8,9-Dihydroxy-2,3,7,11b-tetrahydro-1*H*-naph[1,2,3-*de*]isoquinoline: A Potent Full Dopamine D_1 Agonist Containing a Rigid *β*-Phenyldopamine Pharmacophore

Debasis Ghosh, Scott E. Snyder, Val J. Watts,† Richard B. Mailman,† and David E. Nichols*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Brain & Development Research Center Departments of Psychiatry, Pharmacology, and Medicinal Chemistry, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7250

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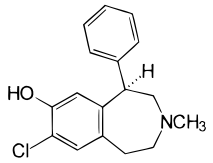
The present work reports the synthesis and preliminary pharmacological characterization of 8,9-dihydroxy-2,3,7,11b-tetrahydro-1*H*-naph[1,2,3-*de*]isoquinoline (**4**, dinapsoline). This molecule was designed to conserve the essential elements contained in our D_1 agonist pharmacophore model (i.e., position and orientation of the nitrogen, hydroxyls, and phenyl rings). It involved taking the backbone of dihydrexidine [**3**; (±)-*trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine], the first high-affinity full D_1 agonist, and tethering the two phenyl rings of dihydrexidine through a methylene bridge and removing the C(7)−C(8) ethano bridge. Preliminary molecular modeling studies demonstrated that these modifications conserved the essential elements of the hypothesized pharmacopore. Dinapsoline **4** had almost identical affinity ($K_I = 5.9$ nM) to **3** at rat striatal D_1 receptors and had a shallow competition curve ($n_H = 0.66$) that suggested agonist properties. Consistent with this, in both rat striatum and C-6-mD_1 cells, dinapsoline **4** was a full agonist with an EC_{50} of ca. 30 nM in stimulating synthesis of cAMP via D_1 receptors. The design and synthesis of dinapsoline **4** provide a powerful test of the model of the D_1 pharmacophore we have developed and provide another chemical series that can be useful probes for the study of D_1 receptors. An interesting property of **3** is that it also has relatively high D_2 affinity ($K_{0.5} = 50$ nM) despite having an accessory phenyl ring usually thought to convey D_1 selectivity. Dinapsoline **4** was found to have even higher affinity for the D_2 receptor ($K_{0.5} = 31$ nM) than **3**. Because of the high affinity of **4** for D_2 receptors, it and its analogs can be powerful tools for exploring the mechanisms of "functional selectivity" (i.e., that **3** is an agonist at some D_2 receptors, but an antagonist at others). Together, these data suggest that **4** and its derivatives may be powerful tools in the study of dopamine receptor function and also have potential clinical utility in Parkinson's disease and other conditions where perturbation of dopamine receptors is useful.

Introduction

The accepted classification of dopamine receptors was originally based on a scheme dividing the receptors into two families (D_1 and D_2) based on pharmacological and functional evidence.[1] D_1 receptors preferentially recognize the phenyltetrahydrobenzazepines and lead to stimulation of the enzyme adenylate cyclase, whereas D_2 receptors recognize the butyrophenones and benzamides and are coupled negatively (or not at all) to adenylate cyclase. More recently, it is clear that at least five genes code for subtypes of dopamine receptors: D_1, D_2, D_3, D_4, and D_5. The traditional classification, however, remains useful, with the D_1-like class comprising the D_1 (D_{1A}) and the D_5 (D_{1B}) receptors, whereas the D_2-like class consists of the D_2, D_3, and D_4 receptors.[2−7]

Although there has been a specific focus on the D_2 family for more than two decades, more recently the critical role of the D_1 receptor in nervous system function has become clear.[8,9] The availability of both a selective antagonist[10] and a high-affinity full D_1 agonist[11,12] has led to increased awareness of the manifold functional roles of D_1 receptors. In addition, the potential utility of novel D_1 ligands in various central nervous system (CNS) disorders has also become clear.[13−15]



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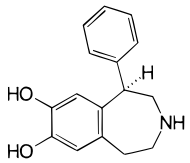

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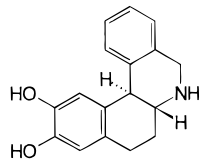

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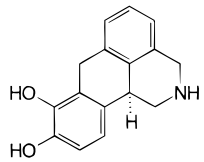

4

The early work on selective D_1 receptor ligands primarily focused on molecules from a single chemical class, the phenyltetrahydrobenzazepines, such as the antagonist SCH 23390 (**1**).[10] In general, the agonists that derived from this class (e.g., SKF 38393, **2**[16]) were of partial efficacy. Even SKF 82958, purported to be a full agonist, recently has been shown not to have full intrinsic efficacy in preparations with decreased receptor reserve.[17] Thus, our laboratories initiated efforts to design ligands that were full agonists, indeed, were of full intrinsic efficacy. This ultimately led to the synthesis of dihydrexidine **3**, a hexahydrobenzo[*a*]phenanthridine, designed as a hybrid of the aminotetralins and the 4-phenyltetrahydroisoquinolines.[12,18] The resulting

* Address all correspondence to: Dr. David E. Nichols, Department of Medicinal Chemistry and Pharmacognosy, Robert Heine Pharmacy Building, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907. (317) 494-1461 (office); (317) 494-6790 (FAX). Internet: drdave@sage.cc.purdue.edu.

† University of North Carolina School of Medicine.

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structure **3** was unique from other D_1 agonists because the accessory ring system is tethered, making the molecule relatively rigid. Molecular modeling studies of **3** have shown that it has a limited number of low-energy conformations, in all of which the aromatic rings are held in a relatively coplanar arrangement.[19] The recent elucidation of the configuration of the active enantiomer of **3**[20] was consistent with predictions from this model. Unlike other high-affinity, high intrinsic activity D_1 agonists like 3-substituted (aminomethyl)-isochromans,[21] **3** provided a semirigid template for developing such models. The essential features of this receptor model include the presence of a transoid β-phenyldopamine moiety, an equatorially oriented electron lone pair on the basic nitrogen atom, and near coplanarity of the pendant phenyl ring with the catechol ring. Interestingly, dopaminergic phenyltetrahydrobenzazepines[22] have a cisoid β-phenyldopamine conformation.

It is important to note that the differentiation between agonists of full and partial efficacy is not only of narrow biochemical interest, but appears to have general importance in terms of complex CNS-mediated events. For example, dihydrexidine **3** and the isochroman full agonist A-77636 have exceptional anti-parkinsonian effects in the MPTP-treated monkey model,[13,21] whereas partial agonists are devoid of, or have significantly less, activity. More recent data suggest that full and partial agonists differ in their effects on other complex neural functions.[14,15] For these reasons, understanding of the molecular mechanisms responsible for D_1 receptor activation are of both heuristic and practical interest.

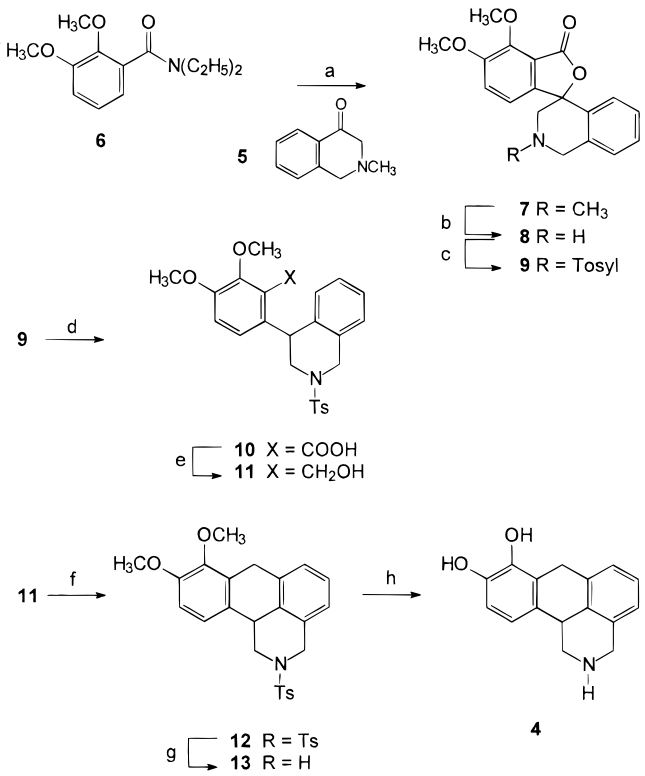
The present report describes the synthesis and pharmacological characteristics of a new rigid β-phenyldopamine analog, 8,9-dihydroxy-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline (**4**). Tethering the two phenyl rings of dihydrexidine through a methylene bridge, and removing the C(7)−C(8) ethano bridge, led to this molecule that conserved the relative orientation of all the essential elements (position and orientation of the nitrogen, hydroxyls, and phenyl rings) of the molecular pharmacophore that we had hypothesized were necessary for high D_1 affinity and full intrinsic efficacy. It was found that **4** was, in fact, a high-affinity ligand at rat striatal D_1 receptors and has full intrinsic activity in at least two preparations of D_1 receptors. Moreover, **4** also has significant affinity for the D_2 receptor ($K_{0.5}$ = 31 nM) despite the presence of its accessory phenyl ring. These data suggest that **4** will have potential clinical utility in Parkinson's disease and in other conditions where perturbation of dopamine receptors can be of importance. On the basis of all the earlier studies of **3**, it is clear that derivatives of **4** will also be powerful tools in the study of dopamine receptor function.

Chemistry

The key starting material for the synthesis of **4**, 2-methyl-2,3-dihydro-4(1*H*)-isoquinolone (**5**), was prepared following the route described by Klein et al.[23]

In Scheme 1, ortho-directed lithiation of 2,3-dimethoxy-*N*,*N*′-diethylbenzamide (**6**) with *sec*-butyllithium/TMEDA in ether at −78 °C and condensation of this lithiated species with **5** followed by reflux with *p*-toluenesulfonic acid, gave spirolactone **7** in modest yield.[24,25] *N*-Demethylation of **7** with 1-chloroethyl chloroformate, followed by methanolysis of the intermediate, afforded **8.**[26] This secondary amine was treated with *p*-toluenesulfonyl chloride and triethylamine to give **9**.

Scheme 1[a]



[a] (a) (i) *sec*-Butyllithium, TMEDA, Et_2O, −78 °C; (ii) **7**; (iii) TsOH, toluene, reflux; (b) (i) 1-chloroethyl chloroformate, $(CH_2Cl)_2$; (ii) CH_3OH; (c) TsCl, Et_3N; (d) H_2/Pd−C, HOAc; (e) BH_3·THF; (f) concentrated H_2SO_4, −40 °C to −5 °C; (g) Na/Hg, CH_3OH, Na_2HPO_4; (h) BBr_3, CH_2Cl_2.

An early attempt to synthesize **9** directly involved condensation of 2-(*p*-tolyl sulfonyl)-2,3-dihydro-4(1*H*)-isoquinolone[27] with lithiated **6** in THF or ether, followed by lactonization with acid; this provided only minute amounts (<5%) of **9**. Enolization of 2-(*p*-tolylsulfonyl)-2,3-dihydro-4(1*H*)-isoquinolone under the basic reaction conditions is one obvious explanation for the poor yield.[28]

Hydrogenolysis of **9** in glacial acetic acid in the presence of 10% palladium on carbon gave **10**,[24] which on reduction with diborane afforded the key intermediate **11**. Cyclization of **11** with concentrated sulfuric acid at low temperature provided **12**. *N*-Detosylation of **12** with Na/Hg in methanol buffered with disodium hydrogen phosphate gave **13**.[29] Finally, **13** was treated with boron tribromide to effect methyl ether cleavage yielding **4** as its hydrobromide salt.

Pharmacology

Binding and Functional Effects at D_1 Receptors. We first examined the affinity of **4** for D_1 receptors in rat striatal homogenates. As shown in Figure 1, **4** competed with high affinity at this receptor, having almost identical affinity to **3**, the first full D_1 agonist. As can be seen in Figure 1, both **4** and **3** had shallower slopes for their competition curves than did the prototypical D_1 antagonist **1**. These binding data are summarized in Table 1.

We next determined whether **4**, as predicted by our model, would be a full agonist at D_1 receptors in the rat striatum. As is shown in Figure 2, saturating

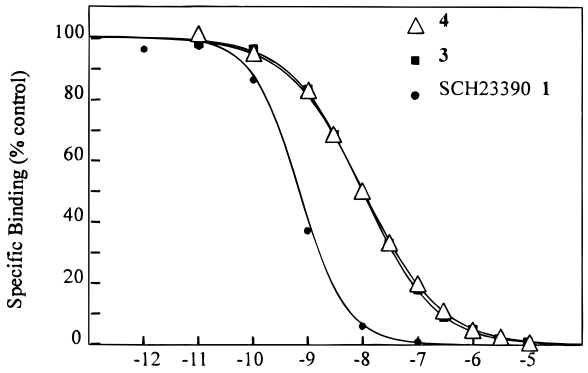


Figure 1. Affinity of compound (±)-**4** at striatal D_1 receptors. The high-affinity full agonist (±)-**3** and the prototype antagonist (+)-**1** were included for comparison purposes.

Table 1. Summary of Affinities of (±)-**3** and (±)-**4** at Dopamine Receptors in Rat Brain[a]

	D_1 binding		D_2 binding	
compound	$K_{0.5}$ (nM)	n_H	$K_{0.5}$ (nM)	n_H
(±)-**4**	5.93 ± 0.45	0.66 ± 0.01	31.3 ± 4.4	0.71 ± 0.03
(±)-**3**	4.59 ± 0.28	0.65 ± 0.01	43.2 ± 3.2	0.72 ± 0.04
(+)-**2**	17 ± 4	0.75 ± 0.08	NT[b]	
(+)-**1**	0.30 ± 0.01	1.05 ± 0.01	NT[b]	
chlorpromazine	–[b]	–	0.92 ± 0.12	0.93 ± 0.01

[a] Radioligand binding studies for dopamine receptors were conducted in rat striatal homogenates, using 0.3 nM [^{3}H]SCH23390 (D_1 sites) and 0.07 nM [^{3}H]spiperone in the presence of 50 nM unlabeled ketanserin (D_2 sites). Competition curves were analyzed by non linear regression to determine estimates for the $K_{0.5}$ and Hill slope (n_H). Data represent the mean and standard error from three independent assays for each test compound. [b] Not tested.

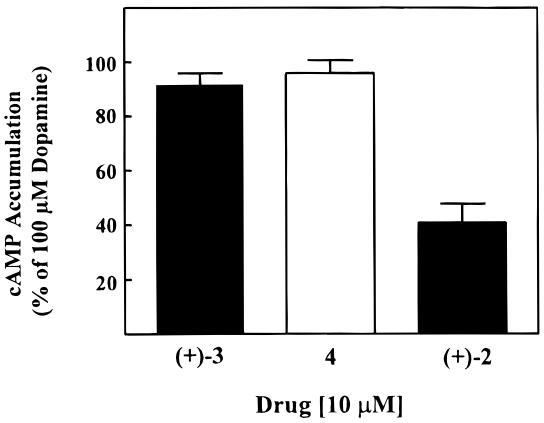


Figure 2. Compound (±)-**4** is a full agonist at D_1 receptors in the rat striatum. The high-affinity full agonist (±)-**3** and the partial agonist (+)-**2** were included for comparison purposes. The ability of the test compounds to stimulate cAMP accumulation was examined in rat striatal homogenates (see Methods for details). Data represent means ± SEM from at least three experiments [**4**, 95.8 ± 4.7; **3**, 91.3 ± 4.6; **2**, 40.7 ± 7.0].

concentrations (10 μM) of both **4** and **3** caused the same degree of increase in cAMP synthesis as did a maximally effective concentration of dopamine (100 μM). Conversely, the partial agonist (+)-**2** caused less than 50% stimulation. These effects were blocked by the D_1 antagonist SCH23390 (1 μM). The functional efficacy of **4** was also tested in cloned primate D_{1A} receptors expressed in C-6 glioma cells. As is shown in Figure 3, compound **4** also was of full efficacy in this preparation, with an EC_{50} of ca. 30 nM. As has been previously shown, (±)-**3** also was of full efficacy in this preparation, whereas (+)-**2** was only of partial efficacy.[17]

Binding at D_2 Receptors. Because **3** had been shown, unexpectedly, to have significant D_2 affinity despite its accessory hydrophobic ring,[12,19] the ability of **4** to compete for D_2 receptors in rat striatal homogenates also was investigated. As is shown in Figure 4 and Table 1, the affinity of **4** for D_2-like receptors in rat striatal homogenates is actually higher than for **3**. As can also be seen in Figure 4, the slope of the competition curves for both **4** and **3** was shallower than for the prototypical D_2 antagonist chlorpromazine.

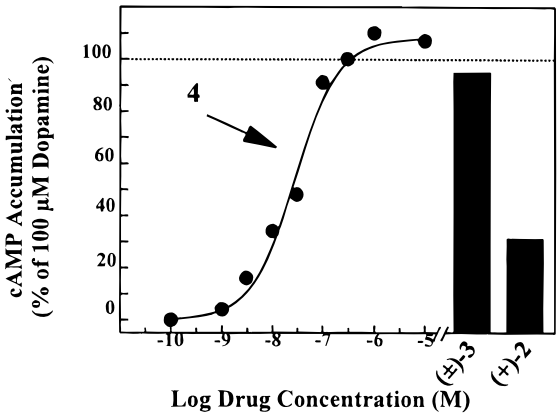


Figure 3. Compound (±)-**4** is a high-potency (EC_{50} ca. 30 nM) full agonist at the primate D_{1A} receptor expressed in C-6 cells. The full agonist (±)-**3** and the partial agonist (+)-**2** (SKF38393) were included for comparison purposes. The ability of the test compounds to stimulate cAMP accumulation was examined in C-6-mD_1 cell membranes (see Methods for details). Data represent the average of two experiments conducted in duplicate.

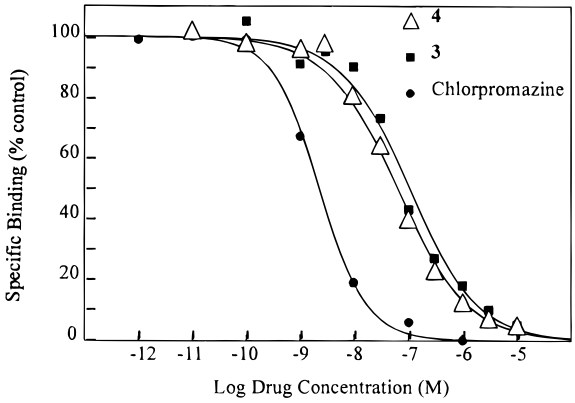


Figure 4. Affinity of compound (±)-**4** at striatal D_2 receptors. The high affinity full agonist (±)-**3** and the prototype antagonist chlorpromazine were included for comparison purposes.

Results and Discussion

As shown clearly by the pharmacological data, compound **4** has high affinity for dopamine D_1 receptors labeled with [^{3}H]SCH23390, almost identical to that of (±)-**3** (dihydrexidine). Moreover, (±)-**4**, in both rat striatal membranes and in cloned expressed primate D_{1A} receptors, was a full agonist relative to dopamine, like (±)-**3** but unlike the partial agonist (+)-**2** that caused less than half the maximal response in this assay (e.g., see Figures 3 and 4). On the basis of our underlying model of the D_1 pharmacophore, we would predict that both the affinity and intrinsic activity of racemic **4** reside in only one of its enantiomers, that with the 11b*R* absolute configuration. Resolution of the racemate would therefore yield one isomer with approximately twice the D_1 affinity obtained here, thus making its affinity for the D_1 receptor similar to (+)-**3**.

While the D_1 affinity and functional efficacy of **4** were

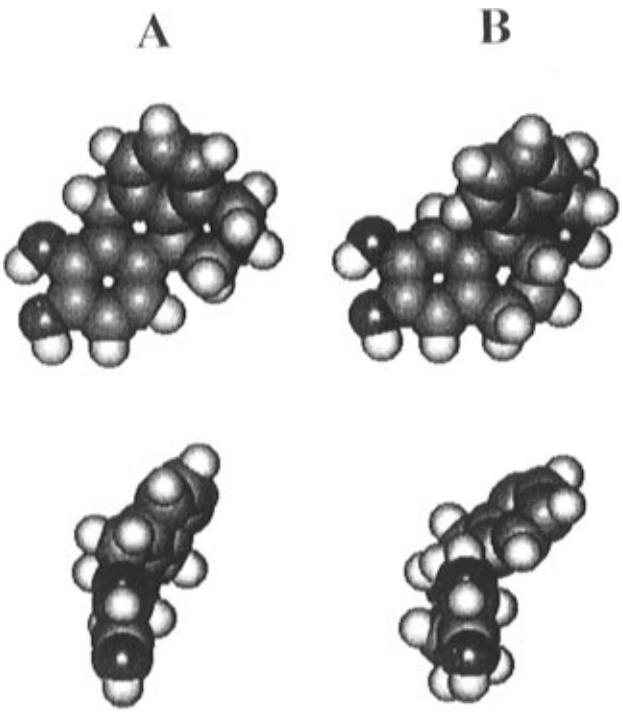


Figure 5. Front and edge-on views of space-filling models comparing minimum energy conformations of the proposed active enantiomer of molecule **4** (A, left, top and bottom) and (+)-dihydrexidine (**3**, B).

as predicted by our model, of equal interest (although less well rationalized) were the actions of **4** at the D_2 receptor. As was shown in Figure 4 and Table 1, (±)-**4** has greater affinity for D_2 receptors than does (±)-**3**. This property, we believe, will in itself be a useful research tool. As was noted in the Introduction, when **3** was synthesized some years ago, it was anticipated that it would have been fairly selective for the D_1 vs D_2-like receptors. It was somewhat surprising, therefore, to find that **3** was only about 10-fold D_1:D_2 selective.[12,19] This observation became more interesting when it was found that **3**, while expected to behave as a typical dopamine agonist, had an unusual property that we have termed "functional selectivity". Specifically, in rats (*in vivo* or *in vitro*), **3** acts as an agonist at D_2-like receptors located postsynaptically, but as an antagonist at D_2-like receptors located presynaptically.[30,31] This phenomenon is now hypothesized to be due to differences in the ligand−receptor-G protein complex, depending on the specific G proteins in a given cellular milieu. We have shown that these D_2 properties of **3** reside in the *same* enantiomer (i.e., 6a*R*,12b*S*) that is the high-affinity full agonist at the D_1 receptor.[20] On this basis, we would predict that both the D_1 and D_2 properties of **4** also reside in the same enantiomer. The optical isomers of **4**, and appropriate analogs, should be powerful tools to study this "functional selectivity" hypothesis.

Figure 5 presents space-filling representations of the low-energy conformations for (+)-**3** and the 11b*R* enantiomer of **4** that is homochiral to (+)-**3** at its 12b*S* chiral center. Overall, the two compounds appear remarkably similar in shape. Two major structural features are readily evident. First, the steric bulk provided by the C(7)−C(8) ethano bridge in **3** has been removed. Second, the angle of the pendent phenyl ring with respect to the plane of the catechol ring is changed slightly. In the top, face-on views, this is most evident, where the aromatic hydrogen H(1) in **3** projects above the catechol ring. In **4**, however, this position is used to tether the pendent phenyl ring through a methylene unit to the catechol ring; this forces the pendent phenyl ring to twist in a clockwise direction, relative to **3**, when viewed from above. The amino groups, which are not readily visible in this figure, are in similar positions, given the degree of conformational flexibility of the heterocyclic rings. In addition, both molecules can present an N-H vector in an equatorial orientation, a feature of the pharmacophore that we have proposed is important for D_1 receptor agonists.[12,18] From these results, one would predict that the pharmacological properties of these two molecules would be similar, and this is exactly what is observed. Furthermore, prior to completion of the synthesis of this molecule, it was fit into a receptor model that we have developed for dopamine D_1 agonists using the active analog approach.[19] Compound **4** gave an ideal fit to the model. The present results help to validate the predictive ability of that model.

Conclusions

The predicted high affinity and full efficacy of **4** provide additional support for our hypothesis that β-phenyldopamine, locked into a properly oriented rigid framework, can serve as the pharmacophore for potent dopamine D_1 agonists.[12] Clearly, on the basis of the results presented here, the pharmacophore takes an optimal conformation when it is disposed in the relative orientations provided by both **3** and **4**. We have previously reported that **3** demonstrated profound antiparkinsonian effects in the MPTP model of Parkinson's disease, and we would anticipate that **4** should show similar effects. Moreover, **4**, like **3**, should also have utility both as a research tool and clinical drug in cases where full intrinsic efficacy D_1 ligands are useful. As noted above, the high affinity of **4** for D_2-like receptors fortuitously provides additional important research tools. Our recent work has shown that it is possible to tailor **3** by appropriate molecular modifications such that the resulting analogs can be targeted to specific subpopulations of the dopamine receptor family.[17,20] It seems obvious that similar strategies with **4** should result in compounds that can have either novel receptor subtype selectivity or atypical functional profiles.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian VXR 500S (500 MHz) NMR instrument, and chemical shifts were reported in δ values (ppm) relative to TMS. The IR spectra were recorded as KBr pellets or as a liquid film with a Perkin-Elmer 1600 series FTIR spectrometer. Chemical ionization mass spectra (CIMS) were recorded on a Finnigan 4000 quadruple mass spectrometer. High-resolution CI spectra were recorded on Kratos MS50 spectrometer. Elemental analysis data were obtained from the microanalytical laboratory of Purdue University, West Lafayette, IN. THF was distilled from benzophenone−sodium under nitrogen immediately before use; 1,2-dichloroethane was distilled from phosphorous pentoxide before use.

2′,3′-Dihydro-4,5-dimethoxy-2′-methylspiro[isobenzofuran-1(3*H*),4′(1′*H*)-isoquinolin]-3-one (7). To a solution of **6** (14.94 g, 63 mmol) in ether (1400 mL) at −78 °C under nitrogen was added sequentially, dropwise, *N*,*N*,*N*′,*N*′-tetramethylenediamine (9.45 mL, 63 mmol) and *sec*-butyllithium (53.3 mL, 69 mmol, 1.3 M solution in hexane) through a rubber septum via syringe. After 1 h, freshly distilled **5** (10.1 g, 62.7 mmol) was added to the heterogeneous mixture. The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature over 9 h. Saturated NH_4Cl

solution (400 mL) was then added a,nd the mixture was stirred for 15 min. The ether layer was separated, and the water layer was extracted with dichloromethane (4 × 100 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated to a brown oil. The oil was dissolved in toluene (500 mL), heated at reflux for 8 h with 3.0 g of *p*-toluenesulfonic acid, cooled, and concentrated under vacuum. The residue was dissolved in dichloromethane, washed with dilute aqueous $NaHCO_3$ and water, then dried (Na_2SO_4), filtered, and evaporated to a gummy residue. On trituration with ethyl acetate−hexane (50:50), a solid precipitated. Recrystallization from ethyl acetate−hexane afforded 12.75 g (63%) of **7**: mp 193−194 °C; IR (KBr) 1752 cm^{-1} (C═O); ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H, NCH_3), 2.88 (d, 1H, J = 11.6 Hz), 3.02 (d, 1H, J = 11.7 Hz), 3.76 (d, 1H, J = 15.0 Hz), 3.79 (d, 1H, J = 15.1 Hz), 3.90 (s, 3H, OCH_3), 4.17 (s, 3H, OCH_3), 6.83 (d, 1H, J = 8.4 Hz, ArH), 7.03 (d, 1H, J = 8.2 Hz, ArH), 7.11 (m, 3H, ArH), 7.22 (m, 1H, ArH); MS (CI) m/z 326 (100). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

2′,3′-Dihydro-4,5-dimethoxyspiro[isobenzofuran-1(3*H*),4′(1′*H*)-isoquinolin]-3-one (8). Following the method of Olofson et al.,[26] to a suspension of **7** (6.21 g, 19.2 mmol) in 100 mL of 1,2-dichloroethane was added dropwise 1-chloroethyl chloroformate (5.1 mL, 46.3 mmol) at 0 °C under nitrogen. The mixture was stirred for 15 min at 0 °C and then heated at reflux for 8 h. The mixture was cooled and concentrated under reduced pressure. To this mixture was added 75 mL of methanol, and the reaction was heated at reflux overnight. After cooling, the solvent was evaporated under reduced pressure to afford the hydrochloride salt of **8** in nearly quantitative yield. It was sufficiently pure to use in the next step without further purification: mp (HCl) 220−222 °C; mp (base) 208−210 °C; IR (film) 1754 cm^{-1} (C═O); ^1H NMR ($CDCl_3$, base) δ 3.18 (d, 1H, J = 13.5 Hz), 3.30 (d, 1H, J = 13.5 Hz), 3.84 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.02 (s, 2H, CH_2N), 6.67 (d, 1H, J = 7.5 Hz, ArH), 7.12 (m, 2H, ArH), 7.19 (d, 1H, J = 7.5 Hz, ArH), 7.26 (t, 1H, J = 7.5 Hz, ArH), 7.41 (d, 1H, J = 8.5 Hz, ArH); MS (CI) m/z 312 (100); HRCIMS calcd for $C_{18}H_{17}NO_4$ 312.1236, found 312.1198. Anal. ($C_{18}H_{17}NO_4$) H, N; C: calcd, 69.44, found, 68.01.

2′,3′-Dihydro-4,5-dimethoxy-2′-(*p*-tolylsulfonyl)spiro[isobenzofuran-1(3*H*), 4′(1′*H*)isoquinolin]-3-one (9). To a mixture of *p*-toluenesulfonyl chloride (3.6 g, 18.9 mmol), **8** (as the HCl salt, obtained from 19.2 mmol of **7**), and chloroform (100 mL) was added 7 mL of triethylamine, dropwise, at 0 °C under nitrogen. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at room temperature for 1 h. It was then acidified with 100 mL of cold aqueous 0.1 N HCl and extracted with dichloromethane (2 × 100 mL), and the organic extract was dried ($MgSO_4$), filtered, and evaporated under vacuum to afford a viscous liquid that on trituration with ethyl acetate−hexane at 0 °C gave a solid. Recrystallization from ethyl acetate−hexane afforded 8.74 g (97%, overall from **7**) of **9**: mp 208−210 °C; IR (KBr) 1767 cm^{-1} (C═O); ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 3.22 (d, 1H, J = 11 Hz), 3.88 (d, 1H, J = 11 Hz), 3.90 (s, 3H, OCH_3), 3.96 (d, 1H, J = 15 Hz), 4.17 (s, 3H, OCH_3), 4.81 (d, 1H, J = 15 Hz), 6.97 (d, 1H, J = 7.7 Hz, ArH), 7.16 (m, 3H, ArH), 7.26 (m, 1H, ArH), 7.38 (d, 2H, J = 8 Hz, ArH), 7.72 (d, 2H, J = 8 Hz, ArH); MS (CI) m/z 466 (100). Anal. ($C_{25}H_{23}NO_6S$) C, H, N.

3,4-Dimethoxy-6-(2-(*p*-tolylsulfonyl)-1,2,3,4-tetrahydroisoquinolin)-4-yl)benzoic Acid (10). Using a procedure modified from de Silva and Snieckus,[24] a solution of **9** (2.56 g, 5.51 mmol) in glacial acetic acid (250 mL) with 10% palladium on activated carbon (6.30 g) was shaken on a Parr hydrogenator at 50 psig for 48 h at room temperature. The catalyst was removed by filtration, and the solvent was evaporated to afford 2.55 g (99%) of **10** that was sufficiently pure to carry into the next step. An analytical sample was recrystallized from ethanol−water: mp 182−184 °C; IR (KBr) 1717 cm^{-1} (COOH); ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 3.12 (m, 1H), 3.51 (dd, 1H, J = 5, 11.5 Hz), 3.71 (s, 6H, OCH_3), 4.10 (m, 1H, Ar_2CH), 4.23 (s, 2H, $ArCH_2N$), 6.52 (d, 1H, J = 7.5 Hz, ArH), 6.78 (d, 1H, J = 7.5 Hz, ArH), 6.90 (m, 1H, ArH), 7.07 (t, 1H, J = 8 Hz, ArH), 7.14 (t, 1H, J = 6.5 Hz, ArH), 7.20 (d, 1H, J = 7.5 Hz, ArH), 7.38 (d, 2H, J = 8 Hz, ArH), 7.63 (d, 2H, J = 8.5 Hz, ArH); MS (CI) m/z 468 (16), 450 (63), 296 (100); HRCIMS calcd for $C_{25}H_{25}NO_6S$ 468.1481, found 468.1467. Anal. ($C_{25}H_{25}NO_6S$) C, H, N.

2-*N*-(*p*-Tolylsulfonyl-4-(2-(hydroxymethyl)-3,4-dimethoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (11). To a solution of **10** (1.4 g, 2.99 mmol) in dry tetrahydrofuran (30 mL) was added 1.0 M borane−tetrahydrofuran (8 mL) at 0 °C under nitrogen. After the addition was complete the mixture was stirred at reflux overnight. Additional diborane (4 mL) was added, and stirring was continued for another 30 min. After cooling and evaporation under reduced pressure, methanol (30 mL) was carefully added, and the solvent was removed at low pressure. The process was repeated three times to ensure the methanolysis of the intermediate borane complex. Evaporation of the solvent gave 1.10 g (81%) of crude **11**. An analytical sample was purified by flash chromatography (silica gel, EtOAc−hexane) followed by recrystallization from ethyl acetate−hexane: mp 162−164 °C; ^1H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.18 (dd, 1H, J = 7.5, 11.9 Hz), 3.67 (dd, 1H, J = 4.5, 11.8 Hz), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.27 (d, 1H, J = 15 Hz), 4.40 (d, 1H, J = 15 Hz), 4.57 (t, 1H, J = 6 Hz, $CHAr_2$), 4.71 (s, 2H, CH_2OH), 6.58 (d, 1H, J = 8.5 Hz, ArH), 6.74 (d, 1H, J = 8.6 Hz, ArH), 6.84 (d, 1H, J = 7.7 Hz, ArH), 7.08 (t, 2H, J = 7.6 Hz, ArH), 7.14 (t, 1H, J = 6.6 Hz, ArH), 7.27 (d, 2H, J = 8 Hz, ArH), 7.65 (d, 2H, J = 8 Hz, ArH); MS (CI) m/z 454 (2.57), 436 (100). Anal. ($C_{25}H_{27}NO_5S$) C, H, N.

8,9-Dimethoxy-2-(*p*-tolylsulfonyl)-2,3,7,11b-tetrahydro-1*H*-napth[1,2,3-*de*]isoquinoline (12). To 50 mL of cold concentrated sulfuric acid (50 mL) at −40 °C under nitrogen was added with vigorous mechanical stirring powdered **11** (427 mg, 0.98 mmol) in several portions. After the addition, the reaction mixture was warmed to −5 °C over 2 h and then poured onto crushed ice (450 g) and left stirring for 1 h. The product was extracted with dichloromethane (2 × 150 mL), washed with water (2 × 150 mL), dried ($MgSO_4$), filtered, and evaporated to afford an oil that on trituration with ether at 0 °C yielded **12** (353 mg, 82%) as a white solid that was used for the next step without further purification. An analytical sample was prepared by centrifugal rotary chromatography using 50% ethyl acetate−hexane as the eluent, followed by recrystallization from EtOAc−hexane: mp 204−206 °C; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 2.80 (m, 1H, H-1a), 3.50 (dd, 1H, J = 4.5, 17.5 Hz, H-1b), 3.70 (dd, 1H, J = 7, 14 Hz, H-3a), 3.828 (s, 3H, OCH_3), 3.832 (s, 3H, OCH_3), 3.9 (m, 1H, H-11b), 4.31 (d, 1H, J = 17.6 Hz, H-7a), 4.74 (ddd, 1H, J = 1.7, 6.0, 11.2 Hz, H-7b), 4.76 (d, 1H, J = 14.8 Hz, H-3b), 6.77 (d, 1H, J = 8.3 Hz, ArH), 6.87 (d, 1H, J = 8.4 Hz, ArH), 6.94 (d, 1H, J = 7.6 Hz, ArH), 7.13 (t, 1H, J = 7.5 Hz, ArH-5), 7.18 (d, 1H, J = 7.2 Hz, ArH), 7.33 (d, 2H, J = 8.1 Hz, ArH), 7.78 (d, 2H, J = 8.2 Hz, ArH); MS (CI) m/z 436 (55), 198 (86), 157 (100); HRCIMS calcd for $C_{25}H_{25}NO_4S$ 436.1583, found 436.1570. Anal. ($C_{25}H_{25}NO_4S$) C, H, N.

8,9-Dimethoxy-2,3,7,11b-tetrahydro-1*H*-napth[1,2,3-*de*]-isoquinoline (13). Following the procedure of Pyne et al.,[29] a mixture of **12** (440 mg, 1.01 mmol), dry methanol (10 mL), and disodium hydrogen phosphate (574 mg, 4.04 mmol) was stirred under nitrogen at room temperature. To this mixture was added 6.20 g of 6% Na/Hg in three portions, and the reaction mixture was heated at reflux for 2 h. After cooling, water (200 mL) was added and the mixture was extracted with ether (3 × 200 mL). The ether layers were combined, dried ($MgSO_4$), filtered (Celite), and evaporated to give an oil that solidified under vacuum. After rotary chromatography, 142 mg (50%) of **13** was obtained as an oil. The oil quickly darkened on exposure to air and was used immediately for the next step. A small portion of the oil was treated with ethereal HCl, and the hydrochloride salt of **13** was recrystallized from ethanol−ether: mp (HCl salt) 190 °C dec; ^1H NMR ($CDCl_3$, base) δ 3.13 (dd, 1H, J = 10.8, 12 Hz, H-1a), 3.50 (dd, 1H, J = 3.4, 17.4 Hz, H-1b), 3.70 (m, 1H, H-11b), 3.839 (s, 3H, OCH_3), 3.842 (s, 3H, OCH_3), 4.03 (dd, 1H, J = 6, 12 Hz, H-7a), 4.08 (s, 2H, H-3), 4.33 (d, 1H, J = 17.4 Hz, H-7b), 6.78 (d, 1H, J = 8.24 Hz, ArH), 6.92 (m, 2H, ArH), 7.11 (t, 1H, J = 7.5 Hz, ArH), 7.18 (d, 1H, J = 7.5 Hz, ArH); MS (CI) m/z 282 (100); HRCIMS calcd for $C_{18}H_{19}NO_2$ 282.1494, found 282.1497.

8,9-Dihydroxy-2,3,7,11b-tetrahydro-1*H*-napth[1,2,3-*de*]-isoquinoline (4). To a solution of **13** (25 mg, 0.089 mmol) in

dichloromethane (5 mL) at −78 °C was added boron tribromide (0.04 mL, 0.106 g, 0.42 mmol). After stirring at −78 °C under nitrogen for 2 h, the cooling bath was removed and the reaction mixture was left stirring at room temperature for 5 h. It was then cooled to −78 °C, and methanol (2 mL) was carefully added. After stirring for 15 min at room temperature, the solvent was evaporated under reduced pressure. More methanol was added, and the process was repeated three times. The resulting gray solid was recrystallized from ethanol−ethyl acetate to yield a total of 12 mg (41%) of the hydrobromide salt of **4**: mp 258 °C dec; ^{1}H NMR (HBr salt, CD_3OD) δ 3.43 (t, 1H, J = 12 Hz, H-1a), 3.48 (dd, 1H, J = 3.5, 18 Hz, H-1b), 4.04 (m, 1H, H-11b), 4.38 (dd, 2H, J = 5.5, 12 Hz, H-7), 4.44 (s, 2H, H-3), 6.58 (d, 1H, J = 8.5 Hz, ArH), 6.71 (d, 1H, J = 8.5 Hz, ArH), 7.11 (d, 1H, J = 7.5 Hz, ArH), 7.25 (t, 1H, J = 7.5 Hz, ArH), 7.32 (d, 1H, J = 7.5 Hz, ArH); MS (CI) m/z 254 (100); HRCIMS calcd for $C_{16}H_{15}NO_2$ 254.1181, found 254.1192.

Pharmacological Methods. Subjects. Adult male Sprague-Dawley rats (200−250 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were killed by decapitation, and the whole brains removed and chilled briefly in ice-cold saline. Brains were sliced with the aid of a dissecting block,[32] and central striata were then dissected from two coronal sections containing the majority of this region. Tissue was frozen immediately on dry ice and stored at −70 °C until the day of the assay. The animal protocols used in the present experiments were consistent with current NIH principles of animal care and were approved by the University of North Carolina Institutional Animal Care and Use.

Cell Cultures. C-6 glioma cells expressing the rhesus macaque D_1A receptor, (C-6-mD_{1A}; Machida et al., 1992) were grown in DMEM-H medium containing 4500 mg/L glucose, L-glutamine, 5% fetal bovine serum and 600 ng/mL G418 or 2 μg/mL puromycin. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2.

Membrane Preparation. Cells were grown in 75 cm^2 flasks until confluent. The cells were rinsed and lysed with 10 mL of ice cold hypoosmotic buffer (HOB) (5 mM Hepes, 2.5 mM $MgCl_2$, 1 mM EDTA; pH 7.4) for 10 min at 4 °C. Cells were then scraped from the flasks using a sterile cell scraper from Baxter (McGaw Park, IL). Flasks received a final rinse with 5 mL of HOB. The final volume of the cell suspension recovered from each flask was ca. 14 mL. Scraped membranes from several flasks were then combined. The combined cell suspension was homogenized (10 strokes), 14 mL at a time, using a 15 mL Wheaton Teflon-glass homogenizer. The cell homogenates were combined and spun at 43000g (Sorvall RC-5B/SS-34, Du Pont, Wilmington, DE) at 4 °C for 20 min. The supernatant was removed, and the pellet was resuspended (10 strokes) in 1 mL of ice cold HOB for each original flask of cells homogenized. This homogenate was then spun again at 43000g at 4 °C for 20 min. The supernatant was removed, and the final pellet was resuspended (10 strokes) in ice cold storage buffer (50 mM Hepes, 6 mM $MgCl_2$, 1 mM EDTA; pH 7.4) to yield a final concentration of ca. 2.0 mg of protein/mL. Aliquots of the final homogenate were stored in microcentrifuge tubes at −80 °C. Prior to their use for adenylate cyclase assays, protein levels for each membrane preparation were quantified using the BCA protein assay reagent (Pierce, Rockford, IL) adapted for use with a microplate reader (Molecular Devices; Menlo Park, CA).

Dopamine Receptor Binding Assays. Frozen rat striata were homogenized by seven manual strokes in a Wheaton Teflon-glass homogenizer in 8 mL of ice cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$ (pH 7.4). Tissue was centrifuged at 27000g for 10 min, the supernatant was discarded, and the pellet was homogenized (five strokes) and resuspended in ice cold buffer and centrifuged again. The final pellet was suspended at a concentration of 2.0 mg wet weight/mL. The amount of tissue added to each assay tube was 1.0 mg, in a final assay volume of 1.0 mL. D_1 receptors were labeled with [^{3}H]SCH23390 (0.30 nM); D_2 receptors were labeled with [^{3}H]-spiperone (0.07 nM); and unlabeled ketanserin (50 nM) was added to mask binding to 5-HT_2 sites. Total binding was defined as radioligand bound in the absence of any competing drug. Nonspecific binding was estimated by adding unlabeled SCH23390 (1 μM) or unlabeled chlorpromazine (1 μM) for D_1 and D_2 receptor binding assays, respectively. As an internal standard, a competition curve with six concentrations of unlabeled SCH23390 (D_1 binding) or chlorpromazine (D_2 binding) was included in each assay. Triplicate determinations were made for each drug concentration. Assay tubes were incubated at 37 °C for 15 min, and binding was terminated by filtering with ice cold buffer on a Skatron 12-well cell harvester (Skatron, Inc., Sterling, VA) using glass fiber filter mats (Skatron no. 7034). Filters were allowed to dry, and 1.0 mL of Optiphase HI-SAF II scintillation fluid was added. Radioactivity was determined on an LKB Wallac 1219 Rack-Beta liquid scintillation counter (Wallac, Gaithersburg, MD). Tissue protein levels were estimated using the BCA protein assay reagent (Pierce, Rockford, IL).

Data Analysis for Radioreceptor Assays. Binding data from each assay were analyzed separately. Data were normalized by expressing the average dpm at each competitor concentration as a percentage of total binding. These data were then subjected to nonlinear regression analysis using the algorithm for sigmoidal curves in the curve-fitting program InPlot (Graphpad Inc.; San Francisco, CA) or EBDA and LIGAND software, as adapted for the IBM-PC by McPherson,[33] to generate $K_{0.5}$ values and a Hill coefficient (n_H) for each curve. Analysis of the residuals indicated an excellent fit; r values were above 0.99 for all curves in the present experiments.

Adenylate Cyclase Activity in Rat Striatum. The automated HPLC method of Schulz and Mailman[34] was used to measure adenylate cyclase activity by separating cAMP from other labeled nucleotides. Briefly, striatal tissue from rat was homogenized with eight manual strokes in a Wheaton Teflon-glass homogenizer in 5 mM HEPES buffer (pH 7.5) containing 2 mM EGTA (50 mL/g tissue). Following the addition and mixing of 50 mL/g of 50 mM HEPES buffer (pH 7.5) containing 2 mM EGTA, a 20 μL aliquot of this tissue homogenate was added to a prepared reaction mixture (final volume of 100 μL) containing 0.5 mM ATP, 0.5 mM isobutylmethylxanthine, [^{32}P]-ATP (0.5 μCi), 1 mM cAMP, 2 mM $MgCl_2$, 100 mM HEPES buffer, 2 μM GTP, 0−100 μM dopamine, DHX, or SKF38393, 10 mM phosphocreatine, and 5 units of creatine phosphokinase. Triplicate determinations were performed for each drug concentration. The reaction proceeded for 15 min at 30 °C and was terminated by the addition of 100 μL of 3% sodium dodecyl sulfate (SDS). Proteins and much of the noncyclic nucleotides were precipitated by addition of 300 μL each of 4.5% $ZnSO_4$ and 10% $Ba(OH)_2$. Samples were centrifuged (10000g for 8−9 min), and the supernatants were injected on an HPLC system (Waters Z-module or RCM 8 × 10 module equipped with a C18, 10 micron cartridge). The mobile phase was 150 mM sodium acetate (pH 5.0) with 23% methanol. A UV detector (254 nm detection) was used to quantify the unlabeled cAMP added to the samples to serve as internal standard. The radioactivity in each fraction was determined by a flow-through radiation detector (Inus Systems, Tampa, FL) using Cerenkov counting. Sample recovery was based on UV measurement of total unlabeled cAMP peak areas quantified using PE Nelson (Cupertino, CA) Model 900 data collection modules and TurboChrom software. Tissue protein levels were estimated using the BCA protein assay reagent (Pierce, Rockford, IL).

Adenylate Cyclase Assay in C-6m-D_1A Cells. Frozen membranes were thawed and added to assay tubes (10 μg of protein/tube) containing a prepared reaction mixture [100 mM Hepes, (pH 7.4), 100 mM NaCl, 4 mM $MgCl_2$, 2 mM EDTA, 500 μM isobutylmethylxanthine (IBMX), 0.01% ascorbic acid, 10 μM pargyline, 2 mM ATP, 5 μM GTP, 20 mM phosphocreatine, 5 units of creatine phosphokinase (CPK), 1 μM propranolol] and selected drugs. The final reaction volume was 100 μL. Basal cAMP activity was determined by incubation of tissue in the reaction mixture with no drug added. Tubes were assayed in duplicate, and after a 15 min incubation at 30 °C, the reaction was stopped by the addition of 500 μL of 0.1 N HCl. Tubes were vortexed briefly and then spun in a BHG HermLe Z 230 M microcentrifuge for 5 min at 15000g to precipitate particulates.

Radioimmunoassay (RIA) of cAMP. The concentration of cAMP in each sample was determined with an RIA of acetylated cAMP, modified from that previously described.[35] Iodination of cAMP was performed using a method reported

by Patel and Linden.[36] Assay buffer was 50 mM sodium acetate buffer with 0.1% sodium azide (pH 4.75). Standard curves of cAMP were prepared in buffer at concentrations of 2−500 fmol/assay tube. To improve assay sensitivity, all samples and standards were acetylated with 10 μL of a 2:1 solution of triethylamine−acetic anhydride. Samples were assayed in duplicate. Each assay tube (total volume 300 μL contained 25 μL of each sample, 75 μL of buffer, 100 μL of primary antibody (sheep, anti-cAMP, 1:100000 dilution with 1% BSA in buffer) and 100 μL of [^{125}I]cAMP (50 000 dpm/100 μL of buffer). Tubes were vortexed and stored at 4 °C overnight (ca. 18 h). Antibody-bound radioactivity was separated by the addition of 25 μL of BioMag rabbit, anti-goat IgG (Advanced Magnetics, Cambridge, MA), followed by vortexing and incubation at 4 °C for 1 h. To these samples was added 1 mL of 12% polyethylene glycol/50 mM sodium acetate buffer (pH 6.75), and tubes were centrifuged at 1700*g* for 10 min. Supernatants were aspirated, and radioactivity in the pellet was determined using an LKB Wallac gamma counter (Gaithersburg, MD).

Data Analysis for Adenylate Cyclase Studies. Data for each sample were expressed initially as pmol/(mg/min) cAMP. Baseline values of cAMP were subtracted from the total amount of cAMP produced in each drug condition. Data for each drug were expressed relative to the stimulation produced by 100 μM DA.

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