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New detection of brain dopamine receptors with [^{3}H]dihydroergocryptine

(neuroleptics/ergots/neurotransmitters/norepinephrine/schizophrenia)

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ABSTRACT **Because dihydroergocryptine (DHE) and closely related ergots are dopamine-mimetic agonists, we tested [^{3}H]DHE as a possible ligand for [^{3}H]dopamine receptors in the calf caudate. In order to avoid [^{3}H]DHE from tagging α-adrenergic receptors, an excess of 500 nM phentolamine was used to block these sites, permitting the dopamine receptors to be measured separately. Specific binding of [^{3}H]DHE was defined as total binding minus that occurring in the presence of 1 μM (+)-butaclamol. Excess phentolamine reduced the specific binding of [^{3}H]DHE from 328 down to 138 fmol/mg, the difference presumably representing α-receptors. The K_D for [^{3}H]DHE was 0.55 nM (with or without phentolamine), and this high affinity site was blocked (in the presence of phentolamine) by 250 nM apomorphine, 650 nM dopamine, and 1200 nM (−)-norepinephrine, indicating that [^{3}H]DHE was binding to dopamine receptors. All neuroleptics blocked specific [^{3}H]DHE binding in direct relation to the clinical potency of the neuroleptic. The displacement of specific [^{3}H]DHE binding by dopamine or by norepinephrine (in the presence of phentolamine) revealed two subsets of dopamine receptors.**

Although it is now possible to use [^{3}H]dopamine, [^{3}H]apomorphine, and [^{3}H]haloperidol for identifying dopamine receptors in biological tissues (1–7), it is desirable to have additional radioligands in order to test the two-state agonist–antagonist receptor hypothesis (3, 5). Recently, [^{3}H]dihydroergocryptine ([^{3}H]DHE) has been introduced for detecting α-noradrenergic receptors (8–11), but it has also been stated that [^{3}H]DHE binds to serotonin receptors (ref 10; see ref. 12 on [^{3}H]ergotamine). Furthermore, Caron *et al.* (13, 14) have used [^{3}H]DHE as a specific dopamine ligand in rat pituitary tissue. Thus, because [^{3}H]DHE can bind to many types of neurotransmitter sites, previous results obtained by using [^{3}H]DHE on brain tissue (8–11) are difficult to interpret.

Caron *et al.* (13, 14) have found that DHE acts as a pure dopamine-like agonist on pituitary cells in culture. It is also known that 2-bromocryptine, which is chemically similar to DHE, has dopamine-like agonist activity on rat behavior (15), mimicks the effect of L-3,4-dihydroxyphenylalanine on patients with Parkinson disease (16), and reduces prolactin secretion in patients in a fashion similar to other dopamine-mimetic drugs (17).

These previous studies on the ergot compounds (13–17) indicate that it should be possible to use [^{3}H]DHE as a ligand for dopamine receptors in the brain, providing the right conditions can be established. Because the pituitary contains primarily dopamine receptors and few if any noradrenaline receptors, [^{3}H]DHE specifically binds to those sites (13, 14). In order to use [^{3}H]DHE to label dopamine receptors in brain tissue, however, it would first be necessary to block the adrenergic receptors. The present study indicates that this is possible. With excess phentolamine to block the α-adrenergic brain receptors, the binding of [^{3}H]DHE exhibits all the properties expected of a ligand for dopamine receptors. Furthermore, the displacement of [^{3}H]DHE by dopamine or norepinephrine reveals *in vitro* evidence for two types of dopamine receptors.

METHODS AND MATERIALS

Preparation of Calf Caudate Homogenates. The experiments were done on crude homogenates of calf caudate, prepared as described (6). In order to retain all the dopamine receptors, the homogenates were not purified or subfractionated. Calf brains were obtained fresh from the Canada Packers Hunnisett plant (Toronto, Canada). The caudates were removed within 2 hr after death, pooled, sliced into small cubes, and suspended in buffer at an approximate concentration of 50 mg of wet weight per ml of buffer. The buffer contained 15 mM Tris·HCl (pH 7.4), 5 mM Na_2EDTA, 1.1 mM ascorbate, and 12.5 μM nialamide. A preliminary crude homogenate of the suspension was made with a glass homogenizer with a Teflon piston (0.13–0.18 mm clearance). This piston, rotating at 500 rpm, was passed up and down 20 times in the homogenizer. The crude homogenate was first incubated at 37° for 60 min and then stored in 3-ml aliquots at −20° for future use. Before using, the samples were thawed, resuspended in the glass–Teflon homogenizer and homogenized by hand (10 up-and-down passes), and centrifuged at 39,000 × *g* for 15 min at 4°; the supernatant was discarded and the pellet was resuspended in 10 ml of buffer. The suspension was finally homogenized by a Polytron homogenizer (Brinkman Instrument Co.) at a setting of 7 (full range = 10) for 20 sec, by using a PT-10 homogenizer probe and a 50-ml polycarbonate tube to contain the suspension. Homogenization at settings higher than 7 led to a loss of protein through the glass fiber GF/B filters used in the radioreceptor assays. Except were indicated, the homogenates were always kept chilled on ice.

[^{3}H]DHE Binding Assays. The [^{3}H]DHE was purchased from New England Nuclear Corp. (Boston, MA) and used without further purification; the specific activity was 21 Ci/mmol. The material was stored in ethanol at −20°.

The [^{3}H]DHE binding assays were done in glass test tubes (12 × 75 mm), in which the following aliquots were placed (using Eppendorf Brinkmann pipettes with polypropylene tips): 0.2 ml of [^{3}H]DHE (final concentration ranging from 0.2 to 2 nM, but with the majority at 0.7 nM); 0.2 ml of brain homogenate (always added last and containing between 0.2 and 0.3 mg of protein); and 0.2 ml of buffer or 0.1 ml of buffer and 0.1 ml drug, or 0.1 ml of drug and 0.1 ml of another drug. Each determination was always done in sextuplicate. After the

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Abbreviations: DHE, dihydroergocryptine; IC_{50}, concentration of drug inhibiting specific [^{3}H]DHE binding by 50%.

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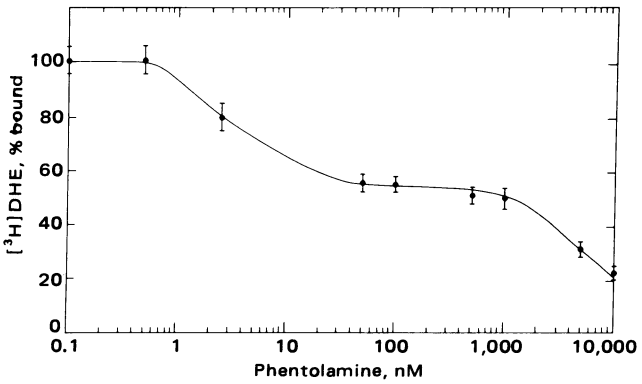

FIG. 1. Effect of phentolamine on the specific binding of 0.7 nM [^{3}H]DHE to calf caudate crude homogenate. The ordinate represents the percentage of the total (+)-butaclamol-displaceable radioactivity displaced at the given concentration of phentolamine. The curve is biphasic, one phase occurring between 0.5 nM and 50 nM and the other phase starting at 1 μM. The bars represent SEM.

samples was incubated for 60 min (22°), an aliquot of 0.5 ml was removed (polypropylene pipette tip) from the mixture and filtered under reduced pressure through a glass fiber filter (GF/B, Whatman; 24-mm diameter) using a Millipore stainless steel mesh support for the filter; the filtration took less than 1 sec. The filter was then washed twice with 7.5 ml of buffer per wash. The wash buffer was delivered by gravity from a syringe Re-pipette over a period of 4 sec. The filters were not blotted or dried but placed directly into liquid scintillation vials; 8 ml of Aquasol (New England Nuclear Corp.) was added and the samples were monitored for ^{3}H after they were stored at 4° for at least 6 hr to allow temperature equilibration and to permit the glass fiber filters to become uniformly translucent.

Specific binding of [^{3}H]DHE was defined as the total amount bound in the absence of (+)-butaclamol minus that bound in the presence of 1 μM (+)-butaclamol, in accordance with previous work on dopamine receptors, (1–7, 13, 14). The exact protein concentration in each homogenate was measured (4, 6) and the amount of [^{3}H]DHE specifically bound was expressed in terms of fmol of [^{3}H]DHE bound per mg of homogenate protein.

RESULTS

Properties of Specific [^{3}H]DHE Binding. As mentioned in the *Introduction*, in order to use [^{3}H]DHE as a ligand for dopamine receptors, it was first necessary to block α-adrenergic receptor sites. Phentolamine was chosen for this, and the effect of this α blocker on the specific binding of [^{3}H]DHE is shown in Fig. 1. These data indicate that [^{3}H]DHE was bound to two sites (at about 10 nM and about 1.5 μM) from which it could be displaced by phentolamine. The site at 10 nM was similar to that for phentolamine displacing the α-antagonist [^{3}H]WB-4101 (18), whereas that at 1.5 μM was similar to that for phentolamine displacing [^{3}H]haloperidol (19).

The specific binding of [^{3}H]DHE over a range of DHE concentrations in both the presence and absence of excess (500 nM) phentolamine is shown in Fig. 2*A*. This concentration of phentolamine was chosen because it saturated the high-affinity α-adrenergic sites (10 nM in Fig. 1) without inhibiting the binding of [^{3}]DHE to the low-affinity sites (1.5 μM in Fig. 1). A Scatchard analysis of these data (Fig. 2*B*) indicated that phentolamine had no effect on the dissociation constant of the high-affinity site for [^{3}H]DHE, because the K_D was 0.54 nM in the presence of phentolamine compared to 0.56 nM in the absence of phentolamine. Phentolamine did, however, reduce

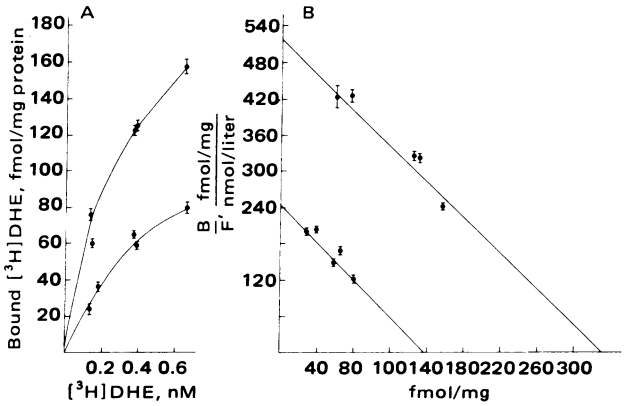

FIG. 2. Characteristics of (+)-butaclamol-specific [^{3}H]DHE binding to homogenate in the presence and absence of 500 nM phentolamine. (*A*) Adsorption isotherm for [^{3}H]DHE obtained by adding increasing concentrations of [^{3}H]DHE. The upper curve represents specific binding in the absence of 500 nM phentolamine, and the lower curve represents specific binding in the presence of 500 nM phentolamine. (*B*) Scatchard analysis of data in *A*. The left-hand curve represents binding in the presence of 500 nM phentolamine (K_D = 0.54 nM), and the right-hand curve represents binding in the absence of phentolamine (K_D = 0.56 nM). The bars represent SEM.

the total number of sites for [^{3}H]DHE binding from 328 down to 138 fmol/mg, the difference presumably representing the blockade of the α-adrenergic sites.

Effects of Agonists on Specific [^{3}H]DHE Binding. Fig. 3 illustrates the displacement of [^{3}H]DHE by dopamine, norepinephrine, and epinephrine. The order of potency (apomorphine > dopamine > norepinephrine > epinephrine) was the same as that found for the effect of agonists on [^{3}H]haloperidol binding and represented the order of potency expected of a dopamine receptor (4, 5). The biphasic nature of the dopamine and norepinephrine data strongly suggests that [^{3}H]DHE (in the presence of 500 nM phentolamine) bound to two sites, high- and low-affinity receptor sites for dopamine.

Effects of Neuroleptics on Specific [^{3}H]DHE Binding. The displacement by various neuroleptics on the specifically bound [^{3}H]DHE is shown in Fig. 4 and Table 1, all experiments being done in the presence of 500 nM phentolamine. The order of potency (haloperidol > pimozide > chlorpromazine > promazine) was identical to that found for neuroleptic inhibition of binding of [^{3}H]haloperidol (4). Fig. 5 shows the correlation between these neuroleptic IC_{50} values (i.e., concentrations

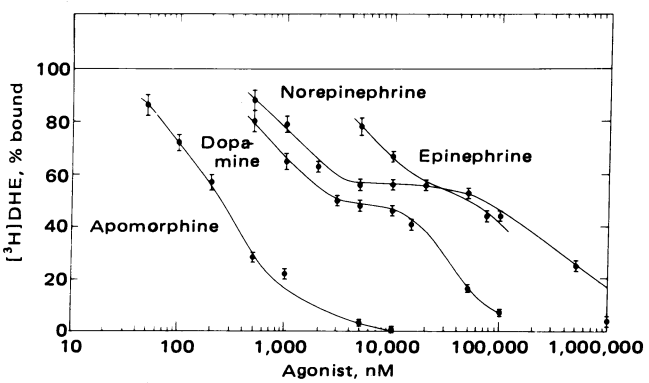

FIG. 3. Effect of various agonists on (+)-butaclamol-specific [^{3}H]DHE binding in the presence of 500 nM phentolamine (see Table 1). Dopamine and (−)-norepinephrine produced biphasic curves, whereas apomorphine produced a monophasic curve. The epinephrine data were insufficient to determine if the curve was monophasic or biphasic. The bars represent SEM.

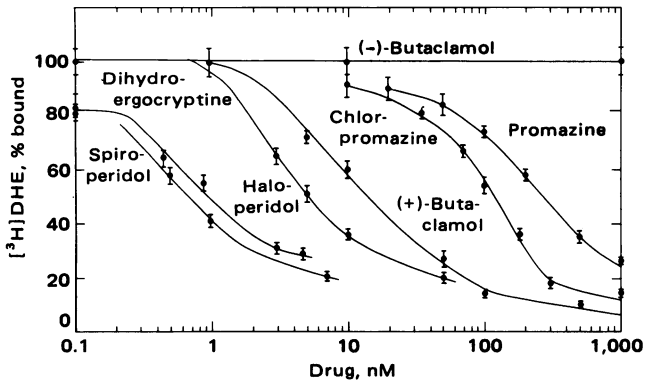

FIG. 4. Effect of various neuroleptic drugs on the (+)-butaclamol-specific component of [3H]DHE binding in the presence of 500 nM phentolamine in calf caudate homogenate (see Table 1). The bars represent SEM.

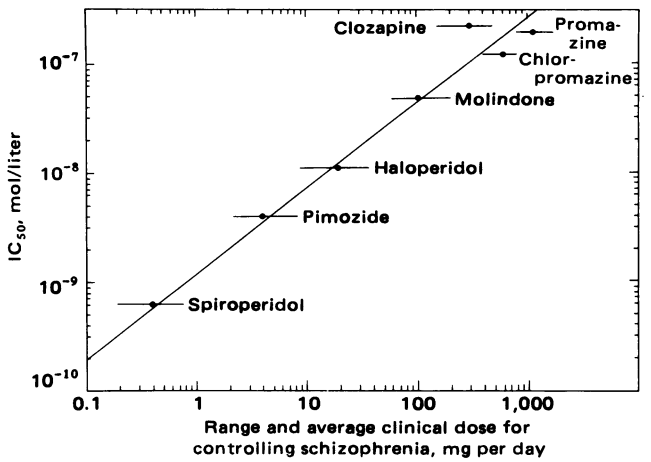

FIG. 5. Correlation of IC_{50} values of neuroleptic drugs on (+)-butaclamol-specific [3H]DHE binding, in the presence of 500 nM phentolamine, with the range and average clinical doses for controlling schizophrenia.

inhibiting specific [3H]DHE binding by 50%) versus the average clinical doses used for neuroleptics. The correlation was similar to that obtained with specific [3H]haloperidol binding (4).

DISCUSSION

These results indicate that [3H]DHE, in the presence of 500 nM phentolamine, binds to a population of receptors with identical properties to those for [3H]haloperidol; the binding is saturable, has a low K_D (0.5 nM), shows strict stereospecificity for both agonists and antagonists, has an order of potency for neuroleptics identical to that of [3H]haloperidol, and shows an order of potency for agonists characteristic of the dopamine receptor.

Davis *et al.* (10) reported [3H]DHE binding to serotonin receptors in the calf caudate with a K_D of 8 nM. It has been found (11) that, at lower concentrations of [3H]DHE, α-adrenergic receptors were apparently first saturated with a K_D of 0.7 nM by [3H]DHE. The data of this present study indicate that, in the caudate, [3H]DHE binds to both dopamine and α-adrenergic sites with equal affinity. Thus, it would appear that, unless excess phentolamine is used, [3H]DHE will tag receptors for both dopamine and noradrenaline and the order of potencies for various agonists and antagonists will be anomalous.

The K_D values for the neuroleptics and agonists on specific [3H]DHE binding in the calf caudate (in the presence of 500 nM phentolamine) are almost identical to those values found by Caron *et al.* (13, 14) using specific [3H]DHE binding in the pituitary to label dopamine receptors. Because the pituitary primarily contains dopamine receptors and few if any other catechol receptors (13, 14), the present data indicate that the dopamine receptors in the pituitary and the caudate are very similar.

Table 1. Inhibition of specific [3H]DHE binding in the presence of 500 nM phentolamine

	IC_{50}, nM
Neuroleptics	
Spiroperidol	0.7
Pimozide	2.4
α-Flupenthixol	3
(+)-Butaclamol	5.2
Haloperidol	16
Molindone	50
Chlorpromazine	100
Clozapine	200
Promazine	275
Agonists	
DHE	1
Apomorphine	250
Dopamine	650; 33,000 (biphasic)
(−)-Norepinephrine	1,200; 450,000 (biphasic)

The biphasic nature of dopamine (as well as norepinephrine) in displacing specific [3H]DHE binding (in the presence of 500 nM phentolamine) strongly suggests the presence of two types of dopamine receptors in the calf caudate. A careful examination of more such displacement data, with other radioligands, may also reveal the presence of two dopamine receptors in the caudate. Apparently the neuroleptics (i.e., [3H]haloperidol) have a similar affinity for both dopamine receptors, making the separate resolution of such sites difficult if one uses radioactive neuroleptics.

Thus, this appears to be *in vitro* evidence for two dopamine receptors, although clinical and behavioral experiments have long indicated the presence of two dopamine receptors in the caudates of humans (20), rats (21), mice (22), and cats (23). Separate characterization of each of these two dopamine receptors requires further study.

The neuroleptic IC_{50} values obtained with [3H]haloperidol are about 20% of those obtained by using [3H]DHE. One possible explanation for this is that the [3H]DHE induces a conformation of the receptor for which the neuroleptics have less affinity.

In conclusion, [3H]DHE possesses excellent physical properties for use as a radioligand for assaying dopamine receptors. It is not easily washed from the receptor when specifically bound and thus gives a highly reproducible measurement for dopamine receptors in the presence of 500 nM phentolamine.

Note Added in Proof. Isoproterenol, propranolol, or serotonin (all at 1 μM) did not affect [3H]DHE binding.

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