of the antagonist [^{3}H]WB-4101, while antagonists have greater potency in reducing the binding of [^{3}H]WB-4101 than that of the [^{3}H]agonists (1-4). Mixed ago-

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nist-antagonists have similar potencies in inhibiting the binding of [^{3}H]agonists and [^{3}H]antagonists (1–4). These findings are consistent with the existence of discrete "agonist" and "antagonist" states of *alpha* noradrenergic receptors, analogous to the agonist and antagonist receptor sites for opiates (5) and dopamine (6). Labeling of *alpha* receptors with a mixed agonist-antagonist might help to clarify the relationship between binding sites for [^{3}H]agonists and [^{3}H]antagonists. Ergot alkaloids containing a peptide side chain display mixed agonist-antagonist activity at *alpha* receptors in peripheral smooth muscle (7). In brain membranes the absence of a measurable receptor-coupled response makes it difficult to demonstrate directly that ergots interact with central *alpha* receptors in an identical fashion. However, the similar affinities of ergots for [^{3}H]*alpha* agonist and antagonist binding sites in the brain are highly suggestive of a mixed role for these agents at central *alpha* adrenergic sites. Williams *et al.* (8, 9) used the mixed agonist-antagonist [^{3}H]dihydroergokryptine to label *alpha* receptors in rabbit uterine membranes. In one study [^{3}H]DHE[3] appeared to bind to dopamine and serotonin receptors as well as to *alpha* receptors in brain membranes (10, 11). Apparent labeling of serotonin receptors in brain membranes has also been reported with the ergot alkaloid [^{3}H]dihydroergotamine (12). Recently we observed that low concentrations of [^{3}H]DHE can be used to label *alpha* receptors selectively in rat brain membranes (13). In the present study we describe detailed characteristics of the binding of [^{3}H]DHE associated with *alpha* receptors in rat cerebral cortical membranes.

MATERIALS AND METHODS

Experiments were conducted as described previously (13). Cerebral cortices of adult male Sprague-Dawley rats killed by decapitation were placed on ice immediately following dissection. Tissue was homogenized with a Tekmar model SDT homogenizer in 20 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°). Homogenates were centrifuged twice in a Sorvall RC-2B preparative centrifuge for 10 min at 50,000 × *g*, with resuspension of the pellet in fresh buffer between spins. The final pellet was homogenized in 50 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°).

[9,10-^{3}H(*N*)]Dihydroergokryptine, 24.1 Ci/mmole, was purchased from New England Nuclear and stored in ethanol at −10°. Dilutions to 40–50 nM were prepared fresh daily in 1 μM HCl.

The standard [^{3}H]DHE binding assay was conducted by incubating 1 ml of 50 mM Tris-HCl buffer (pH 7.7 at 25°), 1 ml of tissue suspension (containing 20 mg of the original wet weight of the tissue), and 15 μl of [^{3}H]DHE in culture tubes at 25° for 60 min in the dark. The final incubation volume of about 2 ml contained 0.3–0.4 nM [^{3}H]DHE. Displacers (20–60 μl) were added where indicated. Following incubation to equilibrium, tubes were filtered under vacuum through Whatman GF/B glass fiber filters with three 5-ml washes of ice-cold buffer. Preliminary studies showed that this wash volume afforded maximal reduction of nonspecific binding without a loss of specific binding. Filters were placed in vials containing 10 ml of Formula 947 (New England Nuclear), cooled overnight, and counted in a Packard Tri-Carb liquid scintillation counter (model 3385) at 40% efficiency.

Specific [^{3}H]DHE binding was defined as binding in excess of blanks determined in the presence of 0.1 mM (−)-norepinephrine.

Ergot alkaloids were obtained from Sandoz; WB-4101, from W. B. Pharmaceuticals; phenylethylamine isomers, from Winthrop; and indoramin (3-[2-(4-benzamidopiperid-1-yl)ethyl]indole hydrochloride), from Wyeth. Other drugs were from commercial sources or the pharmaceutical company of origin.

RESULTS

Saturation of [^{3}H]DHE binding. Specific binding of [^{3}H]DHE is saturable. At 0.3 nM, the concentration of [^{3}H]DHE used

[3] The abbreviation used is: DHE, dihydroergokryptine.

in routine binding assays, the ratio of total binding to nonspecific binding measured in the presence of 0.1 mM (−)-norepinephrine is about 3. Nonspecific binding increases linearly up to 4 nM, the highest concentration examined (Fig. 1). Specific binding, the difference between total and nonspecific binding, reaches a plateau between 2 and 4 nM [^{3}H]DHE. Half-maximal binding is apparent at about 1.0 nM [^{3}H]DHE. Scatchard analysis of data from five experiments indicates a single population of binding sites with a dissociation constant (K_D) of 1.6 ± 0.3 nM (mean ± SE). The calculated maximal number of binding sites is 20.9 ± 1.7 pmoles/g of wet tissue weight, which is similar to the sum of the values obtained for sites bound by [^{3}H]clonidine (14 ± 1.3 pmoles/g) and [^{3}H]WB-4101 (11 ± 1.0 pmoles/g) in rat brain membranes (1, 2). A Hill plot of [^{3}H]DHE saturation data is linear, with a Hill coefficient (n_H) of 1.13 ± 0.09, indicating that [^{3}H]DHE binds in a noncooperative manner to a population of sites for which it has uniform affinity.

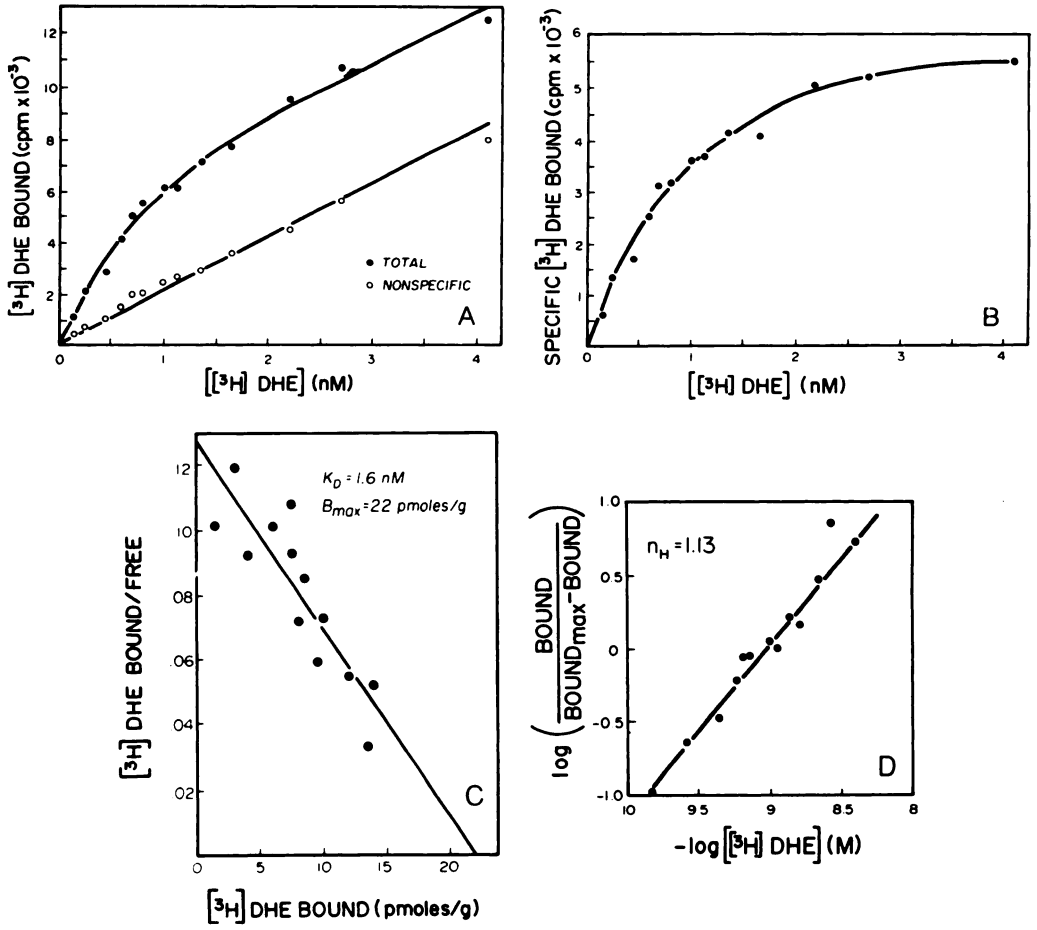

FIG. 1. *[^{3}H]DHE binding with increasing concentrations of [^{3}H]DHE*

Homogenates of rat cerebral cortex were incubated for 60 min at 25°, as described in MATERIALS AND METHODS, in the presence of various concentrations of [^{3}H]DHE. Nonspecific binding was measured in the presence of 0.1 mM (−)-norepinephrine. The data shown are from a single experiment, performed in triplicate and repeated four times. A. Total and nonspecific binding. B. Specific binding, determined by subtracting nonspecific from total binding at each concentration. C. Scatchard analysis of saturation data. The slope obtained by linear regression analysis gives a K_D value of 1.6 nM and receptor density (B_{max}) of 22 pmoles/g of original wet tissue weight. D. Hill plot of specific binding data, with slope (n_H) = 1.13.

Kinetics of [³H]DHE binding. The rates for association and dissociation of [³H]DHE binding at 25° are slower than the corresponding rates for the binding of [³H]-clonidine and [³H]WB-4101 (2). Binding does not appear to reach equilibrium until about 35 min of incubation at this temperature (Fig. 2). Half-maximal binding occurs at about 10 min. The second-order rate constant for association (k_1) was determined as described by Williams *et al.* (9). using the equation $k_1 = (k_{obs} - k_2)/$ [[³H]DHE], where k_{obs} is the pseudo-first-order rate constant for association (Fig. 2) and k_2 is the rate constant for dissociation (Fig. 3). This formula presupposes "zone A" behavior (14) for the ligand-receptor system. The method takes into account the simultaneous association and dissociation of the reversible ligand-receptor complex and the ligand concentration dependence of k_{obs}, and gives a value for k_1 of 0.1 nM^{-1} min^{-1}.

The rate of dissociation of specifically bound [³H]DHE was determined by incubating membranes to equilibrium at 25° in the presence of the ³H-labeled ligand and subsequently adding 1 mM (−)-norepinephrine to prevent rebinding of dissociated [³H]DHE (Fig. 3). The specific [³H]DHE binding remaining was measured at various time intervals up to 2 hr. Dissociation proceeds slowly at 25°, with a value for $t_{1/2}$ of 60 ± 5 min. Dissociation is linear with time when plotted on a semilogarithmic scale, with a first-order rate constant for dissociation (k_2) of 0.01 min^{-1}. A similar rate constant for dissociation was obtained when 0.1 μM unlabeled DHE was used to prevent rebinding. The dissociation constant (K_D) calculated from the ratio of k_2 to k_1 is 0.1 nM, which is much less than the value of 1.6 nM obtained in saturation experiments. The most likely explanation for this disparity is an underestimation of the value for k_2, making the K_D value from saturation data more reliable. The kinetic K_D value for binding of [³H]DHE to *alpha* receptor sites in rabbit uterine membranes is similarly discrepant with respect to the value calculated from saturation data (9).

Effects of drugs on [³H]DHE binding. A variety of agents with potent effects on *alpha* receptors in sympathetic end organs and high affinity for sites labeled by tritiated *alpha* noradrenergic agonists and antagonists in brain membranes also com-

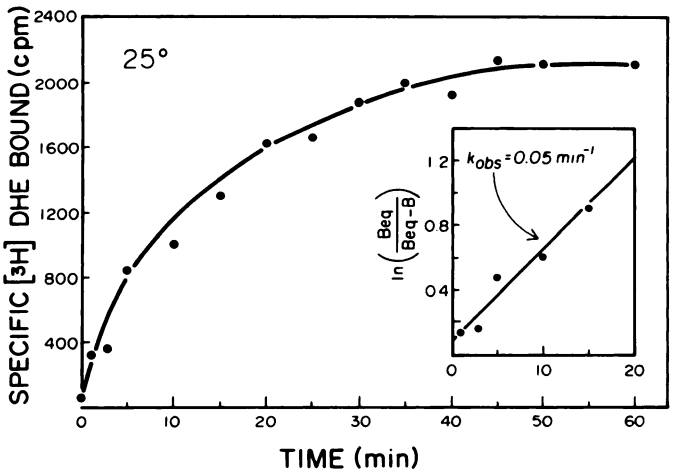

FIG. 2. *Time course of association of [³H]DHE binding*

Rat cerebral cortical membranes, prepared as described in MATERIALS AND METHODS, were incubated with 0.4 nM [³H]DHE at 25° for various time intervals. Binding in the absence and presence of 0.1 mM (−)-norepinephrine was measured simultaneously, the difference between these values representing specific binding. Association was begun by addition of tissue and terminated by rapid filtration. The data shown are from a single experiment, performed in triplicate and replicated twice. The pseudo-first-order rate constant for association (k_{obs}) was determined by plotting ln (specific binding at equilibrium/specific binding at equilibrium minus specific binding at a given time) vs. time, and using the resulting linear slope.

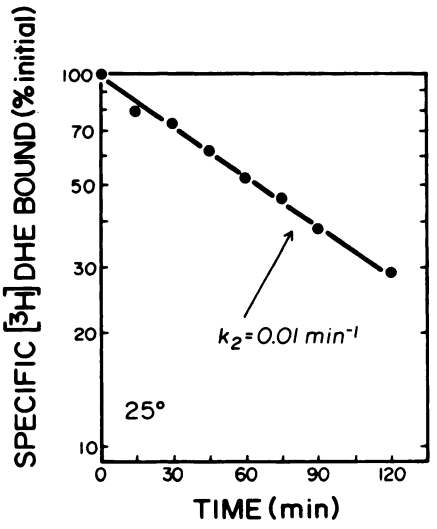

FIG. 3. *Time course of dissociation of [³H]DHE binding*

Rat cerebral cortical membranes, prepared as described in MATERIALS AND METHODS, were incubated to equilibrium (60 min) at 25° in the presence of 0.4 nM [³H]DHE. Dissociation was begun by addition of 1 mM (−)-norepinephrine to tubes at zero time, and terminated by rapid filtration at various intervals. The data presented are from a single experiment, performed in triplicate, which was replicated twice.

pete avidly for [³H]DHE specific binding sites, but fail to affect nonspecific binding. Mixed agonist-antagonists, including DHE itself and other ergot alkaloids, are substantially more potent inhibitors of [³H]DHE binding than of [³H]clonidine or [³H]WB-4101 binding (2). This contrasts with the effects of pure agonists and antagonists, which reduce [³H]DHE binding with potencies intermediate between those observed at [³H]clonidine and [³H]WB-4101 sites (Table 1).

The relative affinities of various sympathomimetic amines for [³H]DHE binding sites resemble their relative effects on the binding of [³H]WB-4101 and [³H]clonidine in brain membranes (2) and [³H]DHE in uterine membranes (9) and correspond to the relative potencies of these agents in producing *alpha* receptor-activated contractions in smooth muscle (17, 18). Thus the ability of phenylethylamines to inhibit [³H]DHE binding diminishes in the order (−)-epinephrine > (−)-norepinephrine > (−)-phenylephrine > (−)-isoproterenol (Table 1). (−)-Epinephrine is about 2.6 times more active than (−)-norepinephrine, compared with its 2.8-fold greater potency in reducing [³H]DHE binding to rabbit uterine membranes (9). (±)-α-Methyl-(±)-norepinephrine exhibits almost 4-fold greater affinity than (−)-phenylephrine for [³H]DHE binding sites, which corresponds to the relative potencies of these agents at *alpha* receptor sites labeled by [³H]DHE in the uterus (9) and by [³H]clonidine (2) and [³H]catecholamines (4) in the brain. In peripheral smooth muscle systems, however, the relative potencies of these two agents at postsynaptic *alpha* receptors varies, with phenylephrine more potent than α-methylnorepinephrine in rabbit aortic strips (19), equipotent in rabbit pulmonary artery (20), and less potent in the isolated rabbit intestine (21). Optically active catecholamines display stereoselectivity in inhibiting [³H]DHE binding, with (−)-epinephrine and (−)-norepinephrine being 60 and 25 times more potent, respectively, than their (+) isomers. These findings correspond to the stereoselectivity of catecholamine isomers in competing for *alpha* receptor binding of [³H]WB-4101, [³H]clonidine, [³H]epinephrine, and [³H]norepinephrine in the brain (2, 4), and of [³H]DHE in the uterus (9).

The most potent *alpha* agonist is oxymetazoline, which is about 3.5 times more potent than the related imidazoline agonist clonidine. The greater potency of oxymetazoline than clonidine is also reflected in the ratio of activities of these agents in competing for [³H]WB-4101 and [³H]clonidine binding (2). Clonidine is itself approximately equipotent with (−)-epinephrine in inhibiting [³H]DHE binding, in agreement with the similar affinities of the two agents for central nervous system *alpha* receptor sites bound by [³H]clonidine (2), [³H]WB-4101 (2), and [³H]catecholamines (4). While the high potency of clonidine at sites labeled by these [³H]ligands might suggest that the receptors being examined are located presynaptically, earlier studies in which adrenergic nerve terminals were destroyed with 6-

TABLE 1

Inhibition of [³H]DHE binding by pharmacological agents

Cerebral cortical membranes were incubated with 0.3–0.4 nM [³H]DHE and five or more concentrations of each drug, using the standard binding assay described in MATERIALS AND METHODS. IC_{50} values, defined as the concentration of drug producing 50% inhibition of specific binding [total binding minus binding in the presence of 0.1 mM (−)-norepinephrine], were determined by log probit analysis. K_i values were calculated from the equation (15).

$$K_i = \frac{IC_{50}}{1 + c/K_D}$$

where c and K_D represent the concentration of [³H]DHE used and the dissociation constant for [³H]DHE from saturation data, respectively. The assumptions underlying the use of this formula and the limitations thereof have been reviewed (16). Values given are the means of three to seven separate experiments, each performed in triplicate, whose results varied by less than 30%.

Agent	K_i	Agent	K_i
	nM		nM
Alpha agonists		Ergonovine	150
(−)-Epinephrine	46	Methysergide	680
(−)-Norepinephrine	120	*Alpha* antagonists	
(±)-α-Methyl-(±)-norepinephrine	320	Phentolamine	6.2
(−)-Phenylephrine	1,200	WB-4101	29
(+)-Epinephrine	2,700	Phenoxybenzamine[a]	31
(+)-Norepinephrine	3,000	Yohimbine	62
Oxymetazoline	15	Piperoxan	110
Clonidine	52	Indoramin	240
Ergot alkaloids		Dibenamine[a]	260
Ergocristine	1.7	Other agents	
Ergocornine	2.1	(−)-Isoproterenol	18,000
Ergotamine	2.2	(−)-Propranolol	3,200
Ergokryptine	2.2	Dopamine	720
2-Bromo-α-ergokryptine	6.4	Chlorpromazine	110
Dihydroergocristine	1.0	Haloperidol	130
Dihydroergotamine	1.6	Serotonin	7,500
Dihydroergocornine	1.7	*d*-Lysergic acid diethylamide	75
Dihydroergokryptine	1.7		

[a] Noncompetitive inhibitors.

hydroxydopamine indicated that clonidine binds to postsynaptic sites with high affinity, and is approximately equipotent with (−)-epinephrine at such sites (2).

Ergot alkaloids are the most potent inhibitors of [³H]DHE binding of all agents examined. Ergocristine, ergocornine, ergotamine, and ergokryptine have similar potencies, while 2-bromo-α-ergokryptine is about one-third as active. The 9,10-dihydrogenated ergots tend to be slightly more potent than their corresponding unsaturated analogues. Dihydrogenated ergots are also more potent than the parent alkaloids in competing for sites labeled by [³H]WB-4101 and [³H]clonidine in the brain (2) and by [³H]DHE in the uterus (9). Ergonovine and methysergide, ergots with amine side chains, are much weaker than the peptide ergots in competing for binding, which corresponds to their lesser effects at *alpha* receptors in smooth muscle (22).

Among the *alpha* antagonists, phentolamine is most potent, with about 4.7 times greater activity than WB-4101. The high potency of WB-4101 in inhibiting the binding of [³H]DHE is consistent with its potent sympatholytic effects in peripheral systems (23, 24) and with the high affinity of [³H]WB-4101 for *alpha* receptor sites in rat brain membranes (1, 2). Piperoxan and indoramin inhibit [³H]DHE binding with potencies intermediate between their activities at [³H]WB-4101 and [³H]clonidine binding sites. The noncompetitive *alpha*

antagonists phenoxybenzamine and dibenamine are also potent inhibitors of [^{3}H]DHE binding, although the irreversible nature of receptor blockade by these agents prevents quantitative comparison with reversible antagonist affinities. Indoramin, the least potent reversible *alpha* antagonist examined, still displays more than 10 times greater affinity than the *beta* antagonist (−)-propranolol for [^{3}H]DHE binding sites. While yohimbine is classically described as an *alpha* antagonist in a variety of smooth muscle systems, its approximately equal effect on [^{3}H]WB-4101 and [^{3}H]clonidine binding resembles more closely the profile of mixed agonist-antagonist ergots than that of pure agonists or antagonists (2).

The weak effects of serotonin and dopamine on [^{3}H]DHE binding under the present experimental conditions coincide with our earlier observations that, at low concentrations, [^{3}H]DHE selectively labels *alpha* receptor sites in rat cerebral cortex (13). The 6-fold lower potency of dopamine than of (−)-norepinephrine is similar to the ratio observed for inhibition of *alpha* receptor binding of [^{3}H]clonidine (2) and [^{3}H]catecholamines (4) in the brain and for contraction of rat vas deferens *in vitro* (25). In rabbit aorta (19) and at [^{3}H]DHE sites in rabbit uterine membranes (9), however, the potency of dopamine relative to that of (−)-norepinephrine is much less. The neuroleptics chlorpromazine and haloperidol are as potent as some classical *alpha* antagonists in competing for sites bound by [^{3}H]DHE. The finding is consistent with the potent activity of these and other neuroleptic agents as sympatholytics in rat vas deferens and rabbit intestine (26) and as inhibitors of the binding of [^{3}H]WB-4101 in rat brain membranes (27). The low affinity of (−)-isoproterenol and (−)-propranolol for [^{3}H]DHE binding sites confirms that DHE does not label *beta* noradrenergic receptor sites in these studies.

The concept that [^{3}H]epinephrine, [^{3}H]norepinephrine, and [^{3}H]clonidine label an "agonist" state of the *alpha* receptor, while [^{3}H]WB-4101 labels an "antagonist" state, suggests that mixed agonist-antagonists such as the ergots might bind to both states. If this is the case, agonists and antagonists should have affinities for [^{3}H]DHE binding sites intermediate between their affinities for sites labeled by [^{3}H]agonists and [^{3}H]antogonists. The K_i values for a variety of *alpha* agonists and antagonists given in Table 1 conform to this prediction.

If [^{3}H]WB-4101 labels antagonist sites selectively, [^{3}H]clonidine labels agonist sites selectively, and [^{3}H]DHE is approximately equipotent at the two sites, then the estimated affinity constant of a drug for [^{3}H]DHE sites should be the mean of its affinity constants for [^{3}H]WB-4101 and [^{3}H]clonidine sites on a logarithmic scale, assuming that the two populations of sites are approximately equal in numbers. Accordingly, we compared the $\log_{10}$ of the nanomolar K_i values of various *alpha* adrenergic agents in competing for [^{3}H]DHE binding with the mean of the $\log_{10}$ (K_i) values for reducing [^{3}H]WB-4101 and [^{3}H]clonidine binding (Table 2). For a series of agonists and antagonists of diverse structure and widely varying potencies, there is close agreement between these values. For the ergot alkaloids, however, the same relationship does not hold. The ergots are in all instances more potent in inhibiting the binding of [^{3}H]DHE than would be predicted from their affinities for [^{3}H]clonidine and [^{3}H]WB-4101 binding sites. This enhanced potency may result from ligand-induced alterations at the binding site that increase the receptor affinity for substrates of similar structure, in analogy to the "induced fit" hypothesis for enzyme-substrate interactions (28).

In a previous study (2) we found that curves for the inhibition of [^{3}H]WB-4101 or [^{3}H]clonidine binding by agonists or antagonists were all parallel. When plotted on a logit-log scale, the slopes obtained were not significantly different from 1.0. In the case of [^{3}H]DHE binding, however, curves describing binding inhibition by different classes of drugs are dissimilar (Fig. 4). Logit-log slopes for inhibition of [^{3}H]DHE binding by the mixed agonist-antagonist ergots and the Hill coefficient for [^{3}H]DHE binding saturation (Fig. 1D)

TABLE 2

Correlation of inhibition of [³H]DHE, [³H]clonidine, and [³H]WB-4101 binding by alpha noradrenergic agonists and antagonists

K_i values for [³H]DHE binding were determined as described in Table 1. Values for inhibition of [³H]clonidine and [³H]WB-4101 binding are as reported previously (2).

Agent	Log $K_{i\,Clo}$	Log $K_{i\,WB}$	$\frac{\text{Log } K_{i\,Clo} + \log K_{i\,WB}}{2}$	log $K_{i\,DHE}$
	nM	*nM*	*nM*	*nM*
Agonists				
(−)-Epinephrine	0.77	2.77	1.77	1.66
(−)-Norepinephrine	1.23	3.00	2.12	2.08
(±)-α-Methyl-(±)-norepinephrine	1.20	3.83	2.52	2.51
(−)-Phenylephrine	2.43	3.41	2.92	3.08
(+)-Epinephrine	1.81	4.45	3.13	3.43
(+)-Norepinephrine	2.78	4.83	3.81	3.48
Oxymetazoline	0.28	1.38	0.83	1.18
Clonidine	0.76	2.63	1.70	1.72
Antagonists				
Phentolamine	1.34	0.56	0.95	0.79
WB-4101	2.30	−0.22	1.04	1.46
Piperoxan	1.98	2.26	2.12	2.04
Indoramin	3.81	0.77	2.29	2.38
Ergot alkaloids				
Ergocristine	1.26	0.80	1.03	0.23
Ergocornine	1.04	1.00	1.02	0.32
Ergotamine	1.08	1.08	1.08	0.34
Ergokryptine	0.90	0.95	0.93	0.34
2-Bromo-α-ergokryptine	1.20	0.95	1.08	0.81
Dihydroergocristine	1.00	0.38	0.69	0.00
Dihydroergotamine	0.38	0.54	0.46	0.20
Dihydroergocornine	0.95	0.57	0.76	0.23
Dihydroergokryptine	0.85	0.38	0.62	0.23

are about 1.0, indicating that these agents compete in a noncooperative manner for a population of sites for which they have uniform affinity. By contrast, the agonists (−)-epinephrine, (−)-norepinephrine, and (−)-phenylephrine and the antagonists phentolamine, WB-4101, and phenoxybenzamine all display shallower competition curves, with logit-log slopes of approximately 0.6. A similar finding for the muscarinic cholinergic receptor, where agonists inhibit the binding of ³H-labeled antagonists with Hill coefficients of less than 1.0, has been interpreted to reflect competition for binding sites that are heterogeneous with respect to the affinity of the inhibiting agents (29).

Regional distribution of [³H]DHE binding. The binding of [³H]clonidine and [³H]WB-4101 associated with *alpha* receptors is highest in the cerebral cortex, lowest in the cerebellum, and intermediate in other rat brain areas. Levels of [³H]clonidine binding in the corpus striatum are similar to those in the hippocampus, medulla-pons, and thalamus-midbrain, significantly higher than values in the cerebellum, and significantly lower than values in the hypothalamus and cerebral cortex. Although [³H]WB-4101 binding displays regional differences in the same rank order, the magnitude of the variations is less (2). [³H]DHE binding differs from that of [³H]clonidine and [³H]WB-4101 by displaying highest levels in the corpus striatum. Striatal binding is significantly in excess of that seen in cerebral cortex, the next highest region examined (Table 3). Except for the corpus striatum, the relative levels of [³H]DHE binding in various regions are similar to values for [³H]clonidine and [³H]WB-4101. Lowest binding occurs in the cerebellum, while the thalamus-midbrain, hippocampus, hy-

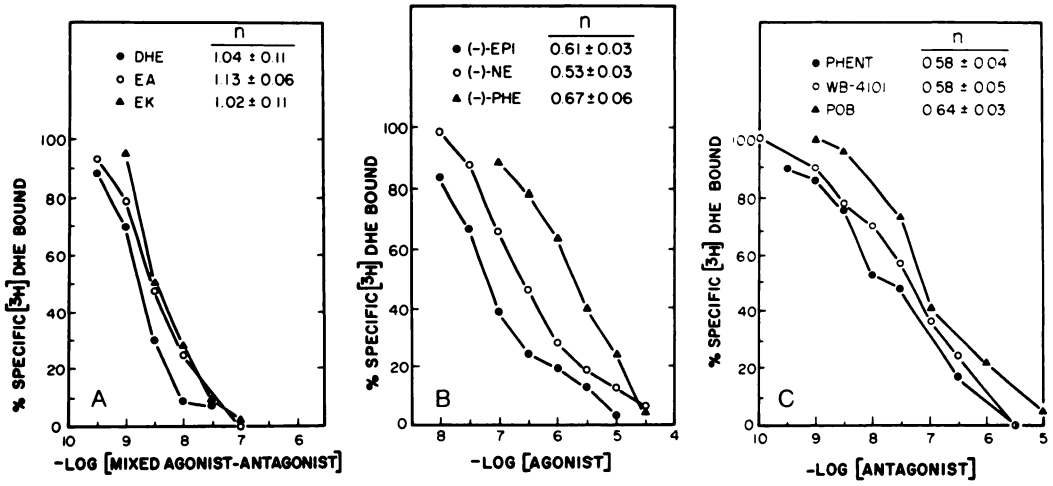

FIG. 4. *Inhibition of [³H]DHE binding by alpha noradrenergic agents*

Various concentrations of inhibiting agents were incubated with 0.3–0.4 nM [³H]DHE under standard assay conditions. Data from a single experiment, which was repeated three to seven times, are shown for each drug, plotted as percentage of specific [³H]DHE remaining bound. Data from each of three to seven experiments for each drug were plotted on a logit-log scale [plotting log [inhibitor] vs. ln (% specific binding/100 minus % specific binding)] to give three to seven values for n, the slope of the logit-log plot, by linear regression analysis. Values given represent slopes ± standard errors. A. Inhibition by mixed agonist-antagonists. B. Inhibition by agonists. C. Inhibition by antagonists. EK=ergokryptine; EA=ergotamine; EPI=(−)-epinephrine; NE=(−)-norepinephrine; PHE=(−)-phenylephrine; PHENT=phentolamine; POB=phenoxybenzamine; WB-4101=2-[(2',6'-dimethoxy)phenoxyethylamino]methylbenzodioxan. Note that phenoxybenzamine is an irreversible *alpha* antagonist.

pothalamus, and medulla-pons have intermediate values, which are somewhat less than those in the cerebral cortex.

DISCUSSION

The specific binding of [³H]DHE to rat cerebral cortical membranes assayed under the present experimental conditions appears to label *alpha* noradrenergic receptor sites selectively, confirming our earlier preliminary observations. Although ergots have substantial activity at central dopamine (31) and serotonin (32) receptor sites, the affinity of [³H]DHE appears to be somewhat greater for *alpha* receptors in the cerebral cortex than for [³H]serotonin or [³H]dopamine binding sites (33), so that at the low concentrations of [³H]DHE utilized in our study (0.3–0.4 nM) cortical binding does not appear to involve dopamine or serotonin receptors. This is supported by the low potency of dopamine and serotonin in competing for [³H]DHE binding, while a variety of *alpha* noradrenergic agonists and antagonists have relative potencies that correspond well with their known influences at classical *alpha* receptors. Using higher concentrations (8 nM) of [³H]DHE, Davis *et al.* (10, 11) observed binding that appeared to involve serotonin and dopamine receptors as well as *alpha* receptors.

The disproportionately high levels of [³H]DHE binding in the corpus striatum compared with binding levels of [³H]WB-4101 and [³H]clonidine in this region suggest that a portion of the striatal binding of [³H]DHE may involve dopamine receptors. Earlier we found that (−)-norepinephrine was about twice as potent as dopamine in reducing [³H]DHE binding to striatal membranes (13), compared with the 10-fold greater potency of dopamine at dopamine receptor sites labeled by [³H]haloperidol and [³H]dopamine in this region (7), suggesting that striatal binding of [³H]DHE involves *alpha* rather than dopamine receptors. However, the high levels of [³H]DHE binding in the corpus striatum observed in the present study,

TABLE 3

Regional distribution of [³H]DHE specific binding in rat brain membranes

Tissue from the various rat brain regions was prepared and assayed for specific [³H]DHE binding as described in MATERIALS AND METHODS. Values given are the means and standard errors of four independent determinations. Tissue from two to five rat brains was pooled for each determination. One-way analysis of variance showed highly significant regional differences in [³H]DHE binding ($F = 13.98, p < 0.001$). Subsequent comparisons of means were performed using the Student-Newman-Keuls test (30).

Region	[³H]DHE Specifically Bound
	pmoles/g original wet tissue wt
Corpus striatum	9.37 ± 0.86[a]
Cerebral cortex	6.67 ± 0.60[b]
Thalamus-midbrain	5.34 ± 0.74
Hippocampus	4.77 ± 0.75
Hypothalamus	4.24 ± 0.62
Medulla-pons	3.38 ± 0.46
Cerebellum	1.85 ± 0.37[c]

[a] Significantly different from all other regions; $p < 0.01$.

[b] Significantly different from medulla-pons; $p < 0.05$.

[c] Significantly different from all other regions except medulla-pons; $p < 0.05$.

taken together with the lesser potency ratio of (−)-norepinephrine to dopamine in this region (13) compared with the cerebral cortex, support the likelihood that a certain portion of [³H]DHE binding in the corpus striatum may involve dopamine receptors. Binding in cerebral cortical membranes, however, appears to be selectively associated with *alpha* noradrenergic receptors, as indicated by the close resemblance of relative drug activities at cortical [³H]DHE binding sites to those at sites labeled by [³H]epinephrine, [³H]norepinephrine, [³H]clonidine, and [³H]WB-4101 in brain membranes, and by [³H]-DHE in rabbit uterus. Involvement of dopamine receptors in the binding of low concentrations of [³H]DHE to striatal but not cortical membranes may be explained by the abundance of dopamine receptors in corpus striatum and negligible levels in cerebral cortex, as demonstrated by binding techniques using [³H]dopaminergic ligands (6, 34).

Our earlier findings that *alpha* agonists have preferential high affinity for [³H]clonidine and [³H]catecholamine binding sites and that antagonists compete most avidly for sites bound by [³H]WB-4101 suggest that [³H]agonists and [³H]WB-4101 label agonist and antagonist *alpha* receptor sites, respectively (1, 2, 4). This hypothesis is supported by the similar affinities of mixed agonist-antagonists for [³H]clonidine and [³H]WB-4101 sites (1, 2) and by the present findings that the affinities of agonists and antagonists for [³H]DHE binding sites are intermediate between their affinities for [³H]WB-4101 and [³H]clonidine binding sites. The differential affinities of agonists and antagonists for [³H]clonidine and [³H]WB-4101 binding sites and the logit-log slopes of 1.0 for agonists displacing [³H]WB-4101 and for antagonists displacing [³H]clonidine binding seem most consistent with the existence of discrete, noninterconvertible *alpha* receptor sites with preferential high affinity for agonists and antagonists, respectively. In such a system, competitive antagonism of the effects of *alpha* agonists could occur at the "antagonist" site in the presence of low concentrations of antagonist or at the "agonist" site in the presence of higher concentrations. The recent demonstration of a close correlation between affinity of *alpha* adrenergic agents for [³H]WB-4101 binding sites and influences on *alpha*-stimulated cyclic AMP accumulation in rat cerebral cortex (35) suggests that the "antagonist" (low agonist affinity) site may be responsible for eliciting the physiological receptor-mediated response. The physiological role of the agonist state is unclear. Because of its high affinity for agonists it might represent a desensitized state of the receptor (36–38). The suggestion that *alpha* noradrenergic agonists and antagonists act at nonidentical sites has been made previously, based on the dissimilarity of agonist and antagonist structures (39).

The present study of [³H]DHE binding provides additional data bearing upon a model for receptor functioning and on the relationship of the presumed agonist and antagonist binding sites. Assuming that the *alpha* receptor in brain membranes

exist in discrete, noninterconverting agonist and antagonist forms, there are several ways in which mixed agonist-antagonists such as DHE could interact with the receptor. Findings that relate to the nature of this interaction include the fact that the maximal number of [^{3}H]DHE binding sites is similar to the sum of [^{3}H]clonidine and [^{3}H]WB-4101 sites, and that mixed agonist-antagonists compete for [^{3}H]DHE binding with logit-log slopes close to 1.0, while slopes for agonists or antagonists reducing [^{3}H]DHE binding are significantly less than 1.0. We can conceive of several alternative mechanisms whereby mixed agonist-antagonists such as DHE may interact with the *alpha* receptor. (a) A single molecule of DHE might bind to an agonist and an antagonist site simultaneously. However, this would be inconsistent with the number of [^{3}H]DHE binding sites being greater than the number of [^{3}H]WB-4101 or [^{3}H]clonidine sites, and with the logit-log slopes of less than 1.0 for the inhibition of [^{3}H]DHE binding by agonists or antagonists. (b) DHE could bind to either interconverting agonist or antagonist "state" of the *alpha* receptor. Such an explanation for the action of mixed agonist-antagonists has been suggested previously in the case of the opiate receptor (40). In the present case, however, the unequal maximal number of [^{3}H]DHE, [^{3}H]agonist, and [^{3}H]-antagonist binding sites argues against this interpretation, although inhibition slopes of less than 1.0 might still be expected. (c) The model most consistent with our data is one in which a molecule of DHE can bind to a discrete, noninterconvertible agonist or antagonist site with out interfering with binding at the other type of site (Fig. 5). Inasmuch as an agonist or antagonist would be competing for the binding of [^{3}H]DHE to a combination of agonist and antagonist sites, the logit-log slopes for such competitors would be less than 1.0. In addition, the total number of DHE binding sites would be similar to the sum of clonidine and WB-4101 sites, as is found to be the case. The suggestion that *alpha* receptor agonist and antagonist binding sites are physically distinct entities is supported by the recent finding that the regional distribution of such sites in calf brain is different for [^{3}H]agonist and antagonist ligands (41).

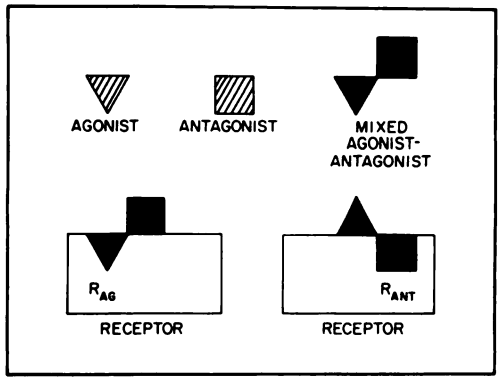

FIG. 5. *Model for alpha receptor binding of [^{3}H]DHE*

According to the model presented, agonists and antagonists bind to discrete, noninterconverting sites with preferential high affinity for agonists and antagonists, respectively. A mixed agonist-antagonist, such as DHE, can bind to either site. A fuller treatment of possible receptor models, and their relationship to the observed experimental data, is presented in the DISCUSSION.

The scheme offered here seeks to explain the actions of agonists, antagonists and mixed agonist-antagonists based on the interaction of such agents with discrete agonist and antagonist receptor sites. By such a formulation, derived from direct binding studies, it is possible to reconcile the all-or-none nature of an individual ligand-receptor interaction with the observed existence of compounds displaying different intrinsic activities (42).

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