Conformational Analysis of D_1 Dopamine Receptor Agonists: Pharmacophore Assessment and Receptor Mapping†

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Computer-aided conformational analysis was used to characterize the agonist pharmacophore for D_1 dopamine receptor recognition and activation. Dihydrexidine (DHX), a high-affinity full agonist with limited conformational flexibility, served as a structural template that aided in determining a molecular geometry that would be common for other more flexible, biologically active agonists. The intrinsic activity of the drugs at D_1 receptors was assessed by their ability to stimulate adenylate cyclase activity in rat striatal homogenates (the accepted measure of D_1 receptor activation). In addition, affinity data on 12 agonists including six purported full agonists (dopamine, dihydrexidine, SKF89626, SKF82958, A70108, and A77636), as well as six less efficacious structural analogs, were obtained from D_1 dopamine radioreceptor-binding assays. The active analog approach to pharmacophore building was applied as implemented in the SYBYL software package. Conformational analysis and molecular mechanics calculations were used to determine the lowest energy conformation of the active analogs (i.e., full agonists), as well as the conformations of each compound that displayed a common pharmacophoric geometry. It is hypothesized that DHX and other full agonists may share a D_1 pharmacophore made up of two hydroxy groups, the nitrogen atom (ca. 7 Å from the oxygen of *m*-hydroxyl) and the accessory ring system characterized by the angle between its plane and that of the catechol ring (except for dopamine and A77636). For all full agonists (DHX, SKF89626, SKF82958, A70108, A77636, and dopamine), the energy difference between the lowest energy conformer and those that displayed a common pharmacophore geometry was relatively small (<5 kcal/mol). The pharmacophoric conformations of the full agonists were also used to infer the shape of the receptor binding site. Based on the union of the van der Waals density maps of the active analogs, the excluded receptor volume was calculated. Various inactive analogs (partial agonists with D_1 $K_{0.5}$ > 300 nM) subsequently were used to define the receptor essential volume (i.e., sterically intolerable receptor regions). These volumes, together with the pharmacophore results, were integrated into a three-dimensional model estimating the D_1 receptor active site topography.

Introduction

Receptors for the neurotransmitter dopamine exist in two general pharmacological classes ("D_1-like" and "D_2-like"). D_1-like receptors consist of products of at least two distinct genes called D_{1A}[1−4] and either D_{1B}[5] or D_5 [6]. While these receptors have been cloned, the molecular mechanisms of ligand binding and of receptor activation of transduction mechanisms are some of the important questions that remain unanswered. It does appear, however, that these D_1-like receptors have selectivity for 1-phenyltetrahydrobenzazepines (e.g., SCH23390) and are often, but not always, positively coupled to the enzyme adenylate cyclase. Conversely, the D_2-like receptors (representing at least four molecular variants termed D_{2long}, D_{2short}, D_3, and D_4) have little affinity for SCH23390 but avidly bind substituted benzamides (e.g., sulpiride) and butyrophenones (e.g., spiperone). As a class, the D_2-like receptors, although having multiple transduction mechanisms, generally are linked to inhibitory G protein mechanisms (e.g., G_i-mediated inhibition of adenylate cyclase activity). Despite the awareness of the critical roles of dopamine receptor function (in both the brain and periphery), it is only recently that the importance of the D_1 receptor has been appreciated widely (see ref **7** for review).

The availability of the selective D_1 antagonist SCH23390 was key in helping to elucidate important functional roles for central D_1 receptors, as well as in showing that D_1 and D_2 receptors have important functional interactions.[8,9] Until recently, however, re-

† Abbreviations: DHX, dihydrexidine [(+)-(6a*R*,12b*S*)-*trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine]; SKF89626, 4-(3′,4′-dihydroxyphenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine; Ro 21-7767, *trans*-6-methyl-9,10-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine; SKF82958, 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; *N*-benz-5,6-ADTN, 1,2,3,4-tetrahydro-2-(benzylamino)-5,6-dihydroxynaphthalene; *N*-benz-6,7-ADTN, 1,2,3,4-tetrahydro-2-(benzylamino)-6,7-dihydroxynaphthalene; A70108, (1*R*,3*S*)-1-(aminomethyl)-5,6-dihydroxy-3-phenylisochroman; A70360, (1*S*,3*R*)-1-(aminomethyl)-5,6-dihydroxy-3-phenylisochroman; A68930, (±)-1-(aminomethyl)-5,6-dihydroxy-3-phenylisochroman; A77636, (1*R*,3*S*) 3-(1′-adamantyl)-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyran.

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search was limited by the fact that none of the available D_1 agonists were full agonists relative to dopamine itself. Specifically, agonists based on the 1-phenyl-3-tetrahydrobenzazepine nucleus (e.g., SKF38393 or fenoldopam) caused a maximal stimulation of adenylate cyclase that is one-half (or less) that caused by dopamine; by the commonly accepted biochemical criterion, these drugs are only partial agonists.

Several years ago, we described the pharmacological characterization and synthesis of dihydrexidine (DHX, (±)-10,11-dihydroxyhexahydrobenzo[*a*]phenanthridine[10−12]). We have shown that this compound not only is a high-affinity ligand for D_1 receptors (racemate $K_{0.5} = 5$ nM, $K_{Dhigh} = 1.5$ nM, $K_{Dlow} = 38$ nM) but is as efficacious as dopamine (and much more potent) in stimulating adenylate cyclase in rat striatum.[12] Subsequent functional studies supported the hypothesis that a full D_1 agonist had significantly different actions than the available partial agonists and therefore was of pragmatic pharmacological importance.[13−16]

The design of dihydrexidine, like other rationally designed drugs, evolved from hypotheses concerning the pharmacophore geometry required for molecular reactivity. Early work by Cannon[17] and McDermed et al.[18] led to a working model of the dopamine pharmacophore. Later, Nichols[19] noted several differences between the D_1 versus D_2 pharmacophores and suggested that agonist activity required proper positioning of an additional accessory ring system, as well as proper orientation of the lone pair of electrons on the nitrogen atom. The limited D_1 receptor activation inherent in the 1-phenyltetrahydrobenzazepine class led Nichols and coworkers to investigate members of the tetrahydroisoquinoline series. From these structure−activity relationships (SARs), it was hypothesized that the D_1 pharmacophore could be described as "*β*-dopamine"[17] and that the orientation of the pendent phenyl ring (i.e., the accessory ring system) was important for receptor activation.[19] These hypotheses led to the development of the thienopyridine derivative 4-(3′,4′-dihydroxyphenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (later named SKF89626), the first full D_1 agonist.[20,21] These data demonstrated that the receptor region interacting with the accessory "phenyl" group could also accommodate a thiophene moiety. Although SKF89626 was a full D_1 agonist, it had relatively modest D_1 affinity and modest D_1:D_2 selectivity and also would not cross the blood−brain barrier.[21]

Such data provided the foundation for a search for a relatively rigid molecule in which the conformation of the pharmacophore would be restricted. This led to the design and synthesis of a novel class of more structurally rigid dopamine ligands (hexahydrobenzo[*a*]phenanthridines). As noted earlier, one member of this series (dihydrexidine) is a high-affinity, bioavailable, full D_1 agonist and, as such, has become a very useful pharmacological probe.[12−16] Moreover, because of the rigid nature of this structural class, DHX and its congeners can be extremely useful for identifying the three-dimensional molecular recognition characteristics of D_1 receptors.

Earlier, we had developed a working model of the D_1 antagonist pharmacophore.[22] This model was based on the SARs of a series of tetrahydroisoquinolines and predicted the active conformation of the D_1 antagonist pharmacophore.[23] The present work complements our previous analyses and describes the D_1 receptor agonist pharmacophore based on the SARs for dihydrexidine and various related full agonists. Several papers published recently were concerned with the conformational analysis and SARs of various ligands of D_1 receptor.[24−29] These papers have suggested preferred conformations for interaction of the D_1 receptor ligands with the receptor, and several have agreed with the notion that that the preferred conformation of the pendent benzene ring is almost coplanar with the catechol ring.[11] In this paper, we have applied the active analog approach (AAA)[30] as the modeling strategy to obtain the 3D pharmacophore model of the D_1 receptor; this involves using analogs that have been determined to be pharmacologically "active" (i.e., full D_1 agonists) and "inactive" (i.e., partial agonists with D_1-binding affinity $K_{0.5}$ > 300 nM) ligands. The implementation of the AAA method was significantly aided by the availability of the highly active and relatively rigid full agonist dihydrexidine developed in these laboratories.[10−12] Thus, this study defines the pharmacophore geometry shared by all full D_1 agonists and proposes a three-dimensional D_1 receptor active site model.

Materials and Methods

Materials. [^{3}H]SCH23390 (ca. 70 Ci/mmol) was synthesized as described by Wyrick and Mailman.[31] [^{3}H]Spiperone was purchased from Amersham Corp. (Arlington Heights, IL). [^{32}P]ATP was supplied by New England Nuclear (Boston, MA), and HEPES buffer was purchased from Research Organics Inc. (Cleveland, OH).

SCH23390 was a gift from Schering Corp. (Bloomfield, NJ). SCH23390 also was purchased from Research Biochemicals Inc. (Natick, MA), as were SKF82958 and SKF38393. Quinpirole (LY171555) was a gift from Lilly Research Laboratories (Indianapolis, IN). Ro 21-7767 was a gift from Hoffmann La Roche Inc. (Nutley, NJ). Dopamine and chlorpromazine were purchased from Sigma Chemical Co. (St. Louis, MO). Ketanserin and domperidone were gifts of Janssen Pharmaceutica (Beerse, Belgium, and New Brunswick, NJ). A68930 (the racemic mixture that contains A70108 as its active component) was a gift of Abbott Pharmaceuticals (Chicago, IL). The other compounds were synthesized by published methods.[11,20,32]

Tissue Preparation. Male Sprague−Dawly rats weighing 200−400 g were decapitated and the brains quickly removed and placed into ice cold saline. After a brief chilling period, brains were sliced into 1.2 mm coronal slices with the aid of a dissecting block similar to that described by Heffner et al.[33] The striatum was dissected from two slices containing the majority of this region, and the tissue was either used immediately or stored at −70 °C until the day of the assay.

Radioligand Receptor Binding. [^{3}H]SCH23390-Labeled D_1 Receptors. Radioligand binding followed the method of Schulz et al.[34] with minor modifications. After dissection, rat striata were homogenized by seven manual strokes in a Wheaton Teflon−glass homogenizer with ice cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$, pH 7.4 (25 °C). Tissue was centrifuged at 27000*g* (Sorvall RC-5B/SS-34 rotor; DuPont, Wilmington, DE) for 10 min; the supernatant was discarded. The pellet was homogenized (five strokes), resuspended in ice cold buffer, and centrifuged again. The final pellet was suspended at a concentration of ca. 2.0 mg of wet weight/mL. Assay tubes (1 mL final volume) were incubated at 37 °C for 15 min. Nonspecific binding of [^{3}H]SCH23390 (ca. 0.25 nM) was defined by adding unlabeled SCH23390 (1 *μ*M). Binding was terminated by filtering with 15 mL of ice cold buffer on a Skatron or Brandel cell harvester (Skatron Inc., Sterling, VA, or Brandel Inc., Gaithersburg, MD) using glass fiber filter mats (Skatron no. 7034, or Brandel GF/B). Filters were allowed to dry, and 2−4 mL of Scintiverse E (Fischer Scientific Co., Fair Lawn, NJ) was added. After shaking for

30 min, radioactivity was determined on an LKB-1219 Rack-Beta liquid scintillation counter. Tissue protein levels were estimated using the Folin reagent method of Lowry et al.[35] adapted to a Technicon Autoanalyzer I (Tarrytown, NY).

[^{3}H]Spiperone-Labeled D_2 Receptors. The binding procedure and protein analysis were identical with that described for [^{3}H]SCH23390 binding. Nonspecific binding of [^{3}H]spiperone (ca. 0.07 nM) was defined by adding unlabeled chlorpromazine (1 μM). Ketanserin tartrate (50 nM) was used to mask binding of [^{3}H]spiperone to serotonin receptors.

Data Analysis. The radioreceptor and functional data were analyzed using the program Inplot (GraphPad Inc., San Diego, CA). A nonlinear regression program was used to calculate a $K_{0.5}$ and a Hill coefficient. As expected, the Hill coefficients for all of the active compounds (all agonists) were significantly <1. Therefore, drug affinities are expressed as $K_{0.5}$ values rather than K_i values. The $K_{0.5}$ corrects for intraexperimental differences in the amount of radioligand used and thus permits better interassay and interlaboratory comparisons; in the case where $n_H = 1$, $K_{0.5} = K_i$. Adenylate cyclase assays were used to determine the D_1 receptor functional activity of the drugs, and the results are expressed as their 50% effective concentration (EC_{50}) as well as stimulation relative to maximal dopamine activity (100 μM). Values are the average of at least two separate experiments.

Dopamine-Sensitive Adenylate Cyclase Assay. The automated HPLC method of Schulz and Mailman[36] was used to measure the adenylate cyclase activity. Briefly, striatal tissue was removed and homogenized at 50 mL/g of tissue in 5 mM HEPES buffer (pH 7.5) containing 2 mM EGTA. After eight manual strokes with a Wheaton Teflon−glass homogenizer, an additional 50 mL/g 100 mM HEPES−2 mM EGTA was added and mixed with one additional stroke. A 20 μL aliquot of this tissue homogenate was added to a prepared reaction mixture, yielding a final volume of 100 μL containing 0.5 mM ATP, 0.5 mM IBMX, [^{32}P]ATP (0.5 μCi), 1 mM cAMP, 2 mM $MgCl_2$, 0.7 mM HEPES buffer, 2 μM GTP, 0−100 μM dopamine and/or drug, 10 mM phosphocreatine and 5 U of creatine phosphokinase. The reaction was initiated by transferring the samples from an ice bath to a water bath at 30 °C and terminated 15 min later by 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% $Ba(OH)_2$ to each incubation tube. The samples were centrifuged at 10000*g* for 6 min and the supernatants immediately removed and loaded in an ISIS instrument (Isco Inc., Lincoln, NB) to separate cAMP from other labeled nucleotides. The HPLC separations were carried out with a Waters Z-module or RCM 8 × 10 module equipped with a C18 column, 10 mm cartridge, using a mobile phase of 150 mM sodium acetate−20% methanol, adjusted to pH 5.0 with concentrated HCl prior to filtration (0.2 μm) and degassing under vacuum. A flow rate of ca. 4 mL/min was used for separation. The autoinjector was programmed for a 2 min injection interval, with a rinse between samples. A UV detector equipped for 254 nm detection triggered collection of the cAMP fractions via a FOXY fraction collector (Isco Inc., Omaha, NE) with a three-way diversion valve. Unlabeled cAMP added to the samples provided the source of UV absorbance and served as an internal standard. Peak areas were quantified by the Nelson analytical chromatography data system (PE Nelson Systems Inc., Cupertino, CA), and the radioactivity in each fraction was determined by liquid scintillation spectroscopy. Percent stimulation was calculated based on total radioactivity corrected for chromatographic peak areas.

Computer-Assisted Conformational Analysis and Molecular Modeling Methods. The molecular modeling studies presented in this paper involved the following analytical steps. (1) The tentative pharmacophoric elements of the D_1 receptor were determined based on known SARs. (2) A rigorous conformational search on the active compounds was performed to determine their lowest energy conformation(s). (3) Conformationally flexible superimposition of these compounds was done using the MULTIFIT routine (as described below) to determine their common (pharmacophoric) conformation. (4)

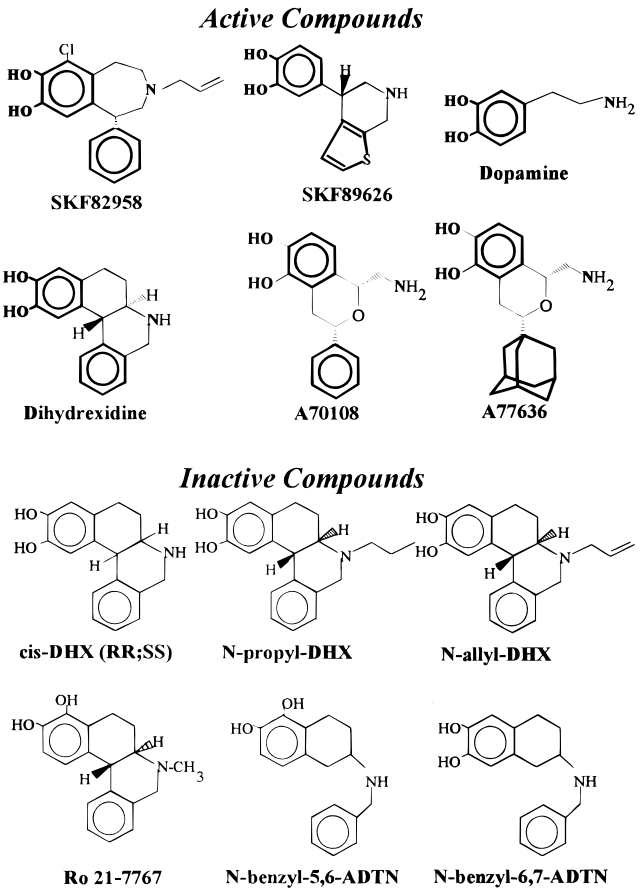

Figure 1. Chemical structures of the ligands used in the molecular modeling study of the D_1 dopamine receptor. The ligands were divided into two groups (active and inactive) based on their pharmacological properties. See Materials and Methods and Table 1 for criteria for this division. The hypothesized pharmacophoric elements are shown in boldface.

Similar conformational analyses were performed for inactive compounds, and the superimposition of inactive compounds in pharmacophoric conformations with the active compounds was made to determine steric limitations in the active site. Where appropriate, the geometry of each inactive molecule was obtained by modifying the chemical structure of the relevant active analogs followed by the energy minimization of the resulting structure. (5) Finally, an evaluation was made of excluded receptor volume and shape as spatial equivalents of the volume and shape of the pharmacophore. All molecular modeling studies were performed with the multifaceted molecular modeling software package SYBYL (Tripos Associates Inc., St. Louis, MO; version 5.5). Implementation of each of these routines is described briefly below.

Pharmacophoric Elements of D_1 Receptor Agonists. The experimental SAR data obtained earlier[11,12] and in the course of this work were used to divide the ligands into two groups based on their affinity and intrinsic activity at rat striatal D_1 receptors: active (full agonists) and inactive (partial agonists with a D_1 $K_{0.5}$ > 300 nM). This division is central to the AAA[30] and its application to D_1 pharmacophore evaluation and receptor site mapping. Based on the experimental SAR data[11,12] and "β-phenyldopamine" hypothesis of D_1 pharmacophore,[19] the following functional groups of agonists were defined as key elements of the D_1 agonist pharmacophore (Figure 1): the two hydroxyl groups of the catechol ring, the nitrogen, and (except for dopamine) an accessory hydrophobic group (e.g., the aromatic ring in dihydrexidine or SKF82958). Thus, the task of molecular modeling analysis was to identify a (pharmacophoric) conformation for each compound where these key pharmacophoric elements were spatially arranged in a way similar for all active compounds.

Lowest Energy Conformations of Active Compounds. Construction of the Pharmacophore. The evaluation of

the D_1 agonist pharmacophore was based on the following three-step routine. Step 1: Perform a conformational search on each of the agonists to identify its lowest energy conformation(s). Depending upon the agonist structure, we applied either a random conformational search routine, a systematic search, or a combination of both as implemented in SYBYL. After some experimenting, the random search (as opposed to the systematic search) was selected as the best searching tool for ring-containing compounds such as DHX or SKF89626, for the following reasons. In a random search process as implemented in SYBYL, the torsional angles in a ring are perturbed randomly, and a resulting conformation is optimized at each step of the search. The optimized conformation is then compared with the previously stored conformations and is stored in the database only if it is different from them. Thus, the conformations produced by the random conformational search are fully optimized and can be used immediately for further analysis. On the other hand, the systematic ring search routine, although more rigorous in nature, produces ring conformations that require further optimization and comparison for similarity.

Step 2: Find common low-energy conformations for all of the compounds. The commonality was assessed by comparing the distances between each of the hydroxyl oxygens and the nitrogen and the angle between the planes of the catechol ring and the accessory ring.

Step 3: Find flexible superimposition of all the agonists in their most common conformations with the MULTIFIT routine as implemented in SYBYL using dihydrexidine as a template compound and a spring force constant of 20 kcal/mol to reinforce superimposition of equivalent pharmacophoric atoms of all the agonists and those of DHX.

D_1 Receptor Mapping. The "pharm" configurations of the active molecules also were used to map the volume of the receptor site available for ligand binding. The steric mapping of the D_1 receptor site, using the MVolume routine in SYBYL, involved the construction of a pseudoelectron density map for each of the active analogs superimposed in their pharmacophore conformations. A union of the van der Waals density maps of the active compounds defines the receptor-excluded volume.[30]

The essential feature of the AAA is a comparison of active and inactive molecules. A commonly accepted hypothesis to explain the lack of activity of inactive molecules that possess the pharmacophoric conformation is that their molecular volume, when presenting the pharmacophore, exceeds the receptor-excluded volume. This additional volume apparently is filled by the receptor and unavailable for ligand binding; this volume is termed the receptor essential volume.[30] Following this approach, the density maps for each of the inactive compounds (in their "pharm" conformations superimposed with those of active compounds) were constructed; the difference between the combined inactive compounds density map and the receptor-excluded volume represents the receptor essential volume. These receptor-mapping techniques supplied detailed topographical data that allowed a steric model of the D_1 receptor site to be proposed.

Results

Pharmacological Data. The classification of ligands as active or inactive was based on data from our laboratory (Table 1) with the exception of those compounds that were unavailable to us (A70168 and A77636; see below). Six compounds were chosen as active based on three criteria: They had affinity for the D_1 receptor ($K_{0.5}$ < 300 nM), they could increase cAMP synthesis in rat striatal membranes to the same degree as dopamine, and this increase could be blocked completely by the D_1 antagonist SCH23390. The compounds that met these criteria were dopamine, DHX, SKF89626, SKF82958, A70168, and A77636. As is shown in Table 1, all of these compounds caused similar (complete) activation of dopamine-sensitive adenylate cyclase in this preparation and had $K_{0.5}$'s ranging from 267 nM (dopamine) to 0.9 nM (A70108). The data for DHX is that for the 6a*R*, 12b*S*-active enantiomer rather than for the racemate as has been previously reported.[10−12]

Table 1. Pharmacological Analysis of DHX and Related Compounds[a]

drug	affinity, $K_{0.5}$ (nM) D_1	affinity, $K_{0.5}$ (nM) D_2	adenylate cyclase EC_{50} (nM)	max stimulation of adenylate cyclase (% vs DA)
dopamine	267	36	5000	100
(+)-DHX	2.3	43.8	30	120
SKF89626	61	142	700	120
SKF82958	4	73	491	94
A70108[a]	0.9	41	1.95	96
A77636[b]	31.7	1290	5.1	92
cis-DHX	$>10^3$	$>10^3$	$>10^4$	17
N-propyl-DHX	326	27	$>10^4$	36
N-allyl-DHX	328	182	$>10^4$	32
Ro21-7767	477	61	$>10^4$	22
N-benz-5,6-ADTN	$>10^3$	$>10^3$	$>10^3$	38
N-benz-6,7-ADTN	$>10^3$	335	$>10^4$	25
quinpirole[c]	$>10^4$	49	*d*	*d*
domperidone[c]	$>10^3$	1.0	*d*	*d*
SCH23390[c]	0.5	600	0	0

[a] See Results section for specific information. Most of these data have been previously reported;[11,12,33] recent reanalysis has verified these values. [b] Values from refs 38 and 41. [c] Included only for reference. [d] Not determined.

As noted above, samples of neither A70108 nor A77636 were available to us when these studies were conducted. We did have a sample of A68930, the racemic mixture consisting of A70108 (the 1*R*,3*S*-enantiomer) and A70360 (1*S*,3*R*-enantiomer) which we evaluated in our assay systems. Consistent with the data of DeNinno et al.,[37−39] we found that A68930 had high affinity for striatal D_1 receptors. On the other hand, we found that this racemic mixture was a *full* agonist in rat striatal homogenates; DeNinno et al.[37,39] had reported that A68930, like SKF38393, was only a partial agonist in this tissue preparation. On the basis of our data and on the following reasoning, we included A70108 as an active compound in our modeling. DeNinno et al.[37,39] reported that it had an affinity more than 1000-fold greater than the less active enantiomer A70360.[37,39] If the higher affinity compound was actually a partial agonist, the racemic mixture would, of necessity, also be a partial agonist (see, for example, Watts et al.[40]). Thus, we have included A70108 as an active compound and have calculated its $K_{0.5}$ based on the $K_{0.5}$ we determined for A68930. It is interesting that this example highlights the pitfalls of performing modeling studies using data from different laboratories.

Pharmacophore Evaluation. Numerous structural analogs of DHX, as well as other related ligands, were assessed for their pharmacological properties at dopamine receptors. Each ligand was tested in radioligand-binding assays to determine its affinity for D_1 ([^{3}H]-SCH23390)- and D_2 ([^{3}H]spiperone)-labeled binding sites. In addition, the ability of each ligand to activate the D_1 receptor was evaluated functionally using adenylate cyclase assays. Together, these variables gave an estimate of the affinity, selectivity, and intrinsic activity of each ligand for D_1 dopamine receptors. The pharmacological data of those compounds that met the requirements for inclusion into the molecular modeling study are summarized in Table 1. These SAR results indicate that D_1 receptor binding is diminished by nitrogen alkylation, while the *N*-propyl substitution

significantly enhances D_2 receptor-binding affinity as has been shown previously.[11,12] Moreover, recognition and activation of the D_1 receptor is also sensitive to the positionings of the *m*-hydroxyl group and the pendent ring system.

To evaluate the D_1 receptor pharmacophore and topography, the AAA requires two groups of ligands: active and inactive. To enhance the quality of the pharmacophore evaluation and receptor mapping, we selected only those D_1 agonists that were able to activate the receptor fully (full agonists) for inclusion into the active group. Likewise, to minimize the risk of ill-defining inaccessible regions of the receptor, the inactive group included only those ligands that were partial agonists and had low affinity for the D_1 receptor ($K_{0.5}$ > 300 nM). Thus, those compounds that possessed moderate to high D_1 affinity, but were only partial agonists, were not used in this study. The structures of the active and inactive ligands that were used in the molecular modeling analysis are shown in Figure 1. The active analogs represented five different structural classes (hexahydrobenzo[*a*]phenanthridines, thienopyridines, phenyltetrahydrobenzazepines, isochromans, and phenethylamines), while inactive analogs were from two classes (hexahydrobenzo[*a*]phenanthridines and aminotetralins).

Prior to undertaking this work, certain predictions had to be made concerning the active enantiomer of DHX, as well as the thienopyridine compound (SKF89626); neither of these full agonists had been resolved at that time. It was known that the absolute configuration of the active enantiomer of the D_1 partial agonist SKF38393 resides in the *R*-enantiomer.[41] In addition, 3′,4′-dihydroxynomifensine, a D_1 agonist structurally similar to SKF89626, had been resolved, and the absolute configuration of its more active enantiomer was known.[42] Using these molecules as stereochemical tools, comparisons were made with DHX and SKF89626, and on the basis of the degree of fit, it was predicted that (6a*R*,12b*S*)-DHX and (*R*)-SKF89626 were the active enantiomers. These predicted active enantiomers subsequently were used for the duration of the modeling study. Recently, we have demonstrated that (6a*R*,-12b*S*)-DHX is, in fact, the active enantiomer.[43]

Conformational Analysis of D_1 Dopamine Receptor Ligands. Simple conformational analysis of cyclic compounds such as DHX or SKF89626 with mechanical models suggests that their stable conformations most likely should be different combinations of (twisted) chairs and boats that could be constructed manually using computer graphics and then optimized. In order to ensure the accuracy of conformational analysis and identify the lowest energy conformations of cyclic compounds rigorously, however, we performed a computerized conformational search as follows.

Due to the apparent similarity of D_1 agonists, we initially performed a conformational search on the most rigid agonists, DHX and SKF89626. Application of the random search on the rings of both structures resulted in four conformers for each of the molecules (Tables 2 and 3) that can be described as different twisted chairs (Figure 2). The four conformers of DHX were compared pairwise with the four conformers of SKF89626. Similarity was based on two criteria: the angle between planes formed by the catechol ring and pendent aromatic ring and the rms (root mean square) deviation between relevant pharmacophoric atoms (two oxygens of catechol rings and the amine nitrogen). From this analysis, DHX-1 (also the lowest energy conformer of DHX) was chosen as the template (Tables 2 and 3). SKF89626-2 was chosen as the propharmacophore conformer of this compound, and it is the second lowest energy conformer of SKF89626 (Table 3). The geometry of all other compounds was optimized, and the resulting

Table 2. Conformers of DHX

conformer	energy (kcal/mol)	distance (Å) 1[a]	distance (Å) 2[b]	θ[c] (deg)
DHX-1	11.5	7.9	7.4	48.2
DHX-2	13.4	7.9	7.3	137.4
DHX-3	13.9	7.7	7.2	125.8
DHX-4	21.4	7.8	7.4	26.0

[a] Distance between nitrogen and oxygen of *m*-hydroxyl group. [b] Distance between nitrogen and oxygen of *p*-hydroxyl group. [c] Angle between planes defined by the catechol ring and the pendent aromatic ring.

Table 3. Conformers of SKF89626[a]

conformer	energy (kcal/mol)	distance (Å) 1	distance (Å) 2	θ (deg)	rms deviation from DHX-1[b]
SKF89626-1	8.5	6.6	5.5	94.0	0.79
SKF89626-2	9.0	7.9	7.1	67.1	0.11
SKF89626-3	9.1	7.9	6.7	113.1	0.29
SKF89626-4	9.4	6.6	6.3	86.0	0.57

[a] See definitions of distances 1 and 2 and angle θ in the legend to Table 2. [b] Defined based on the best fit between the three pharmacophoric atoms (nitrogen and two hydroxyl group oxygens of catechol ring).

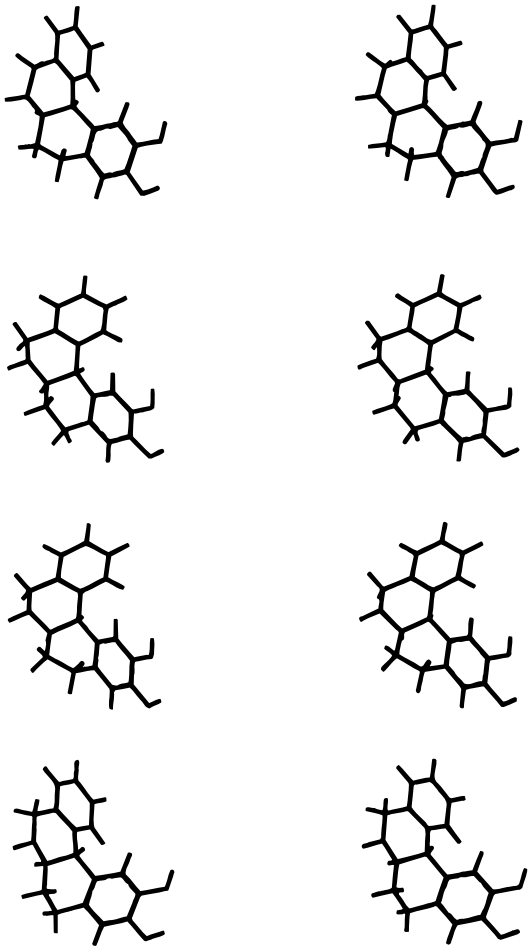

Figure 2. Stereopair view of the four conformers of DHX.

Table 4. Energy and Geometry Calculations of the D_1 Receptor Pharmacophore

	energy (kcal/mol)		d_1 (Å)		d_2 (Å)		angle (deg)	
	global	pharm	global	pharm	global	pharm	global	pharm
dopamine	4.7	9.2	7.9	7.8	7.0	7.4	NA	NA
A77636	12.0	16.3	6.7	8.0	6.4	7.3	NA	NA
SKF82958	13.4	17.9	6.2	7.1	6.4	7.1	94.4	82.0
A70108	12.6	15.4	7.9	7.9	7.2	7.4	94.3	85.0
dihydrexidine	11.5	11.8	7.9	7.8	7.4	7.3	48.2	50.2
SKF89626	8.5	13.0	7.9	8.0	7.1	7.4	67.1	60.1

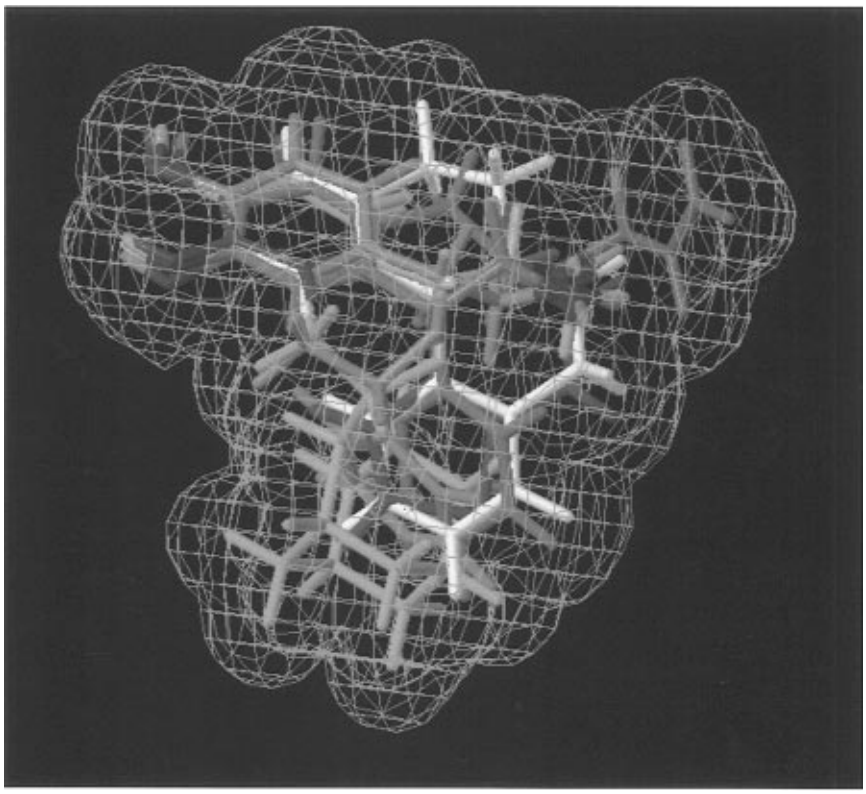

Figure 3. "Pharm" conformations of the six active analogs and the excluded volume map of the D_1 receptor. Note the overlap of the pharmacophoric atoms (*m*- and *p*-hydroxyls, nitrogen, and β-carbon accessory ring substituent). The volume, shown as blue-green wire mesh, is defined by a union of the six active compounds' van der Waals density maps and represents the volume that is available to drugs interacting with the D_1 receptor. The color codes for each of the compounds are DHX, white; SKF89626, purple; SKF82958, green; dopamine, cyano; A70108, orange; and A77636, yellow.

low-energy conformations were compared to the DHX-1 and SKF89626-2 templates for maximum similarity as follows.

The random search analysis of A70108 yielded 21 conformers. Those conformers most resembling DHX-1 and SKF89626-2 were chosen for the subsequent construction of the pharmacophore. These data, along with the lowest energy conformer of each compound, are reported in Table 4. The conformational analysis of SKF82958 that resulted from the random search routine resulted in eight conformers for the seven-membered ring. The conformation of this ring, in which the distance between the two oxygens of the catechol ring and the amine nitrogen was closest to those in DHX-1 and SKF89626-2, was chosen as pharmacophoric. Further conformational search on SKF89626-2 allowed us to obtain the global minimum conformer and the propharmacophoric orientation of the accessory ring (Table 4). The propharmacophore conformation of the adamantyl derivative of A70108 was obtained by replacing the adamantyl radical for the pendent phenyl ring of A70108 and minimizing the potential energy of this compound. Finally, the lowest energy conformation of dopamine was determined via systematic conformational search on the flexible side chain. The propharmacophoric conformation of dopamine (i.e., the one that was used for the subsequent MULTIFIT minimization) was determined by modifying the DHX-1 molecule into dopamine followed by molecular mechanics minimization.

Having determined the lowest energy state of each of the active compounds, the energy of each molecule when it was in the D_1 pharmacophoric configuration was calculated. Since DHX is very active and somewhat rigid, it is logical to assume that the chemical features of the pharmacophore are present in its active spatial relationship. To determine the conformation of each analog that was best able to fit a common pharmacophore geometry, the structures were submitted to MULTIFIT analysis. This routine allows the simultaneous optimization of the geometry of all the molecules while the pharmacophoric atoms are harmonically constrained to their average relative positioning. The best fit pharmacophoric conformers ("pharm") of all six active molecules are shown superimposed in Figure 3, and their "pharm" energies are given in Table 4.

Since the "pharm" conformations of the active analogs represent the optimized orientation adopted by the

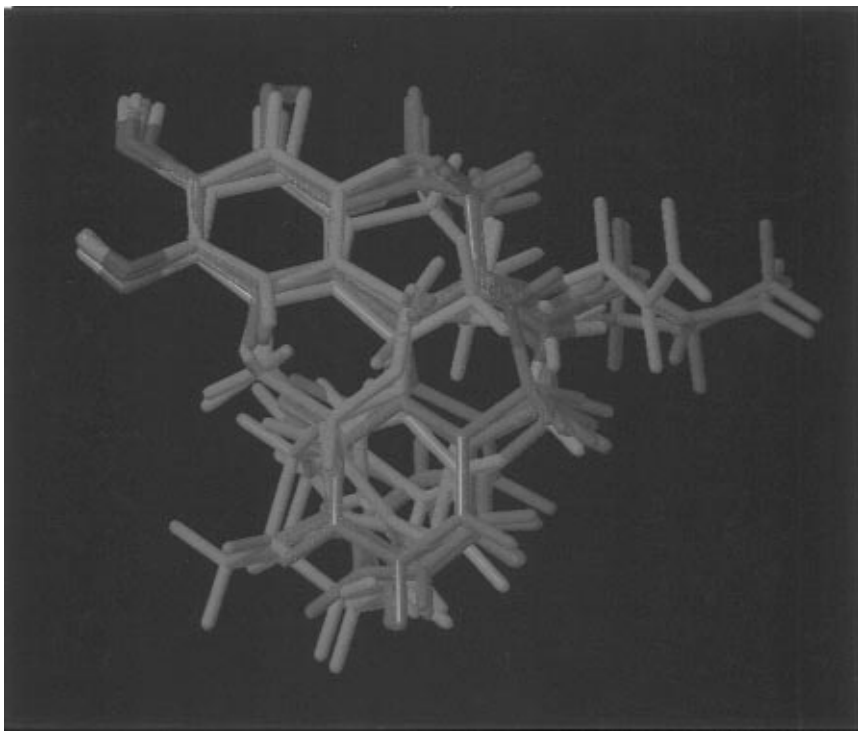

Figure 4. Superimposed pharmacophore conformations of the active and inactive compounds**.** These "pharm" conformations, generated by MULTIFIT calculations, represent low-energy structures produced in Multifit FOLLOWING alignment of the pharmacophoric atoms (*m*- and *p*-hydroxyls, nitrogen, and β-carbon accessory ring substituent) of each compound. See the methods section for additional details of the molecular modeling procedures. The active compounds are shown in green, the inactives in purple, the oxygens in red, and the nitrogens in blue.

molecule to produce a common pharmacophore (i.e., a shared "bioactive" geometry), these conformations were used to evaluate and define the geometry of the D_1 receptor pharmacophore. The parameters used to define the pharmacophore (Table 4) included the distances between pharmacophoric atoms and the orientation of the accessory ring system (e.g., as characterized by the angle between its plane and that of the catechol ring). From these geometric calculations, it thus appears that DHX and other D_1 agonists can share a pharmacophore made up of two hydroxyl groups, a nitrogen atom (ca. 7.8 Å from the *m*-hydroxyl), and an accessory ring system, the latter of which may not necessarily be aromatic (e.g., A77636).

D_1 Receptor Mapping. Once the pharmacophore configuration was defined, the volume available for binding at the receptor site could be mapped. The steric mapping of the D_1 receptor site involved the construction of a pseudoelectron density map for each of the active analogs utilizing Gaussian functions that have been calibrated in terms of van der Waals radii by the MVolume routine in SYBYL. Next, a union of the density maps for the set of active analogs was calculated, and this is termed the "excluded volume map"[30] (Figure 3). This represents that volume that is available to drugs interacting with the receptor (i.e., active volume of the receptor site) as evidenced by the active analogs requiring that volume. By defining the shape of the receptor binding pocket as the complement to the common volume of the active analogs, the receptor regions responsible for recognition and molecular stabilization are naturally positioned in three-dimensional space. This is critical in defining the receptor topography and modeling the active site.

The D_1 receptor site topography was envisioned further by the spatial information described by each of the inactive molecules. The inactive compounds were superimposed with the active compounds as shown in Figure 4. These inactive analogs were used to define the "receptor essential volume" (i.e., inactive volume of the receptor site), a region of the receptor that is not available for interaction with the ligands.[30] For the inactive compounds, it should be noted that even though these molecules possess the pharmacophoric atoms, their inability to be "active" is likely due to some part of their "pharm" conformation interacting with receptor intolerable regions. Therefore, each inactive molecule has a defined receptor essential volume (Figure 5). These receptor essential volumes can be used to define the receptor regions of steric intolerance. For example, it is clear from the receptor essential volumes of *N*-propyl-DHX and *N*-allyl-DHX that the D_1 receptor is intolerable to bulky substitution on this region of the DHX molecule.[11]

An integration of all the inactive volumes with that of the active volume can give a well-defined topographical model of the D_1 receptor. This D_1 receptor site volume model can be complemented with the incorporation of the pharmacophore structural requirements to design a detailed three-dimensional D_1 receptor model (Figure 6). In this hypothetical model of the D_1 receptor, we have labeled the pharmacophoric regions of the D_1 receptor: hydroxyl-binding region, amine-binding region, area of steric occlusion, and accessory-binding region. DHX is shown superimposed on the model to aid the visual and molecular orientation of the pharmacophore. This visual 3D model can provide insights that may be overlooked by conventional 2D modeling. This model also will offer qualitative guidelines that will aid in the design of new ligands; those worthy of synthesis can be used subsequently to further refine the initial model.

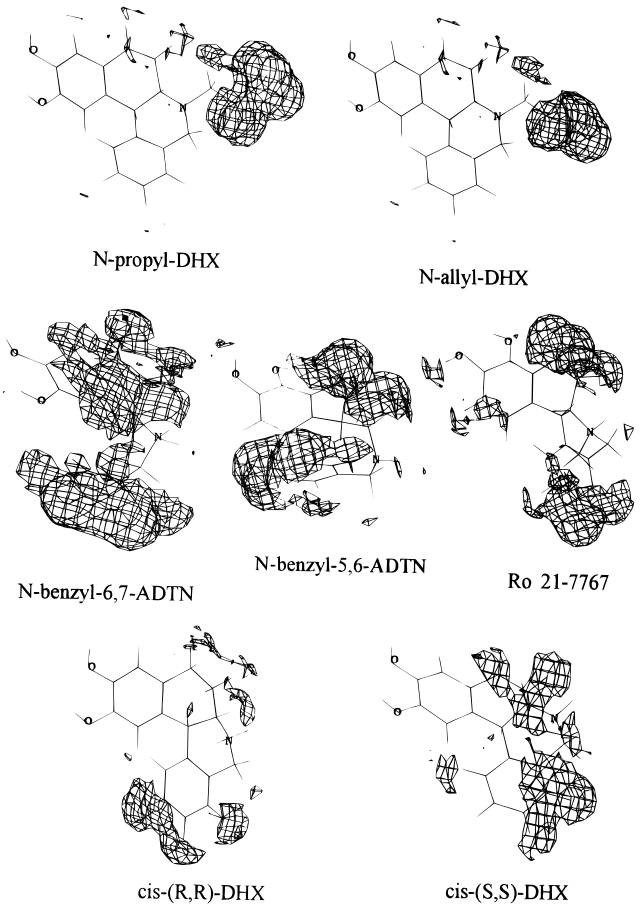

Figure 5. D_1 receptor essential volume. This inactive volume is defined by each of the inactive analogs and displayed as wire mesh. These volumes are calculated by the subtractions of the receptor-excluded volume (Figure 3) from the van der Waals density map of each ligand. These volumes represent regions of the D_1 receptor that may not be available for drug interactions (i.e., are required by the receptor).

Discussion

The goal of this study was to define the geometry of the D_1 dopamine receptor agonist pharmacophore and map the receptor topography. One of the important issues inherent in our approach was the choice of active compounds on which to base our modeling. In this regard, the decision was made to use *only* full D_1 agonists; the most widely used ligand (SKF38393) is a partial agonist. We believed that the *full* D_1 agonists might have more restricted spatial requirements (i.e., needed for full receptor activation) than would partial agonists; the latter might contain aspects of both agonist and antagonist pharmacophores. Although we have attempted to define the criteria we have used rigorously, it should be noted that we have chosen compounds with "full intrinsic activity" (i.e., in the empirical systems for which data are available). Our criteria could be made yet more rigorous by choosing only compounds with "full intrinsic efficacy" (i.e., equal to the endogenous ligand dopamine in *all* systems tested). In a few cases, a compound with full intrinsic activity in some preparations may not have full intrinsic efficacy.[40] Such data, as they become available, should be incorporated into refinements of this model.

While limited in number, the agonists we chose represent five diverse structural classes. Since these drugs can activate the same receptor, it is likely they have a common three-dimensional geometry (pharmacophore) that is responsible for their affinity and functional activity. On the basis of this assumption, it was requisite that we predict the conformation of each agonist that displayed a common pharmacophoric pattern. This pharmacophoric conformation was compared to the conformation of the molecule that was energetically most favorable, to determine whether the pharmacophoric conformation was a feasible entity or a modeling artifact.

In this study, all active compounds were able to attain a common pharmacophoric configuration with a moderate energy penalty (<5 kcal) that represents the difference in energy of the pharmacophoric and the lowest energy conformations. In a biological environment (e.g., the receptor binding site) this penalty should be easily accommodated due to favorable interactions with the receptor. Among other compounds, the pharmacophoric geometry of SKF82958 was the most different from the common pharmacophore (cf. distances d_1 and d_2, Table 4). Although this ligand was included because it is reported to be a full D_1 receptor agonist,[44] in our hands the pharmacology of its D_1 activation is complex (i.e., extremely shallow dose−response curves). The modeling data were thus consistent with the biological data suggesting that SKF82958 possesses an atypical and/or indirect mechanism(s) of action at D_1 dopamine receptors. The contribution of SKF82958 to this modeling analysis, however, was retained until either the pharmacology of this ligand can be explained or appropriate analogs invalidate its regional contribution to the excluded volume map. (It should be noted that our recent work has demonstrated that SKF82958 may not, in fact, have full intrinsic activity.[40])

Inherent in the comparison of the pharmacophoric conformation versus the energetically most favorable conformation is the concept that the biologically active conformation of a molecule need not be the one of lowest energy. As noted above, this concept seems reasonable because a consideration must be made for conformers within energetic reach of perturbations due to receptor interactions. Therefore, determination of the absolute low-energy conformations and associated concerns (e.g., multiple energy minima) become of less importance in describing the pharmacophore geometry.[30]

An important feature that strengthens the accuracy of this molecular modeling study is the use of the structurally rigid, high-affinity, full agonist dihydrexidine. After conclusion of the work described here, and while this manuscript was in preparation, the enantiomers of DHX were resolved chemically and tested pharmacologically.[43] X-ray crystallographic analysis confirmed that the absolute configuration of the active enantiomer was as predicted by this modeling [i.e., (6a*R*,12b*S*)-DHX]. These recent data support the use of DHX as a pharmacophoric template and strengthen the validity of results derived from molecular modeling studies.

The low-energy conformation of each agonist that met the pharmacophoric requirements (i.e., "pharm") was used to define the geometry of the pharmacophore. Our decision to use the two hydroxyls, the nitrogen atom, and the β-carbon substituent (i.e., the accessory ring) as the pharmacophoric atoms was based on our idea that a "β-phenyldopamine" backbone is critical for a

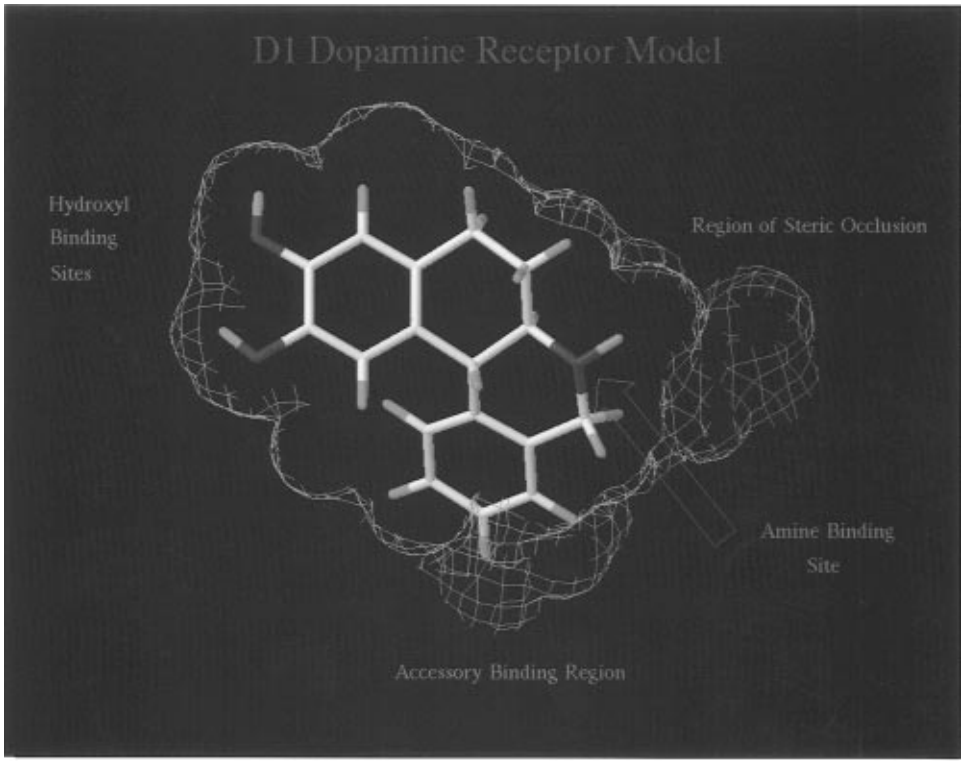

Figure 6. Proposed three-dimensional D_1 receptor model**.** The blue-green mesh volume (cross section of the excluded volume) represents the receptor binding pocket. DHX is shown superimposed on the receptor pocket. The orange mesh displays the summation of the receptor essential volumes of all the inactive analogs. The hydroxyl-binding, amine-binding, and accessory-binding regions are labeled, as is the steric region originally described by McDermed et al.[18]

potent, full D_1 receptor agonist. In fact, it was this hypothesis that led to the design of dihydrexidine. Clearly, a β-phenyl group is not requisite *per se* (e.g., SKF89626 incorporates a thiophene moiety). Our D_1 receptor agonist pharmacophore may be described more accurately as dopamine in the *trans*-β-rotamer (extended) conformation with a β-accessory ring system, "*trans*-β-dopamine$-\beta$-ring". The geometry of the pharmacophore (Table 4) can be defined by two hydroxyls, a nitrogen atom (ca. 7 Å from the *m*-hydroxyl), and an accessory ring system (ca. 5 Å from the catechol ring, slightly above and close to planarity with it). This pharmacophore geometry is consistent with that proposed earlier by Nichols.[19]

Since the process of molecular recognition involves a three-dimensional interaction between a drug and a receptor, the topography of the binding site must be complementary to the arrangement of electron density that is common to all the drugs capable of fully activating the receptor. This complementarity allows the active site of the receptor to be mapped based on (1) the electron density volumes of the active ligands and (2) receptor intolerable regions identified by the volumes required by inactive molecules that lie outside the active site. Following this AAA to receptor mapping, the pharmacophoric conformations of the six active D_1 analogs were used to define the receptor-excluded volume (Figure 3). This entity displays the minimum volume that the receptor must accommodate if it were to be able to bind all active drugs using the pharmacophore as a common frame of reference for molecular orientation.

Mapping of the D_1 receptor was extended further by using the spatial information defined in the inactive compounds that are capable of presenting the common D_1 pharmacophore. Thus, even though these ligands possess the appropriate pharmacophoric atoms, and even though they are capable of presenting the pharmacophore, their receptor affinities are very low. Assuming this is due to negative steric interactions, by using the conformations of the inactive analogs that presented the pharmacophore, the volume required by each molecule that was not common to the excluded receptor volume was calculated (Figure 5). This volume is termed the receptor essential volume and can be used to define the steric boundaries of the active site. It is obvious from this type of approach that the more modifications that are done to one specific region, the better the sterically intolerable region will be defined. In this study, all the inactive analogs did not share one common modification site, and therefore the areas of their receptor essential volumes varied across the receptor surface. The receptor essential volume of the inactive compounds can be summed to generate various boundaries of the D_1 receptor. While this modeling approach is extremely insightful, it must be remembered that for inactive analogs that display more than one distinct receptor essential region, inactivity may result from interaction at only one site. Therefore, analogs must be logically designed to refine the initial model and to rectify any regions of uncertainty.

As noted earlier, one concern when using the AAA is the definition of "active" and "inactive" analogs. In this regard, the utility of the resulting model (and its predictive power) depends on the pharmacological criteria (e.g., affinity and intrinsic activity) that are used. In using the AAA, any reasonable pharmacological criteria will result in some ligands that fall between "active" and "inactive" (in the present case, e.g., they might have been partial agonists with $K_{0.5}$ < 300 nM).

Spatial information of considerable importance may be lost in omitting ligands that do not meet the criteria for inclusion in the AAA. New approaches are being developed to tap the information of all ligands in a tested series (e.g., comparative molecular field analysis (CoMFA)), and together with the AAA, these should aid and strengthen future modeling analyses.

Although the AAA routine has such limitations, the approach is logical and highly informative. The excluded volume map and the receptor essential volumes can be integrated to derive a detailed D_1 receptor topography model (Figure 6). The picture of this three-dimensional model of the receptor binding site is viewed as a cross section, with the excluded volume defining the active site and the receptor essential volume defining intolerable boundaries of the receptor. This D_1 receptor model incorporates our earlier model[11] and serves to define it on a three-dimensional ligand reconstructive basis.

Based on this proposed model, two specific regions of the active receptor volume may be novel sites for DHX modifications. The region juxtaposed to the nitrogen-binding domain, which accommodates the allyl group of SKF82958, may be accessible by modifications to the C-ring of DHX (e.g., alkylation of the 5-position). Secondly, lack of intolerable space defined by the inactive analogs in parts of the hydrophobic accessory region of the model suggests that this area may be able to tolerate modifications, specifically to the 2- and 3-positions of DHX. Such structural modifications to DHX may affect its interaction with the D_1 receptor, thereby altering pharmacological activity (affinity and/or selectivity). Such approaches help to provide insight that can lead to novel new ligands and better understanding of receptor topography.

As has been mentioned earlier, the present study was based on the strategy of AAA and therefore hinges on our pharmacological criteria. Thus, the proposed pharmacophore and receptor model reflect the D_1 receptor of the rat striatum. Characterization of a cloned D_1 receptor in molecular expression systems suggests that the cloned and native receptors are essentially identical, both pharmacologically and functionally.[1−4] Thus, it would be expected that the active ligands used in the present study would have similar affinity and intrinsic activity in such expression systems. If significant differences are found between rat striatum and expression systems, the models we have developed may need to be altered. It is also noteworthy that at least one other mammalian "D_1-like" receptor (the D_5 or alternatively D_{1B}) also has been cloned.[5,6] On the basis of the differences in deduced primary amino acid sequence between D_1 and D_5/D_{1B}, it is likely that the pharmacophores may also be subtly different. Moreover, other "D_1-like" receptors have been hypothesized,[45] making the present work an excellent starting point for design of new subtype selective ligands.

The area that we term the "hydrophobic accessory region" of the D_1 receptor may be one site where the molecular determinants differ among "D_1-like" receptor subtypes. This region appears to be able to accommodate moieties larger than the phenyl ring of DHX. For example, DeNinno et al.[37] recently showed this accessory-binding region could accept numerous structural modifications in an isochroman series of D_1 agonists; A70108 served as the prototype in this series. Their SAR analysis of the isochromans was both interesting and informative, revealing that the pharmacology of a number of derivatives was unusually complex. In part, it may be that such complex pharmacology results from interactions with multiple D_1 receptor subtypes. Thus, innovative pharmacological and SAR analyses, together with modeling analysis, may promote the design of D_1 subtype selective agents.

As noted earlier, the pharmacological profiles of the novel structural agonist A70108 and its enantiomers are unusually complex in terms of their D_1 receptor-binding properties and D_1 functional activity.[37−39] A70108 (the 1*R*,3*S*-enantiomer) and the pharmacologically inactive A70360 (1*S*,3*R*-enantiomer) were tested in the present study to determine if they could meet the conformational requirements of our D_1 pharmacophore and receptor model. Even though A70108 was from a different structural class than dihydrexidine, the pharmacophoric atoms were able to take on a similar geometric arrangement, incurring only a minor energetic cost (<5 kcal/mol, Table 4). The molecular regions where A70108 deviated from DHX (and the other agonists) were the ring adjacent to the catechol and the positioning of the accessory phenyl ring. This difference in the ring adjacent to the catechol ring of A70108 is largely attributed to the carbon atom in the 4-position, which has no such corresponding atom in any of the ligands originally tested.

As can be seen from Figure 3, the pendent phenyl and adamantyl rings of A70108 and its adamantyl derivative, respectively, do not overlap well with aromatic pendent rings of DHX, SKF89626, and SKF82958. Thus, the conglomerate of the accessory rings of all active compounds in the D_1 pharmacophore covers a substantial area of the receptor-excluded volume (Figure 3), formally equivalent to the volume that would be occupied by two phenyl rings of, say, naphthyl derivatives. It is not clear yet whether there exists such an extensive excluded volume of the receptor, or whether the receptor can accommodate either one of the two alternative conformations of the accessory ring system of the D_1 agonists. Future studies of chimeric compounds (e.g., naphthyl derivative of DHX) may shed additional light on the spatial organization of the pendent ring system-binding subsite of the receptor.

It was interesting that the accessory phenyl ring of A70108 was positioned in the region of the DHX molecule that we previously had postulated to be capable of accepting additional structural bulk.[11] We hypothesized, however, that a planar orientation of the accessory phenyl ring relative to the catechol ring would be advantageous in this hypothetical binding region. The accessory ring in (1*R*,3*S*)-A70108 was more noncoplanar relative to the catechol, similar to the situation with phenyltetrahydrobenzazepines and phenyltetrahydroisoquinolines, as modeled in the D_1 receptor antagonist-like pharmacophore.[23] Although we were not able to test this compound ourselves in rat striatum, we believe it is a full D_1 receptor agonist in the caudate model based on our studies of the racemic A68930 (see Results for details). This would suggest that the receptor may be able to accommodate a more noncoplanar orientation of the ring system in this location (ca. 6.5 Å from catechol). If, however, this enantiomer remains a

partial agonist in the caudate as it was in the carp retina, this would provide further support for our receptor models. A70360, containing the 1*S*,3*R*-configuration, appears to position the accessory ring away from the accessory-binding domain defined by DHX. Perhaps this positioning prevents the accessory phenyl ring from interacting with the receptor, rendering a dopamine backbone that may account for the full intrinsic activity, but very low affinity, of this ligand. In support of this idea is the fact that A70360 is not selective for the D_1 vs D_2 receptor.[37] The pharmacological analysis of these enantiomers of A70108 in the caudate model of dopamine-sensitive adenylate cyclase will provide great insight into the mechanism of action of these novel structural ligands. As a new structural class, the SAR analysis of A70108 and related isochromans is very important in aiding our understanding of the structural requirements of the D_1 receptor. This information undoubtedly will guide future drug design and should allow further probing of the geometric requirements of this hydrophobic pocket of the D_1 receptor. It should be noted, however, that it remains unclear whether these subtle structural differences between A70108 and the DHX series can account for the induction of seizure-like activity in rats treated with A70108 or the racemic A68930.[37−39]

Recently, Seiler et al.[46] showed that the benzergolines were the first structural class of potent D_1 receptor agonists lacking a catechol group. While these agents displayed moderate D_1 affinity with the indole modification, none of the derivatives were full D_1 agonists. This finding supports the contention that a catechol moiety is an important pharmacophoric functional group for full D_1 agonists.

Recent molecular biological data concerning the amino acid sequence of dopamine receptors can aid our understanding of SARs, supplement the molecular modeling analysis, and, together, promote novel predictions of receptor structure and activity. For example, the amino acids thought to be critical for ligand interactions at the D_2 receptors (and homologous receptors) are an aspartic acid residue in the third transmembrane region, for the electrostatic interaction with the nitrogen atom, and two serine residues in the fifth transmembrane region, for hydrogen bonding to the hydroxyl groups.[47] For the D_1 receptor, the amine-binding region may focus on aspartic acid residue 103, and the hydroxyl-binding region may be related to serine residues 197, 199, and 201. While this is consistent with accepted ideas, it is possible that these serine residues are not critical; our initial attempts to dock our D_1 pharmacophore to the D_1 receptor using this attachment model were unsatisfactory (Tropsha and Mailman, unpublished observations). In any event, based on the arrangement of the seven transmembrane regions of rhodopsin, it is interesting to speculate that the D_1 receptor accessory-binding region may be derived from the highly hydrophobic area of transmembrane helix 6 (residues 287−297). Moreover, this area presents itself close to the extracellular domain. Although portions of this stretch are conserved among other dopamine receptors, seven of the last nine amino acids are conserved for the "D_1-like" (D_1 and D_5) receptors. The "D_2-like" receptors have a different pattern of amino acid substitutions in this region, some of which are conserved among the "D_2-like" group. Perhaps point mutations directed at various residues in this region of the D_1 receptor will result in a loss of the appropriate D_1 pharmacology.

In summary, the structural (and pharmacological) properties of dihydrexidine were used in the present study to define better the geometry of the D_1 dopamine receptor pharmacophore and map the receptor topography. Together, these results were employed to generate a three-dimensional model of the D_1 receptor binding site. This model offers structural insights to aid the design of additional novel D_1 ligands and provides a frame of reference to assess novel ligands prior to their synthesis. The resulting SAR data can then be used to refine this model. This modeling approach can complement studies based on molecular modeling and analysis of receptor structure (eventually including X-ray crystallography) to define more clearly the molecular determinants critical for D_1 receptor recognition and activation. From such models, the pharmacophore of D_1 receptor subtypes can evolve, permitting the design of new subtype selective ligands.

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