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PHARMACOLOGY OF [^{3}H]APOMORPHINE BINDING TO BOVINE BRAIN TISSUE

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Abstract—Activity against high-affinity (nM), and over 80 per cent dopamine (DA)-agonist-displaceable binding of [^{3}H]apomorphine (APO) to a "subsynaptosomal" membrane fraction of bovine caudate nucleus was evaluated using amino-6,7-dihydroxytetralin (ADTN) as a blank. Aporphines with 10,11-catechol and small *N*-alkyl groups were potent inhibitors ($IC_{50} < 25$ nM) with parallel inhibition curves ($n_H = 0.8$ to 1.0). For phenethylamines, catechol and amine moieties were important structural requirements; α or β substitution markedly reduced potency. Thus, epinephrine and isoproterenol were weak while DA and its *N*-methylated congeners were potent; (−)norepinephrine (NE) and fluoro-DAs were intermediate in potency and (+)NE, other catechols and hydroxylated phenethylamines were virtually inactive; *m*-tyramine, while weak, was more active than *p*-tyramine. Other DA agonists [ADTN > lergotrile > *N*-*n*-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP) > bromocriptine] were active. Neuroleptics competed relatively weakly, with imperfect correspondence to *in vivo* activities. Stereoselectivity was found with several aporphines, phenethylamines, and antipsychotic drugs. Many other neuropharmacologically active agents, including inhibitors of amine uptake and adrenergic receptors, were inactive. These characteristics strongly suggest that APO interacts with a DA agonist binding site in mammalian brain tissue.

In recent attempts to label putative dopamine (DA) receptors, use of tritiated apomorphine (APO) has been reported by several groups, usually working with crude homogenates of brain tissue which provide relatively low proportions of displaceable binding [1–3]. We have found that the binding of low (nM) concentrations of [^{3}H]APO exhibits many characteristics expected of selective and saturable occupation of specific binding sites in bovine and rodent brain tissue [4–7]. When a partially purified "subsynaptosomal" membrane preparation (P_4) was employed, the proportion of selective or saturable binding exceeded 80 per cent of the total counts of [^{3}H]APO bound. Kinetic analysis of this binding indicated the presence of a high-affinity component with an apparent K_d in the low nM range. The binding was dependent on temperature and pH, was decreased by GTP, and was not altered by physiologic concentrations of cations.

We now report half-maximally effective concentrations (IC_{50}) of various substances that might compete with the binding of [^{3}H]APO, as defined by the addition of excess amino-6,7-dihydroxytetralin (ADTN). These agents included a series of aporphines, the pharmacologic activity of many of which has been reported, as well as some which have not been available previously. In addition, a series of phenethylamine derivatives or analogs and other neuropharmacologically active substances was included to help evaluate the possibility that the high-affinity binding of [^{3}H]APO might manifest structure–activity characteristics expected of an agonist binding site possibly associated with at least one type of DA receptor.

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MATERIALS AND METHODS

Assay methods. Displaceable binding of [^{3}H]APO to calf caudate tissue was evaluated using techniques described elsewhere [4–7]. Briefly, caudate nucleus tissue was dissected from protease-free calf brains within 15 min of removal from the animal and placed on dry ice, pooled, stored at −70° overnight, and homogenized the following day in 0.32 M sucrose at 4° with a glass Potter–Elvehjem homogenizer and a loose-fitting (0.25 mm clearance) Teflon pestle. Homogenates were centrifuged at 1000 *g* for 10 min, and the supernatant fraction (S_1) was centrifuged at 20,000 *g* for 20 min. The resulting pellet (P_2, a crude mitochondrial/synaptosomal fraction) was resuspended, hypo-osmotically shocked in water, and further disrupted in a Polytron (Brinkmann Instruments, Westbury, NY). This homogenate was centrifuged at 8000 *g* for 20 min. The loose intermediate layer of tissue ("buffy coat") was resuspended in a small volume of the supernatant fraction (S_3), combined with the remaining S_3, and finally centrifuged at 48,000 *g* for 20 min. The final pellet (P_4) was resuspended in water; aliquots of 2.0 ml were frozen at −20°. Immediately prior to each assay, an aliquot of frozen P_4 tissue was thawed and washed twice with 10 ml of 50 mM Tris–HCl (pH

7.7), recovered by centrifuging at 48,000 *g*, and resuspended in 5 vol. of assay buffer consisting of 50 mM Tris–HCl, 5 mM Na_2EDTA, 0.1% (w/v) Na-ascorbate, and 12.5 μM nialamide (final pH 7.5). This preparation was given a final homogenization with the Polytron, and transferred to assay tubes.

In the [^{3}H]APO binding assay, the following components were added (1.0 ml, final volume):

(a) 0.25 ml of the assay buffer (to provide a final pH of 7.5), with or without addition of compounds to be tested;
(b) 0.25 ml of the same buffer, with or without the unlabeled amino-6,7-dihydroxytetralin [(±)ADTN], to yield a final concentration of 10 μM, used to define nondisplaceable or "nonspecific" binding;
(c) 0.25 ml of [^{3}H]APO in the assay buffer to yield a final concentration of 0.5 nM;
(d) 0.25 ml of membrane preparation (P_4) in assay buffer, added just prior to incubation (typically containing 0.2 to 0.4 mg protein [8]).

The assay incubation was carried out in disposable 13 × 100 mm borosilicate glass culture tubes (VWR Scientific, Boston, MA), cleaned with an air stream passed through spun glass. Assays were started by adding the membrane preparation and moving the tubes from an ice bath to a slowly agitated 25° water bath for 60 min. Incubation was terminated by adding 5 ml of ice-cold Tris buffer to each tube and rapidly filtering over 2.5 cm diameter Whatman (GF/B) glass fiber filters held in a Millipore (Millipore Co., Bedford, MA) manifold. Filters were washed twice with an additional 5 ml of the cold buffer, placed in polypropylene scintillation counting vials and counted for tritium in a liquid scintillation counter (Tri-Carb, Packard Instruments, Downers Grove, IL) at *ca.* 40 per cent efficiency.

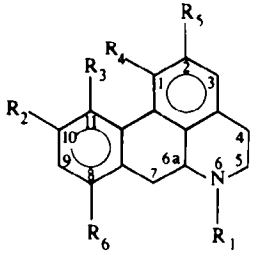
Materials. [8,9-^{3}H]Apomorphine–HCl ([^{3}H]APO, 38–40 Ci/mmole) was obtained from the New England Nuclear Corp., Boston, MA. Recovery of [^{3}H]APO after incubation was evaluated chromatographically and was found to yield a single radioactive peak co-chromatographic with authentic labeled and unlabeled APO, all as described elsewhere [3, 4]. (−)*O,O'*-Dipivaloylapomorphine–HCl, (−)*O,O'*-dibenzoylapomorphine, (−)10,11-methylenedioxyaporphine, and *N,N*-dimethyldopamine were donated by Dr. Robert Borgman of Arnar-Stone Laboratories, McGraw Park, IL. Apomorphine quinone was donated by Professor Robert C. Smith of the University of Texas, Austin, TX. All other aporphine derivatives were prepared at the Department of Medicinal Chemistry of Northeastern University, Boston, MA, by Dr. Say-Jong Law and Professor John L. Neumeyer, and were donated by Professor Neumeyer. Fluorodopamines were provided by Drs. Kenneth Kirk and John Daly, NIH, Bethesda, MD). *N-n*-Propyl-3-(3-hydroxyphenyl)-piperidine-HBr (3-PPP) was provided by Professor Arvid Carlsson of Göteborg, Sweden; ergolines were donated by Sandoz Laboratories, Basel, Switzerland. Neuroleptic agents were donated as follows: (+) and (−)butaclamol (Ayerst Laboratories, New York, NY); chlorpromazine–HCl and trifluoperazine–HCl (Smith, Kline & French, Philadelphia, PA); clozapine (Sandoz Laboratories); haloperidol (McNeil Laboratories, Fort Washington, NY); fluphenazine–HCl (E. R. Squibb & Sons, New Brunswick, NJ); perphenazine (Schering Corp., Bloomfield, NJ); pimozide and domperidone (Janssen Pharmaceutica, Beerse, Belgium); and (±)sulpiride (Societé d'Études Scientifiques et Industrielles de l'Ile de France, Paris, France); (+) and (−)sulpiride were provided by Professor G. U. Corsini of the University of Cagliari, Italy. Miscellaneous drugs were donated as follows: amitriptyline–HCl, (+)amphetamine sulfate, benztropine mesylate, (+) and (−)α-methyldopamine-HCl and (±) metaraminol bitartrate (Merck Laboratories, West Point, PA); desipramine–HCl (Merrell-National Laboratories, Cincinnati, OH); imipramine–HCl and phentolamine–HCl (CIBA–Geigy Corp., Summit, NJ); naloxone (Endo Laboratories, Garden City, NY); nomifensine maleate (Hoechst-Roussel Laboratories, Paris, France); (+)norepinephrine–HCl (Sterling–Winthrop Laboratories, Rensselaer, NY); phenoxybenzamine–HCl (Smith, Kline & French); and propranolol–HCl (Ayerst Laboratories). Authentic (−)-apomorphine–HCl-1/2H_2O was obtained from MacFarlan-Smith (Edinburgh, U.K.); the aminotetralin, (±)amino-6,7-di-hydroxy-1,2,3,4-tetrahydronapthalene-HBr (ADTN, recently prepared and purified) was from Burroughs–Wellcome (Research Triangle, NC). Other drugs and chemicals were obtained from commercial sources in the highest available purity.

RESULTS

The characterization of [^{3}H]APO binding to the P_4 fraction of calf caudate has been presented elsewhere [3, 4]. The P_4 fraction improved the proportion of specific:total cpm bound, as well as the amount bound per mg protein, by several-fold over cruder fractions of tissue. The assay employed 0.5 nM [^{3}H]APO with 10 μM ADTN added as a blank to define specific binding; less than 5 per cent of total free cpm was bound. ADTN produced a monophasic inhibition of binding of [^{3}H]APO that was nearly complete at 0.1 μM and unchanged between 1.0 and 10 μM. Binding was protein dependent and demonstrated a pH optimum of 7.0 to 7.5 and a temperature optimum between 20 and 25° (with pH controlled). Equilibration was complete in 60 min at 20–25°. Kinetic characteristics obtained from association and dissociation constants were very similar to those from saturation isotherms [6, 7]. The apparent K_d was 0.45 nM, and the apparent B_{max} was 270 fmoles/mg protein, as calculated by Scatchard [9] analysis ($r = 0.99$).

Illustrative examples of typical inhibition curves for several aporphines, phenethylamine analogs, ADTN, and neuroleptics are provided in Figs. 1–3. All of these compounds yielded highly linear log-probit plots, the linear correlation coefficients (r) of which were all greater than 0.95. In competition with 0.5 nM [^{3}H]APO, unlabeled APO yielded an IC_{50} of 1.0 nM (compound **1**, Table 1); K_i was 0.47 nM ($K_d = 0.45$ nM) as computed according to Cheng and Prusoff [10]. The Hill coefficient [11], n_H, was 0.95 for concentrations of APO between 0.5 and 20 nM.

Table 1. Inhibition of binding of [³H]apomorphine by aporphines*



Position substituted

Compounds	R_1(N)	R_2(10)	R_3(11)	R_4(1)	R_5(2)	R_6(8)	IC_{50} (nM)
1 (−)Apomorphine–HCl	CH_3	OH	OH	H	H	H	1.0
2 (−)10,11-Dihydroxy-*N*-*n*-propylnorapomorphine–HCl (−NPA)	$CH_2CH_2CH_3$	OH	OH	H	H	H	2.5
3 (±)10,11-Dihydroxy-*N*-*n*-propylnorapomorphine HI (±NPA)	$CH_2CH_2CH_3$	OH	OH	H	H	H	5.0
4 (−)2,10,11-Trihydroxyaporphine	CH_3	OH	OH	H	OH	H	10.0
5 (−)2,10,11-Trihydroxy-*N*-allylnoraporphine–HBr	$CH_2CH{=}CH_2$	OH	OH	H	OH	H	24.0
6 (−)2,10,11-Trihydroxy-*N*-ethylnoraporphine–HBr	CH_2CH_3	OH	OH	H	OH	H	25.0
7 (−)2,10,11-Trihydroxy-*N*-*n*-propylnoraporphine–HBr	$CH_2CH_2CH_3$	OH	OH	H	OH	H	25.0
8 (−)10,11-Dihydroxy-*N*-hydroxyethylnoraporphine–HCl	CH_2CH_2OH	OH	OH	H	H	H	25.0
9 (−)2,10,11-Trihydroxynoraporphine–HBr	H	OH	OH	H	OH	H	30.0
10 (±)11-Hydroxy-*N*-*n*-propylnoraporphine–HI	$CH_2CH_2CH_3$	H	OH	H	H	H	36.0
11 (+)Bulbocapnine	CH_3	CH_3O	OH	O—	OCH_2—	H	70.0
12 (±)10-Hydroxyaporphine–HBr	CH_3	OH	H	H	H	H	112
13 (−)2,10,11-Trihydroxy-*N*-Propynylnoraporphine	$CH_2{\equiv}CH$	OH	OH	H	OH	H	143
14 (−)Morphothebaine	CH_3	OCH_3	OH	H	OH	H	204
15 (−)*N*-Chloroethyl-norapomorphine–HCl (NCA)	CH_2CH_2Cl	OH	OH	H	H	H	394
16 (±)10-Hydroxy-*N*-*n*-propylnoraporphine–HI	$CH_2CH_2CH_3$	OH	H	H	H	H	700
17 (−)10,11-Diacetoxy-*N*-chloroethylnoraporphine	CH_2CH_2Cl	$OCOCH_3$	$OCOCH_3$	H	H	H	1,060
18 (−)10,11-Methylenedioxyaporphine–HCl	CH_3	OCH_2—	O—	H	H	H	1,310
19 (−)Apocodeine	CH_3	OCH_3	OH	H	H	H	2,400
20 (+)2,10,11-Trihydroxyaporphine–HBr	CH_3	OH	OH	H	OH	H	≥5,000
21 (±)*N*-*n*-propylnorapocodeine–HCl	$CH_2CH_2CH_3$	OCH_3	OH	H	H	H	≥5,000
22 (±)10-Methoxyaporphine–HCl	CH_3	OCH_3	H	H	H	H	≥5,000
23 (−)*N*-Chloroethyl-2,11-dihydroxy-10-methoxy-noraporphine	CH_2CH_2Cl	OCH_3	OH	H	OH	H	≥5,000
24 (−)*N*-Chloroethyl-2,11-diacetoxy-10-methoxyaporphine	CH_2CH_2Cl	OCH_3	$COOCH_3$	H	$COOCH_3$	H	≥5,000
25 (−)2,11-Diacetoxy-10-methoxynoraporphine	H	OCH_3	$COOCH_3$	H	$COOCH_3$	H	≥5,000
26 (±)8-Hydroxy-*N*-*n*-propylnoraporphine–HI	$CH_2CH_2CH_3$	H	H	H	H	OH	>10,000
27 (−)*N*-Chloroethylnorapocodeine–HCl	CH_2CH_2Cl	OCH_2	OH	H	H	H	>10,000
28 (−)*O*,*O*′-Dipivaloylapomorphine–HCl	CH_3	$OCOC(CH_3)_3$	$OCOC(CH_3)_3$	H	H	H	>10,000
29 (−)*O*,*O*′-Dibenzoylapomorphine	CH_3	$OCOC_6H_5$	$OCOC_6H_5$	H	H	H	>10,000
30 (−)Apomorphine–quinone	CH_3	=O	=O	H	H	H	>10,000

* Competition by aporphines for the binding of ³H-labeled apomorphine (APO) (0.5 nM) was evaluated with the P_4 fraction of calf caudate homogenates as described in Materials and Methods. Specific binding was defined by use of 6,7-ADTN (10 μM) as a blank to displace [³H]APO. Values for IC_{50} were calculated by log-probit analysis of data from at least triplicate determinations with at least four concentrations of each compound; the S.D. was less than ± 5 per cent of each mean value.

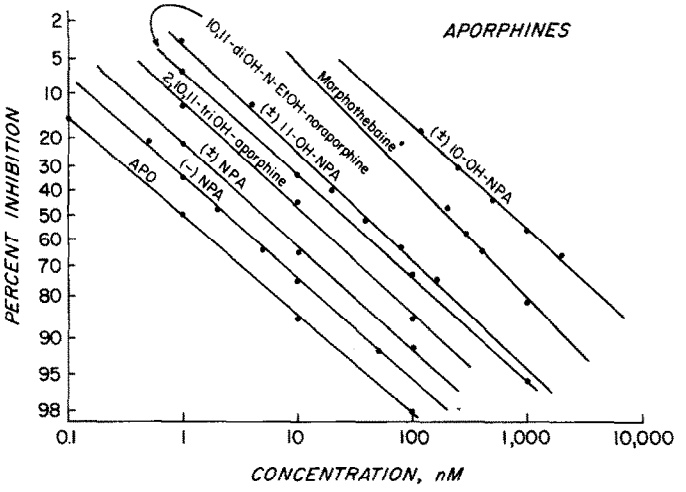
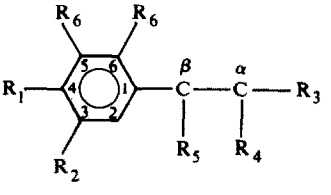


Fig. 1. Inhibition of binding of [^{3}H]apomorphine (APO) by aporphines. Data are presented for representative aporphines shown in Table 1, plotted in log-probits as per cent inhibition of binding of 0.5 nM [^{3}H]APO (with or without 10 μM ADTN to define specific binding) versus the molar concentration of inhibitor on a logarithmic scale. The compounds shown are, from left to right: (−)apomorphine; (−)10,11-dihydroxy-*N*-*n*-propylnorapomorphine (−NPA); (±)10,11-dihydroxy-*N*-*n*-propylnorapomorphine (±NPA); (−)2,10,11-trihydroxyaporphine; (−)10,11-dihydroxy-*N*-hydroxyethylnoraporphine; (±)11-hydroxy-*N*-*n*-propylnoraporphine; (−)morphothebaine; and (±)10-hydroxy-*N*-*n*-propylnoraporphine. Data are means for at least three separate determinations with at least four concentrations of each compound (S.D. < ± 5 per cent).

Table 2. Inhibition of binding of [^{3}H]apomorphine by phenethylamine derivatives*



Compound	Position substituted						
	R_1(4)	R_2(3)	R_3(α)	R_4(α)	R_5(β)	R_6(5, 6)	IC_{50} (nM)
N-Methyldopamine (epinine)	OH	OH	$NHCH_3$	H_2	H_2	H	3.4
Dopamine	OH	OH	NH_2	H_2	H_2	H	4.5
N,N-Dimethyldopamine	OH	OH	$N(CH_3)_2$	H_2	H_2	H	13.0
(−)Norepinephrine	OH	OH	NH_2	H_2	H, OH	H	50.0
5-Fluorodopamine	OH	OH	NH_2	H_2	H_2	F, H	50.0
6-Fluorodopamine	OH	OH	NH_2	H_2	H_2	H, F	61.0
(−)α-Methyldopamine	OH	OH	NH_2	H, CH_3	H_2	H	280
(+)Norepinephrine	OH	OH	NH_2	H_2	H, OH	H	590
m-Tyramine	H	OH	NH_2	H_2	H_2	H	825
(+)α-Methyldopamine	OH	OH	NH_2	H, CH_3	H_2	H	1,800
(−)Epinephrine	OH	OH	$NHCH_3$	H_2	H, OH	H	≥10,000
(−)Isoproterenol	OH	OH	$NHCH(CH_3)_2$	H_2	H, OH	H	≥10,000
(±)Metaraminol	H	OH	NH_2	H, CH_3	H, OH	H	>10,000
(±)*p*-Hydroxynorephedrine	OH	H	NH_2	H, CH_3	H, OH	H	>10,000
(±)Octopamine	OH	H	NH_2	H_2	H, OH	H	>10,000
p-Tyramine	OH	H	NH_2	H_2	H_2	H	>10,000
3-Methoxytyramine	OH	OCH_3	NH_2	H_2	H_2	H	>10,000
L-Dopa	OH	OH	NH_2	H, COOH	H_2	H	>10,000
3,4-Dihydroxyphenylacetic acid	OH	OH	OH	=O	H_2	H	>10,000
Homovanillic acid (HVA)	OH	OCH_3	OH	=O	H_2	H	>10,000
(+)Amphetamine	H	H	NH_2	H, CH_3	H_3	H	>10,000
N-Methylphenethylamine	H	H	NCH_3	H_2	H_2	H	>10,000

* Each value is the mean of triplicate determinations (S.D. < ± 5 per cent) with at least four concentrations of each freshly dissolved compound per experiment, using 0.5 nM [^{3}H]APO with or without 10 μM unlabeled ADTN to define specific binding to calf caudate tissue (P_4 fraction). The IC_{50} was calculated by log-probit analysis. Most compounds were evaluated at least three times.

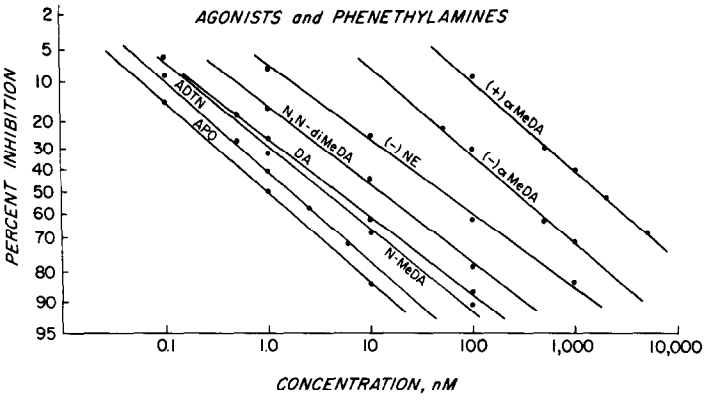


Fig. 2. Inhibition of binding of [3H]APO by DA agonists and phenethylamines. Data are presented for representative dopaminergic agonists and phenethylamine derivatives from Tables 1–3, and are plotted as for Fig. 1. The compounds shown are, from left to right: (−)apomorphine; ADTN; *N*-methyldopamine (epinine); dopamine; *N,N*-dimethyldopamine; (−)norepinephrine; (−)α-methyldopamine; and (+)α-methyldopamine. Data are mean values for at least three separate determinations with at least four concentrations of each compound (S.D. < ± 5 per cent).

Inhibition of binding of [3H]*apomorphine by aporphines.* Table 1 summarizes the IC_{50} values for thirty aporphine derivatives in competition with the binding of 0.5 nM [3H]APO to the calf caudate membrane preparation (P_4), and Fig. 1 presents inhibition curves for some representative aporphines. Several aporphines, especially those with hydroxyl groups at positions 10 and 11 (see structural formula in Table 1), exerted potent competition against the binding of [3H]APO. Thus, in addition to APO itself, several other structurally similar catechol-like 10,11-dihydroxyaporphines displaced [3H]APO at $IC_{50} \leq 25$ nM.

The substituent attached to the nitrogen at the 6 position of the aporphine ring seemed to have only moderate influence on the displacement of [3H]APO. With respect to the alkyl moiety bound at this N-6 position, the following represents a rank-order of potency of displacement of [3H]APO among the 10,11-dihydroxyaporphines tested:

Table 3. Inhibition of binding of [3H]APO by dopamine agonists, antagonists and other agents*

Agent	IC_{50} (nM)
Agonists	
(±)Amino-6,7-dihydroxytetralin (ADTN)	1.8
Lergotrile	45.0
N-n-Propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP)	170
2-Bromo-α-ergocriptine (bromocriptine)	300
Antagonists and other antipsychotic agents	
(+)Butaclamol	230
Trifluoperazine	580
Haloperidol	630
Fluphenazine	770
Perphenazine	800
Chlorpromazine	1,250
Clozapine	2,100
Domperidone	2,310
(−)Sulpiride	3,600
Spiroperidol	4,200
Pimozide	7,600
(±Sulpiride	≥10,000
(+)Sulpiride	≥50,000
(−)Butaclamol	≥100,000

* Data were obtained as for Table 2 by log-probit analyses of triplicate determinations at three or four concentrations of each freshly dissolved compound and are mean values (S.D. < ± 5 per cent), using 0.5 nM [3H]APO and 10 μM unlabeled ADTN to define specific binding to calf caudate (P_4). The following compounds had IC_{50} values ≥ 10,000 nM: acetylcholine (with 1 mM eserine), amitriptyline, γ-aminobutyric acid (GABA), benztropine, desipramine, eserine, 5-hydroxytryptamine (serotonin, 5-HT), imipramine, morphine, naloxone, nomifensine, phenoxybenzamine, phentolamine, propranolol and pyrogallol.

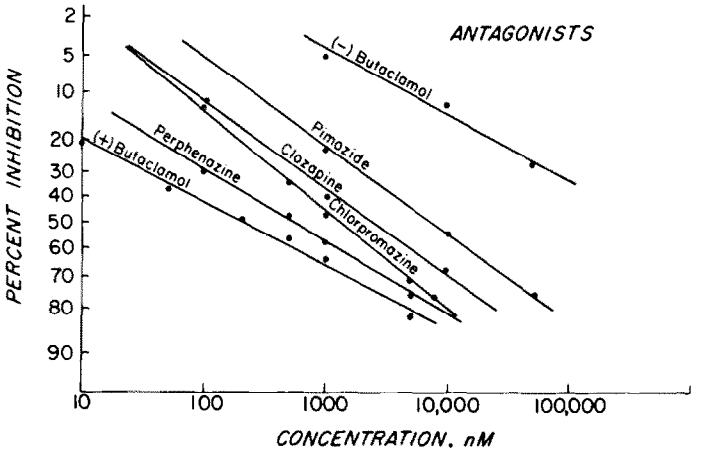


Fig. 3. Inhibition of binding of [^{3}H]APO by DA antagonists. Data are presented for representative DA antagonists from Table 3, plotted as for Figs. 1 and 2. The compounds shown are, from left to right: (+)butaclamol, perphenazine, chlorpromazine, clozapine, pimozide, and (−)butaclamol. Data are mean values for at least three separate determinations with at least four concentrations of each compound (S.D. < ± 5 per cent).

$CH_3 \geq CH_2CH_2CH_3 > CH_2CH_2OH > CH_2CH_2Cl$ (see compounds **1**, **2**, **8** and **15**, Table 1). Two 10-monohydroxylated aporphines, (±)10-hydroxy-aporphine (**12**) and (±)10-hydroxy-*N*-*n*-propylnor-aporphine (**16**), demonstrated a similarly greater potency of the methyl than the *n*-propyl substituent as with the dihydroxylated compounds. In addition, order among the *N*-alkyl-2,10-11-trihydroxylated aporphines was: $CH_3 > CH_2CH{=}CH_2 \geq CH_2CH_3 = CH_2CH_2CH_3 > H > CH_2C{\equiv}CH$ (see compounds **4**, **5**, **6**, **7**, **9**, and **13** in Table 1).

Inhibition of binding of [^{3}H]*apomorphine by phenethylamine analogs.* Table 2 summarizes the potency of phenethylamine derivatives or analogs, and Fig. 2 shows inhibition curves for representative phenethylamine derivatives and other DA agonists. The DA agonists, *N*-methyl-DA, DA, and *N*,*N*-dimethyl-DA, had potent effects (IC_{50} = 3.4, 4.5 and 13 nM). (−)Norepinephrine (NE) was fifty times less potent than DA and its *N*-alkylated congeners, but twelve times more potent than the pharmacologically inactive enantiomer (+)NE. The 5- and 6-F-DA derivatives had potencies of 50 and 61 nM. Other compounds had little ability to displace [^{3}H]APO from the caudate membranes, although *m*-tyramine was slightly active.

Inhibition of binding of [^{3}H]*apomorphine by non-catecholamine DA agonists, antagonists and other agents.* Table 3 summarizes IC_{50} values for compounds considered dopaminergic agonists or antagonists, other putative neuroregulators, or additional miscellaneous agents of interest, and Fig. 3 presents inhibition curves of representative antagonists. The DA agonists ADTN and lergotrile exerted potent inhibition (IC_{50} = 1.8 and 45 nM); the putative agonists 3-PPP and bromocriptine were somewhat less potent (170 and 300 nM).

Among the DA antagonists are compounds known to compete potently against the binding of [^{3}H]neuroleptics in mammalian striatal homogenates [12, 13]. Neuroleptics demonstrated monophasic inhibition of binding of [^{3}H]APO across concentrations from 10 nM to 100 μM, but with Hill slopes (0.5 to 0.7) well below those of agonists. Isomeric differences were found, however: the pharmacologically more active (+)-enantiomer of butaclamol was more than 400-fold more active than (−)butaclamol; similarly, (−)sulpiride was over 20-fold more potent than the pharmacologically inactive (+)-enantiomer.

In addition, the neurotransmitters 5-HT, ACh, and GABA; the β-adrenergic antagonist propranolol and the α-antagonists phentolamine and phenoxybenzamine; the COMT inhibitor pyrogallol; as well as morphine and the opiate antagonist naloxone, all competed negligibly with [^{3}H]APO. Most of these compounds (Table 3) exhibited IC_{50} values two or more orders of magnitude higher than that of APO and its potent analogs (Table 1) or DA and its agonistically potent *N*-alkylated derivatives (Table 2), and higher even than neuroleptics (Table 3).

DISCUSSION

Aporphines versus binding of [^{3}H]*apomorphine.* A catechol moiety was found to be important, but not sufficient for competition with the binding of [^{3}H]APO. Thus, the most potent aporphines were 10,11-catechols (compounds **1** through **9**, Table 1) with IC_{50} values ≤ 30 nM. This catechol position is analogous to the *trans*-α rotamer of DA. Such conformational requirements have also been reported previously for stimulation of DA-sensitive adenylate cyclase in striatal homogenates [14, 15], which may or may not include sites labeled by [^{3}H]APO [16, 17]. The potent interactions of 6,7-ADTN (Table 3), an analog of the *trans*-β conformer of DA, is at variance with this generalization [18, 19].

The nearly 20-fold greater potency of 11- versus 10-OH-NPA (compounds **10** and **16**, Table 1) suggests that the 11-OH group may contribute more to [^{3}H]APO binding. The 11-OH corresponds to the *meta*-OH of catecholamines, and *meta*-OH-phenolethylamines are reportedly more potent adrenergic agonists than *para*-OH congeners

[20, 21]. Also, in binding, *m*-tyramine was 14-fold more potent than *p*-tyramine (Table 2). Bulbocapnine (compound **11**, Table 1), with only 11-OH free, was also relatively potent.

Catechol groups are evidently *not sufficient* to provide potent competition for the binding of [^{3}H]APO, as several 10,11-di-OH-aporphines and 3,4-di-OH-phenethylamine derivatives bound weakly (Tables 1 and 2). Evidently, *N*-substitution can modify interactions with binding sites. For example, CH_2CH_3, $CH_2CH_2CH_3$, CH_2CH_2OH, or CH_2CH_2Cl *N*-substituted hydroxyaporphines (compounds **2**, **6**, **7**, **8**, **12**, **15** and **16**, Table 1) were less potent than their *N*-CH_3 congeners. While *N*-propyl aporphines were somewhat less potent than APO versus binding, they are somewhat more potent *in vivo* [18], possibly due to pharmacokinetic factors. *N*-Chloroethyl-norapomorphine (NCA) may have long-lasting anti-DA actions [5, 22, 23], possibly analogous to effects of phenoxybenzamine (also *N*-chloroethyl-substituted) [21, 24]. The somewhat lower activity of isoproterenol versus epinephrine (Table 2) suggests a modifying influence of an *N*-isopropyl substituent.

Thus, in addition to the presence and conformation of the catechol moiety, the substituent at the 6-*N*-position in the aporphine ring also plays an important part in the binding.

Displacement by phenethylamines. Displacement of [^{3}H]APO by phenethylamines (Table 2, Fig. 2) was also dependent on a free catechol moiety and was influenced by substitutions at the amino-*N* position. Except for DA and its *N*-methylated congeners, other phenethylamines (even the catecholamines, NE, epinephrine and isoproterenol) competed relatively weakly. *N*-Alkylated derivatives of DA are also active stimulators of striatal DA-sensitive adenylate cyclase [14, 25]. Phenethylamines lacking a free catechol group [*N*-methylphenethylamine, (+)amphetamine, *m*- or *p*-tyramine, 3-methoxytyramine, (±)octopamine, metaraminol, *p*-hydroxynorephedrine, and homovanillic acid] were weak inhibitors of [^{3}H]APO binding. Phenethylamines (metaraminol, *p*-hydroxynorephedrine) and even catecholamines (NE, isoproterenol, epinephrine), when β-hydroxylated, were much less potent than their corresponding non-β-hydroxylated analogs (tyramine, DA).

Non-specific binding of labeled catecholamines to a "catechol receptor" may yield misleading impressions concerning hypothetical agonist-receptors for catecholamines [26, 27]. In this regard, only very weak binding interactions were found, with several catechol-phenethylamine analogs lacking a simple ethylamine side-chain [α-methyl-DA, isoproterenol, epinephrine, (+)NE, L-dopa, 3,4-dihydroxyphenylacetic acid, and pyrogallol] (Tables 2 and 3). These observations suggest that both the catechol and ethylamine moieties are critical for potent displacement of [^{3}H]APO from caudate membranes and that non-specific interactions with catechol binding sites are unlikely to account for the interactions of low concentrations of [^{3}H]APO with the present tissue preparation that presumably contains DA receptors [4, 6, 7]. In addition, the lack of potency (Tables 2 and 3) of benztropine, nomifensine, and (+)amphetamine, or desipramine (and other antidepressants), which are effective blockers of DA or NE uptake [28–30], suggests that [^{3}H]APO binding is unrelated to catecholamine transport sites.

Displacement by dopamine antagonists and other compounds. Neuroleptic DA antagonists exhibited relatively weak competition for [^{3}H]APO binding to calf caudate membranes (Table 3, Fig. 3) and had relatively shallow inhibition curves. Except for the phenothiazines (piperazines more potent than chlorpromazine) and the isomer pairs of butaclamol and sulpiride, their potency did not correspond closely to reported *in vivo* potency, or activity against the binding of [^{3}H]neuroleptics [12, 13, 31]. Additional discrepancies in the potency-rankings for neuroleptics versus agonist binding can be found among data presented by other laboratories [1–3]. Several investigators found that DA agonists demonstrate high affinity for [^{3}H]agonist binding ([^{3}H]DA, [^{3}H]ADTN, or [^{3}H]APO) and that antagonists preferentially displace [^{3}H]antagonists (such as [^{3}H]haloperidol or [^{3}H]spiroperidol) [13, 32–34]. Although there has been some consideration of mechanisms that might account for these patterns [3, 16, 31–38], their biochemical basis and pharmacological significance, at present, remain unclear.

Among other DA agonists (Fig. 3, Table 3), 6,7-ADTN exerted potent competition with [^{3}H]APO. The ADTNs are semi-rigid DA analogs [18, 38] found to be potent as DA agonists in behavioral tests [38, 39] and as inhibitors of binding of [^{3}H]ADTN or [^{3}H]DA [40]. The hydroxyphenylpiperidine, 3-PPP, which is reported to have DA-agonist-like activity *in vivo*,* had only moderate potency versus [^{3}H]APO binding. Bromocriptine has been used clinically as a DA agonist [41, 42], but its relatively high binding affinity versus [^{3}H]haloperidol and low affinity versus [^{3}H]DA [33] suggest mixed agonistic/antagonistic characteristics at DA receptors, in accord with its intermediate IC_{50} in the present assay.

Stereoselectivity of [^{3}H]*apomorphine binding.* Stereospecificity, an important criterion for characterizing interactions with putative receptors [43], was found with several compounds in the present study. For example, the 2-fold difference in potency between (−)NPA (6a, R) and (±)NPA indicates that the *laevo*-isomer contains most of the activity of the racemate; similarly (−)2,10,11-tri-OH-aporphine was 530-fold more potent than the (+)isomer (Table 1). There was a 12-fold difference favoring (−)NE over (+)NE, and there was over a 6-fold difference between (−) and (+)α-methyl-DA (Table 2).

The more than 100-fold difference in IC_{50} values for the (+)- and (−)-isomers of butaclamol (Table 3, Fig. 3) accords with the greater potency of the former in displacing binding of [^{3}H]DA or [^{3}H]neuroleptics, in antagonizing adenylate cyclase activity or animal behaviors induced by DA agonists [31, 44], or in clinical antipsychotic activity [45]. The (−)-isomer of sulpiride is active as an antipsychotic

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agent [7] and was more potent versus [^{3}H]APO binding than the (+)-isomer or the racemate (Table 3). This difference was not necessarily to be expected in view of the atypical (not neuroleptic-like) central depressant actions of this agent [46, 47], and the atypically weak competition between [^{3}H]-(−)sulpiride and DA or APO, or even typical neuroleptics such as phenothiazines or haloperidol [47].

In conclusion, the present pharmacological and structure–activity evaluation of the stereoselective, high-affinity binding of [^{3}H]APO to a "subsynaptosomal" membrane fraction of bovine caudate tissue is consistent with that expected of dopaminergic agonist binding [18, 19, 48]. These observations, coupled with the high proportion of "specific" or displaceable high-affinity binding (over 80 per cent) of [^{3}H]APO as was characterized biochemically and reported separately [4–7], suggest that these methods may be applicable to further studies of aporphine receptor–ligand interactions in mammalian nervous tissue. In addition, such binding provides a selective and highly sensitive radiobinding assay of tissue catecholaporphines, results which accord well with alternative biochemical assays and yield close correlations between brain levels and behavioral actions of APO [7].

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