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Regional Variations in *Alpha* Adrenergic Receptor Interactions of [^{3}H]-Dihydroergokryptine in Calf Brain: Implications for a Two-Site Model of *Alpha* Receptor Function

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SUMMARY

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The *alpha* adrenergic agonists [^{3}H]epinephrine and [^{3}H]norepinephrine, the antagonist WB-4101 (2-([2′,6′-dimethoxy]phenoxyethylamino)methylbenzodioxan), and the ergot alkaloid [^{3}H]dihydroergokryptine bind to membranes from various calf brain regions in a fashion that suggests a selective interaction with *alpha* adrenergic receptors. Binding sites for [^{3}H]*alpha* agonists appear to predominate in calf cerebellar cortex, while [^{3}H]WB-4101 sites are more plentiful in the pons. Similar numbers of [^{3}H]agonist and [^{3}H]antagonist sites are found in cerebral cortex. Properties of [^{3}H]dihydroergokryptine binding reflect the relative preponderance of [^{3}H]*alpha* agonist and [^{3}H]antagonist binding sites in these regions. In cerebral cortex, 300 nM (−)-norepinephrine, which completely inhibits binding of [^{3}H]agonists, and 300 nM indoramin, which affords 100% reduction of [^{3}H]antagonist binding, each decrease the maximal number of [^{3}H]dihydroergokryptine binding sites by about half. These agents in combination abolish binding entirely. In contrast, 300 nM (−)-norepinephrine, but not indoramin, eliminates binding to cerebellar membranes. In pons, the maximal number of [^{3}H]dihydroergokryptine binding sites is reduced 64% and 28%, respectively, by 300 nM indoramin and 300 nM (−)-norepinephrine. Compared with cerebral cortex values, the agonists clonidine and (−)-norepinephrine are more potent [^{3}H]dihydroergokryptine inhibitors in cerebellum and less potent in pons; for the antagonists WB-4101 and indoramin, this relationship is reversed. The apparent K_i values for these agents at [^{3}H]dihydroergokryptine binding sites in cerebellum and pons are similar to values obtained for cortical binding of [^{3}H]agonists and [^{3}H]WB-4101, respectively. These findings support the proposed existence of two discrete populations of *alpha* adrenergic receptors with respective high affinities for agonists and antagonists and differential regional distribution in the brain, both of which may be labeled by [^{3}H]dihydroergokryptine.

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INTRODUCTION

Several neurotransmitter receptors in the brain can be labeled by the binding of both radioactive agonists and antagonists. In some cases receptors appear to exist in two distinct but interconvertible states with selective high affinities for agonists or antagonists, respectively. Evidence favoring such a model of receptor functioning has been obtained in the case of dopamine (1), muscarinic cholinergic (2), and γ-aminobutyric acid (3, 4) receptors. In the case of the opiate receptor in the central nervous system[4] (5, 6) and *beta* adrenergic receptors in calf brain, frog erythrocytes, and rat lung (7, 8), radiolabeled agonists and antagonists appear to bind a single form of the receptor under sodium-free conditions.

Alpha adrenergic receptor sites in peripheral tissues and the brain can be labeled with a variety of tritiated ligands (9–12). [^{3}H]Epinephrine, [^{3}H]norepinephrine, and [^{3}H]clonidine label an *alpha* adrenergic site with selective high affinity for agonists and much lower affinity for antagonists, while the antagonist [^{3}H]WB-4101 (2-([2′,6′-dimethoxy]phenoxyethylamino)methylbenzodioxan) labels a site with selective high affinity for antagonists. [^{3}H]Dihydroergokryptine, although a pharmacological *alpha* receptor antagonist, appears to label both agonist- and antagonist-preferring sites (12).

Several lines of evidence suggest that the two *alpha* receptor binding sites are distinct and do not undergo interconversion. No evidence for apparent cooperativity has been obtained for inhibition of [^{3}H]agonist binding by unlabeled antagonists, and vice versa (9, 10). Regional variations in the binding of [^{3}H]agonists and [^{3}H]antagonists to *alpha* receptor sites differ strikingly (13). Sodium selectively reduces the binding of [^{3}H]agonists by an influence on the number of sites. However it alters neither [^{3}H]WB-4101 binding nor the potencies of agonists in competing for sites so labeled (14).

In regional studies in the calf brain, the cerebellum displays negligible binding of [^{3}H]WB-4101 despite the existence of substantial amounts of [^{3}H]epinephrine and [^{3}H]norepinephrine binding, suggesting that the cerebellum contains almost exclusively agonist-preferring sites. In contrast, the pons has a preponderance of [^{3}H]WB-4101 binding sites, while in the cerebral cortex similar proportions of agonist and antagonist binding can be demonstrated (13). In the present study, using [^{3}H]DHE,[5] which in the rat labels both agonist- and antagonist-preferring *alpha* receptor sites (12, 15), we have explored properties of *alpha* receptors in different calf brain areas and obtained further evidence supporting the concept of distinct *alpha* receptor sites.

MATERIALS AND METHODS

Calf brains were obtained at a local slaughterhouse and transferred in ice-cold 0.9% NaCl, and the cerebral cortex, cerebellum, and pons were removed on ice. Tissue was rapidly frozen in an acetone–Dry Ice bath and stored at −70°. Immediately prior to binding assays, tissue was thawed, homogenized in 20 volumes (w/v) of 0.05 M Tris-HCl buffer (pH 7.7 at 25°) with a Brinkmann Polytron PT-10, and centrifuged at 50,000 × *g* for 10 min. The resulting pellet was resuspended and centrifuged in the same manner, and the final pellet was homogenized in 46.5 volumes (w/v) of 0.05 M Tris-HCl buffer (pH 7.7 at 25°) and incubated for 15 min at 25° in the presence of 1 μM pargyline.

[^{3}H]Epinephrine, [^{3}H]norepinephrine, [^{3}H]WB-4101, and [^{3}H]DHE binding assays were performed as described previously (9, 10, 12). Either 5 nM (±)-[^{3}H]epinephrine (11 Ci/mmole) or 5 nM (−)-[^{3}H]norepinephrine (24.4 Ci/mmole) was added to an incubation volume of 1 ml containing 20 mg of tissue, original wet weight, 1 mM pyrocatechol, 0.1 mM disodium EDTA, and 10 μM dithiothreitol. Tubes were incubated at 25° for 60 min ([^{3}H]epinephrine) or 40 min ([^{3}H]norepinephrine) and rapidly filtered under vacuum through Whatman GF/B glass fiber filters, followed by washing with 16 ml of ice-cold buffer containing 1 mM pyrocatechol and 0.1% ascorbic acid. [^{3}H]WB-4101 binding was determined by incubating 0.2 nM [^{3}H]WB-4101 (11.7 Ci/mmole) with 20 mg of tissue, original wet weight, in a volume of 2 ml at 25° for

[4] I. C. Creese, R. Simantov, and S. H. Snyder, manuscript in preparation.

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

20 min. Tubes were filtered as described above, and the filters were washed with 10 ml of ice-cold buffer. For [^{3}H]DHE binding assays, 0.3 nM [^{3}H]DHE (24.1 Ci/mmole) was incubated with 20 mg of tissue, original weight, for 60 min at 25° in the dark. Incubation was terminated by the filtration procedure described above, followed by washing with 15 ml of buffer. In all cases filters were counted by liquid scintillation spectrometry in 10 ml of Formula 947 (New England Nuclear).

Specific binding was determined by subtracting from total binding in the absence of competitors that component of binding not inhibited by 1 μM oxymetazoline (for [^{3}H]epinephrine and [^{3}H]norepinephrine assays) or 0.1 mM (−)-norepinephrine (for [^{3}H]WB-4101 and [^{3}H]DHE assays). These agents produced the same maximal inhibition of binding observed in the presence of high concentrations of all other inhibitors examined. Using the above concentrations of [^{3}H]ligands, in cerebral cortex membranes specific binding of [^{3}H]epinephrine (800 cpm), [^{3}H]norepinephrine (1200 cpm), [^{3}H]WB-4101 (500 cpm), and [^{3}H]DHE (1000 cpm) represented 60–70% of total binding.

[^{3}H]Epinephrine, [^{3}H]norepinephrine, and [^{3}H]DHE were purchased from New England Nuclear; WB-4101 was provided by WB Pharmaceuticals and tritiated at New England Nuclear as previously described (9). Clonidine was obtained from Boehringer/Ingelheim; (−)-norepinephrine, from Sterling-Winthrop; and indoramin (3-[2-(4-benzamidopiperid-1-yl)ethyl] indole hydrochloride), from Wyeth. Other reagents were obtained from commercial sources.

RESULTS

In previous studies using rat brain membranes, [^{3}H]DHE appeared to label both agonist- and antagonist-preferring receptors (12, 15). The distribution of [^{3}H]DHE binding in some calf brain regions likewise is correlated with the sum of [^{3}H]agonist and [^{3}H]WB-4101 binding (Table 1). To determine whether [^{3}H]DHE in calf brain membranes labels two distinct sites, we also evaluated the saturation of [^{3}H]DHE binding in the presence and absence of the potent antagonist indoramin (16) or the agonist (−)-norepinephrine (Figs. 1–4). The concentration of indoramin selected was sufficient to occupy virtually all the [^{3}H]WB-4101 sites while producing negligible effects on the sites labeled by [^{3}H] agonists (9, 10). The concentration of (−)-norepinephrine selected (300 nM) similarly should occupy almost all the [^{3}H]agonist sites with no influence on [^{3}H]WB-4101 sites (9, 10).

In calf cerebral cortex membranes, specific binding of [^{3}H]DHE, in the absence of competitors, is saturable, with half-maximal binding at about 1.5 nM and maximal binding evident at 5–6 nM (Fig. 1). The potencies of drugs in inhibiting this specific binding closely resemble values determined previously in rat cerebral cortex (12), suggesting that this high-affinity, saturable component of [^{3}H]DHE binding in calf cortical membranes represents *alpha* receptor binding. The maximal number of [^{3}H]DHE sites in calf cerebral cortex (15–17

TABLE 1

Regional distribution of [^{3}H]alpha adrenergic ligand binding in calf brain

Standard binding assays for each ligand were performed as described in MATERIALS AND METHODS. Values presented are mean specific binding, expressed as picomoles per gram of original wet tissue weight and as a percentage of frontal cortex binding. As the ligands used have the same affinity in all regions of calf brain studied (13), specific binding at the single [^{3}H]ligand concentration examined is proportional to the maximal number of binding sites present in each region (B_{max}). Values given are the means of five to seven separate experiments, each performed in triplicate, whose results varied by no more than 20%.

Region	[^{3}H]Epinephrine		[^{3}H]Norepinephrine		[^{3}H]WB-4101		[^{3}H]DHE	
	pmoles/g	%	*pmoles/g*	%	*pmoles/g*	%	*pmoles/g*	%
Frontal cortex	3.9	100	2.9	100	2.3	100	2.8	100
Cerebellar cortex	2.3	59	2.0	69	0	0	1.5	54
Pons	0.6	15	0.5	17	1.1	48	1.0	36

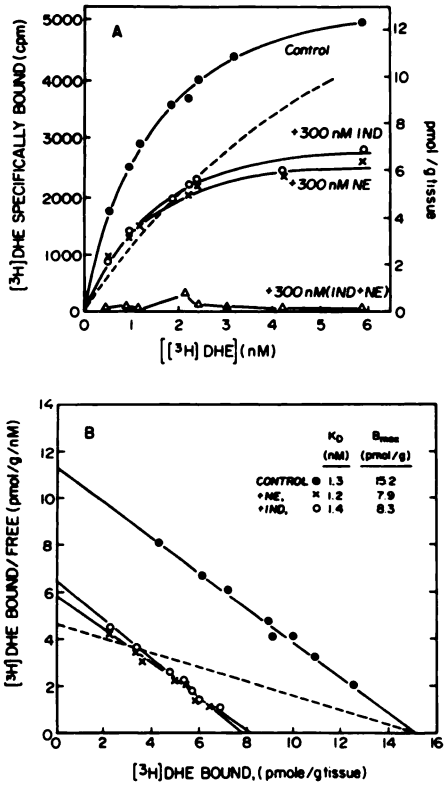

FIG. 1. *[^{3}H]DHE binding saturation in calf cerebral cortex*

Cerebral cortex homogenates (20 mg, original wet tissue weight) were incubated at 25° for 60 min, as described in MATERIALS AND METHODS, with various concentrations of [^{3}H]DHE. Specific binding was determined by subtracting from total binding that portion of binding not inhibited by 0.1 mM (−)-norepinephrine. At each [^{3}H]DHE concentration, specific binding was measured in the absence of inhibitors (●) and in the presence of 300 nM indoramin (IND) (○), 300 nM (−)-norepinephrine (NE) (×), and both 300 nM indoramin and 300 nM (−)-norepinephrine (△). Points shown are the means of triplicate assays in a single experiment. The experiment was replicated three times. The predicted theoretical curve for binding in the presence of 300 nM indoramin or 300 nM (−)-norepinephrine (- - -), assuming competitive inhibition at a homogeneous population of binding sites, was determined by a modification of the equation of Cheng and Prusoff (17). The predicted Scatchard line was derived by setting B_{max} equal to the value (15.2 pmoles/g) obtained for [^{3}H]DHE saturation in the absence of inhibitors, and calculating the apparent K_D of [^{3}H]DHE in the presence of 300 nM indoramin or (−)-norepinephrine as

$$K_{D\,app} = K_D\left(1 + \frac{[inhibitor]}{K_{i\,inhibitor}}\right)$$

where $K_D = K_D$ for [^{3}H]DHE in the absence of inhibitors obtained from saturation data, [inhibitor] = 300 nM, and $K_{i\,inhibitor} = K_i$ for indoramin or (−)-norepinephrine inhibition of [^{3}H]DHE binding (210 nM) (Table 2). Several points were taken from the resulting line and used to construct a binding curve. A. Specific binding. B. Scatchard plot, showing K_D = 1.3 nM and B_{max} = 15.2 pmoles/g for [^{3}H]DHE binding in the absence of inhibitors, and an apparent reduction in B_{max} without alteration of K_D in the presence of 300 nM indoramin or (−)-norepinephrine.

pmoles/g, wet weight) is equal to the sum of [^{3}H]agonist (norepinephrine, epinephrine, or clonidine) sites (8–9 pmoles/g) and [^{3}H]WB-4101 sites (8–9 pmoles/g). This finding accords with observations previously obtained in rat cerebral cortex membranes (12).

If a drug reduces the binding of a [^{3}H]ligand by competitive inhibition at the same binding sites, it should reduce the apparent affinity of the ligand with no alteration in the maximal number of sites bound. We constructed the theoretical curve describing this effect for [^{3}H]DHE saturation in the presence of 300 nM concentrations of the inhibitors indoramin and (−)-norepinephrine (Fig. 1). The experimental data obtained under such conditions, however, do not fit this predicted curve. Both indoramin and (−)-norepinephrine at 300 nM reduced the maximal number of [^{3}H]DHE binding sites (B_{max}) in calf cerebral cortex by about 50%, with no change in the apparent K_D of [^{3}H]DHE. In the presence of both 300 nM indoramin and 300 nM (−)-norepinephrine, there was no detectable [^{3}H]DHE specific binding within the range of concentrations examined (0.5–6 nM) (Fig. 1).

With 3.0 μM concentrations of (−)-norepinephrine or indoramin present, however, there was a 2-fold decrease in the apparent affinity of [^{3}H]DHE in saturation experiments, with no change in the B_{max} values of [^{3}H]DHE obtained in the presence of 300 nM concentrations of either drug (Fig. 2). This suggests that at concentrations greater than 300 nM, both drugs act as competitive inhibitors at the remaining site labeled by [^{3}H]DHE.

In calf cerebellum, where the apparent absence of [^{3}H]WB-4101 binding suggests that very few antagonist-preferring sites exist, the effects of 300 nM (−)-norepineph-

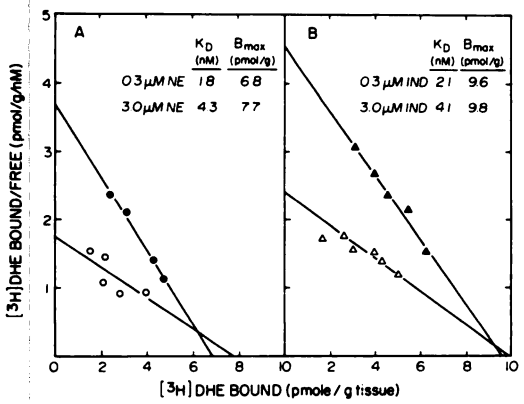

FIG. 2. *Scatchard plots of [^{3}H]DHE binding saturation in calf cerebral cortex in the presence of (−)-norepinephrine (A) or indoramin (B).*

A. ●, 300 nM norepinephrine (NE); ○, 3.0 μM. B. ▲, 300 nM indoramin (IND); △, 3.0 μM. Experiments were conducted as described for Fig. 1. Points shown are the means of triplicate assays in a single experiment.

rine and 300 nM indoramin on [^{3}H]DHE binding saturation differed from those observed in cerebral cortex (Fig. 3). Indoramin at 300 nM had no detectable influence on [^{3}H]DHE saturation parameters, whereas (−)-norepinephrine, both alone (300 nM) and combined with indoramin, eliminated [^{3}H]DHE specific binding over the entire concentration range examined.

Similar [^{3}H]DHE experiments were performed in membrane preparations from the calf pons, where there appear to be about twice as many sites labeled by [^{3}H]WB-4101 as sites labeled by [^{3}H]agonists (13). As was found in the cerebral cortex, the affinity of [^{3}H]DHE was unaffected by the presence of either 300 nM (−)-norepinephrine or 300 nM indoramin, but both drugs appeared to reduce the maximal number of sites bound by [^{3}H]DHE (Fig. 4). However, in this tissue, indoramin abolished binding to approximately two-thirds of the sites, whereas (−)-norepinephrine eliminated only one-third of the sites. The combination of both drugs, as in the cortex and cerebellum, eliminated all [^{3}H]DHE specific binding over the concentration range.

To explore further the possibility that the relative numbers of agonist- and antagonist-preferring sites in various calf brain regions differ, we evaluated the potencies of the agonists clonidine and (−)-norepinephrine and the antagonists WB-4101 and indoramin as inhibitors of the binding of the [^{3}H]agonists epinephrine and norepinephrine, the antagonist [^{3}H]WB-4101, and [^{3}H]DHE in calf cerebral cortex, cerebellum, and pons (Table 2). In all tissues examined, clonidine and (−)-norepinephrine were much more potent in competing for the binding of [^{3}H]agonists than [^{3}H]WB-4101, while the reverse situation held for the antagonists WB-4101 and indoramin.

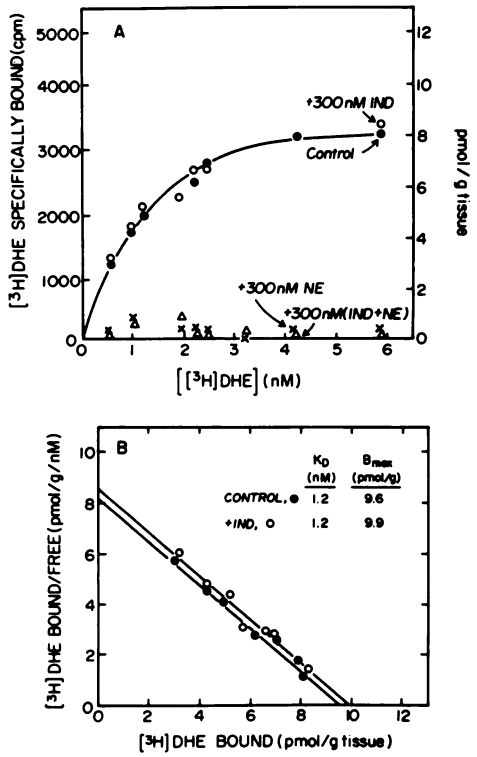

FIG. 3. *[^{3}H]DHE binding saturation in calf cerebellar cortex*

Cerebellar homogenates (20 mg, original wet tissue weight) were incubated at 25° for 60 min, as described in MATERIALS AND METHODS, with various concentrations of [^{3}H]DHE. Specific binding was defined as that component of binding inhibited by 0.1 mM (−)-norepinephrine. At each [^{3}H]DHE concentration, specific binding was measured in the absence of inhibitors (●) and in the presence of 300 nM indoramin (IND) (○), 300 nM (−)-norepinephrine (NE) (×), and both 300 nM indoramin and 300 nM (−)-norepinephrine (△). Points shown are the means of triplicate assays in a single experiment. The experiment was replicated three times. A. Specific binding. B. Scatchard plot, showing K_D = 1.2 nM and B_{max} = 9.6 pmoles/g and 9.9 pmoles/g for binding in the absence and presence of 300 nM indoramin, respectively.

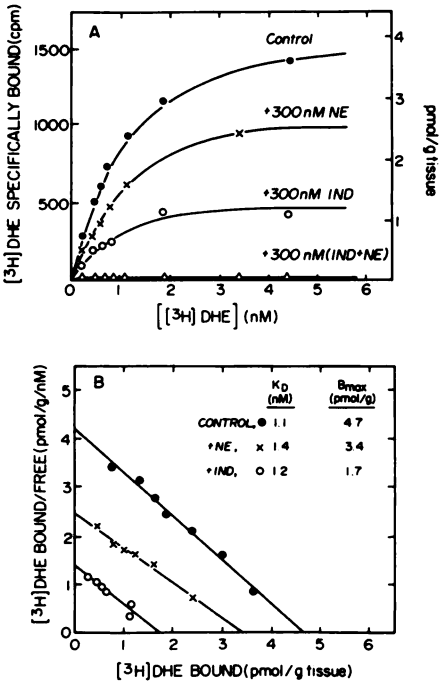

FIG. 4. *[³H]DHE binding saturation in calf pons*
Pontine homogenates (20 mg, original wet tissue weight) were incubated at 25° for 60 min, as described in MATERIALS AND METHODS, with various concentrations of [³H]DHE. Specific binding was defined as that component of binding inhibited by 0.1 mM (−)-norepinephrine. At each [³H]DHE concentration, specific binding was measured in the absence of inhibitors (●) and in the presence of 300 nM indoramin (IND) (○), 300 nM (−)-norepinephrine (NE) (×), and both 300 nM indoramin and 300 nM (−)-norepinephrine (△). Points shown are the means of triplicate assays in a single experiment. The experiment was replicated three times. A. Specific binding. B. Scatchard plot, showing K_D = 1.1 nM and B_{max} = 4.7 pmoles/g in the absence of inhibitors, and 64% and 28% reduction in apparent B_{max} in the presence of 300 nM indoramin and 300 nM (−)-norepinephrine, respectively, without alteration of K_D.

For [³H]epinephrine, [³H]norepinephrine, and [³H]WB-4101, there were no regional differences in the potencies of competing agents.

In the cerebral cortex, the potencies of clonidine, (−)-norepinephrine, WB-4101, and indoramin in reducing [³H]DHE binding were intermediate between their potencies in competing for the binding of [³H]agonists and [³H]WB-4101 (Table 2). In the cerebellum, however, where saturation experiments suggested that [³H]DHE binds only to agonist-preferring sites, the potencies of these drugs in reducing [³H]DHE binding were the same as their potencies in inhibiting the binding of the [³H]agonists epinephrine and norepinephrine. In contrast, in the pons, where the relative amounts of [³H]ligand binding and [³H]DHE saturation experiments suggest that antagonist-preferring sites predominate, the potencies of WB-4101, indoramin, clonidine, and (−)-norepinephrine in reducing [³H]DHE binding approached their potencies in competing for the binding of [³H]WB-4101.

These data are consistent with the concept that [³H]DHE labels both of two distinct *alpha* receptor sites with selective high affinity for agonists and antagonists, respectively. One would expect that in the presence of concentrations of indoramin that occupy all the antagonist-preferring sites in cerebral cortex, [³H]DHE should label only agonist-preferring sites. Similarly, in the presence of concentrations of (−)-norepinephrine that would saturate agonist-preferring sites, [³H]DHE should label only antagonist-preferring sites. When binding of [³H]DHE in cerebral cortex membranes was evaluated in the presence of 300 nM indoramin, the potency of clonidine was enhanced 26-fold, to a value comparable to its potency in competing for the binding of the [³H]agonists epinephrine and norepinephrine (Table 3). In contrast, in the presence of 300 nM indoramin, the potency of WB-4101 was reduced 10-fold, to a value similar to its potency in competing for binding for [³H]agonists. Conversely, when [³H]DHE binding in the presence of 300 nM (−)-norepinephrine was evaluated, the potencies of clonidine and WB-4101 tended to approximate their potencies in competing for [³H]WB-4101 sites (Table 3).

The shapes of the inhibition curves for the various drugs on the [³H]ligands also support the hypothesis that [³H]DHE labels distinct populations of receptors (Figs. 5 and 6). In the cerebral cortex, slopes for inhibition of [³H]DHE binding by the agonists clonidine and (−)-norepinephrine and the antagonists indoramin and WB-4101 are all relatively shallow, with log-logit slopes of approximately 0.5. In contrast, in the cerebellum, where only a single popu-

TABLE 2

Inhibition of [^{3}H]alpha adrenergic ligand binding in calf brain regions by alpha adrenergic agents

Standard binding assays for each ligand were performed as described in MATERIALS AND METHODS. IC_{50} values were determined by log probit analysis of percentage inhibition of specific binding, and apparent K_i values were calculated from the equation $K_i = IC_{50}/(1 + [[^3H]ligand]/K_D)$ (17). Values are means of three or more separate experiments, each performed in triplicate, whose results varied by no more than 30%.

Ligand and region	Apparent K_i			
	Clonidine	(−)-Norepinephrine	WB-4101	Indoramin
	nM	*nM*	*nM*	*nM*
[^{3}H]DHE				
Frontal cortex	62	210	1.3	210
Cerebellar cortex	2.6	21	19	7,700
Pons	134	584	0.3	10
[^{3}H]Epinephrine				
Frontal cortex	1.3	6.4	41	11,000
Cerebellar cortex	1.6	10	23	21,000
Pons	4.8	23	28	8,800
[^{3}H]Norepinephrine				
Frontal cortex	1.4	5.7	26	9,600
Cerebellar cortex		12		26,000
[^{3}H]WB-4101				
Frontal cortex	450	1,300	0.3	5.9
Pons	220	1,200	0.5	3.0

TABLE 3

Inhibition of [^{3}H]DHE binding in calf frontal cortex in the presence and absence of 300 nM (−)-norepinephrine and 300 nM indoramin

The standard [^{3}H]DHE binding assay was conducted as described in MATERIALS AND METHODS. IC_{50} values for clonidine and WB-4101 were determined by incubation with (a) [^{3}H]DHE alone, (b) [^{3}H]DHE plus 300 nM (−)-norepinephrine, and (c) [^{3}H]DHE plus 300 nM indoramin. At 300 nM, these agents completely inhibit [^{3}H]agonist and [^{3}H]antagonist binding, respectively (9, 10). Apparent K_i values were determined according to the equation given in Table 2. Values given are means ± standard errors of three to five separate experiments, each performed in triplicate.

Condition	Apparent K_i	
	Clonidine	WB-4101
	nM	*nM*
[^{3}H]DHE alone	62 ± 17	1.3 ± 0.7
[^{3}H]DHE + 300 nM (−)-norepinephrine	441 ± 55	0.2 ± 0.1
[^{3}H]DHE + 300 nM indoramin	2.4 ± 0.1	13 ± 4

lation of *alpha* receptors appears to exist, inhibition curves for all four drugs are steeper, with log-logit slopes of about 1.0, consistent with the existence of a single population of binding sites. In the pons, inhibition curves appear to involve two components, with the antagonists displaying high affinity for the majority of sites and agonists displaying selective high affinity for a smaller portion of the sites. Thus, in the pons, the agonists clonidine and (−)-norepinephrine inhibited about 20% of the binding with relatively high affinity while indoramin and WB-4101 inhibited the majority of specific binding with high affinity.

The possibility that 300 nM (−)-norepinephrine can fully occupy the agonist-preferring sites in the cerebral cortex, leaving only a single population of antagonist-preferring sites, is supported by the observation that inhibition curves for all agents were rendered steeper, with log-logit slopes of 1.0, in the presence of 300 nM (−)-norepinephrine. Inhibition curves were similarly affected in the presence of 300 nM indoramin.

DISCUSSION

In a detailed evaluation of the regional distribution of the binding of [^{3}H]WB-4101, [^{3}H]epinephrine, and [^{3}H]norepinephrine to *alpha* adrenergic receptors, differences in the relative amounts of binding of [^{3}H]agonists and antagonists in various calf brain regions were observed (13). In the present study we have attempted to repli-

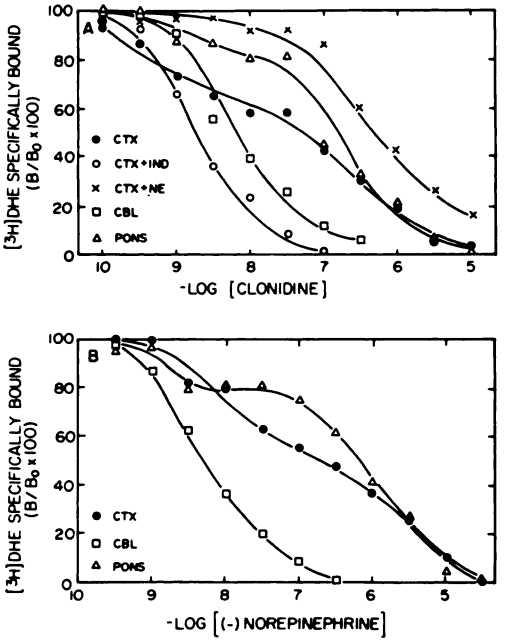

FIG. 5. *Inhibition of [^{3}H]DHE specific binding by alpha adrenergic agonists*

[^{3}H]DHE standard binding assays were performed as described in MATERIALS AND METHODS. Data shown are the means of triplicate assays in a single experiment. The experiment was replicated three times. A. Inhibition of binding by clonidine in cerebral cortex (CTX) (●), cerebral cortex incubated with 300 nM indoramin (IND) (○), cerebral cortex incubated with 300 nM (−)-norepinephrine (NE) (×), cerebellar cortex (CBL) (□), and pons (△). B. Inhibition of binding by (−)-norepinephrine in cerebral cortex (○), cerebellar cortex (□), and pons (△).

cate these observations in the areas that show the greatest difference between agonist and antagonist ligand binding and have also evaluated the binding of [^{3}H]DHE in these areas—frontal cerebral cortex, cerebellar cortex, and pons. As observed earlier, binding of [^{3}H]epinephrine and [^{3}H]norepinephrine in the frontal cerebral cortex tends to be about 1.5 times greater than binding in the cerebellum. The ratio of binding between cerebral cortex and cerebellum for [^{3}H]DHE is slightly higher, about 2.0. In contrast, although substantial binding levels of [^{3}H]WB-4101 are detected in the frontal cortex, no significant binding is observed in the cerebellum. [^{3}H]Epinephrine and [^{3}H]norepinephrine binding in the pons is only 15–17% of values in the frontal cerebral cortex, while [^{3}H]WB-4101 binding in the pons is half that in the frontal cortex. The comparable ratio for [^{3}H]DHE falls between the [^{3}H]agonist and [^{3}H]WB-4101 ratios. Our previous studies established that the regional variations in ligand binding estimated at a single concentration could be attributed to variations in the maximal number of binding sites, as the affinities of [^{3}H]ligands in the various brain regions were the same (13). This suggests that the cerebellum possesses negligible amounts of antagonist-preferring receptors, while in the pons there appear to be more antagonist than agonist sites, and the cerebral cortex possesses similar numbers of the two sites.

In rat brain membranes, [^{3}H]DHE appears to label both agonist- and antagonist-preferring receptors (12, 15). The maximal

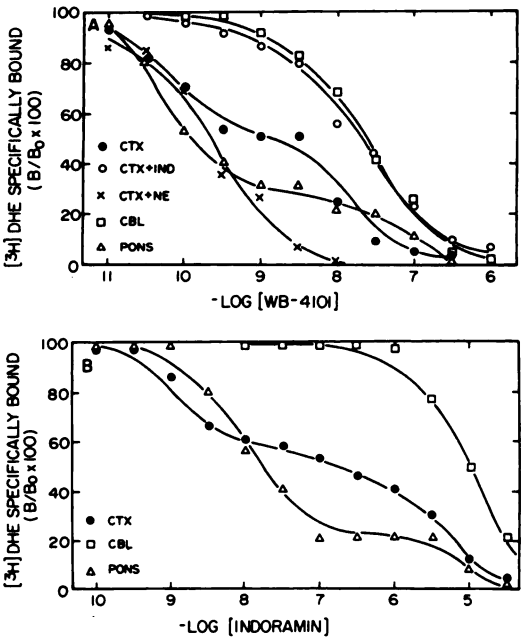

FIG. 6. *Inhibition of [^{3}H]DHE specific binding by alpha adrenergic antagonists*

[^{3}H]DHE standard binding assays were performed as described in MATERIALS AND METHODS. Data shown are the means of triplicate assays in a single experiment. The experiment was replicated three times. A. Inhibition of binding by WB-4101 in cerebral cortex (CTX) (●), cerebral cortex incubated with 300 nM indoramin (IND) (○), cerebral cortex incubated with 300 nM (−)-norepinephrine (NE) (×), cerebellar cortex (CBL) (□), and pons (△). B. Inhibition of binding by indoramin in cerebral cortex (●), cerebellar cortex (□), and pons (△).

number of [^{3}H]DHE binding sites is similar to the sum of the [^{3}H]agonist (clonidine, norepinephrine, or epinephrine) and [^{3}H]-WB-4101 binding sites. Moreover, the potencies of agonists and antagonists in inhibiting [^{3}H]DHE binding are intermediate between their potencies in competing for the binding of [^{3}H]agonists and [^{3}H]WB-4101 (12). Although *alpha* agonists and antagonists inhibit the binding of [^{3}H]agonists and [^{3}H]WB-4101 with log-logit slopes of about 1.0, their curves for inhibition of [^{3}H]DHE binding are shallow (log-logit slopes about 0.5), suggesting that the drugs displace [^{3}H]DHE from at least two sites for which the drugs have differential affinities (12).

The findings of the present study support the view that [^{3}H]DHE labels two distinct sites of roughly equal density in calf cerebral cortex, and that the calf cerebellum possesses only one of these sites, that which apparently prefers agonists. In the pons, as in the cortex, [^{3}H]DHE labels two sites, but the antagonist-preferring site appears to predominate. In analogy to the observed properties of other neurotransmitter receptor binding systems in the brain (1–6), the selective high affinities of agonists for one type of binding site, and of antagonists for the other, had suggested that the two *alpha* sites might represent distinct agonist- and antagonist-preferring conformations of a single *alpha* receptor. The finding that the relative proportions of [^{3}H]agonist and [^{3}H]antagonist sites vary in different brain regions suggests that the two sites might be distinct *alpha* receptor species comparable to the distinct subtypes of *beta* noradrenergic (18, 19), histamine (20), and dopamine (21) receptors observed in many tissues. In physiological studies of peripheral *alpha* noradrenergic responses, there is evidence for the existence of two distinct classes of *alpha* receptors, differing in relative agonist affinities. In inhibiting spontaneous contractions of rabbit gut, *alpha* agonists are about 100 times more potent than in altering contractions of rat vas deferens (22–24). The behavior of clonidine as an antagonist of *alpha* effects in some tissues (25, 26) and as an agonist in others (27–29) also favors the existence of distinct types of *alpha* receptors. Action of *alpha* adrenergic agonists and antagonists at different sites has previously been proposed, based upon their structural disparities (30).

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