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DOPAMINE RECEPTOR TOPOGRAPHY

CHARACTERIZATION OF ANTAGONIST REQUIREMENTS OF STRIATAL DOPAMINE-SENSITIVE ADENYLATE CYCLASE USING PROTOBERBERINE ALKALOIDS

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Abstract—Representative protoberberine-related alkaloids, i.e. tetrahydroprotoberberines (THPB), quaternary protoberberine salts (Quat. PB) and quaternary dehydroprotoberberine salts (Dehyd. Quat. PB), have been used to characterize the geometric and stereospecific requirements of antagonists of the dopamine receptor. The optical isomers of 2, 3, 10, 11-THPB were tested for their ability to antagonize dopamine stimulated adenylate cyclase activity. The results indicate that (±)-2, 3, 10, 11-THPB inhibited the ability of 100 μM dopamine to elevate adenylate cyclase in homogenates of the rat caudate nucleus. The IC_{50} was observed to be 6 μM. The *S*-(-)-isomer of 2, 3, 10, 11-THPB was a more potent antagonist of dopamine-sensitive adenylate cyclase activity than the *R*-(+)-isomer. The IC_{50} for (−)-THPB was 1μM whereas that for the (+)-isomer was 50 μM. The data also show that the positional isomer, 2, 3, 9, 10-THPB, antagonized dopamine activation of adenylate cyclase with the same degree of potency as 2, 3, 10, 11-THPB. Exhaustive *O*-methylation of THPB at all four hydroxyl positions, the 2, 3-position of the "a" ring and the 10, 11-position of the "d" ring as in xylopinine or the 2, 3-position of the "a" ring and the 9, 10-position of the "d" ring as in tetrahydropalmatine, rendered these compounds weak antagonists of the dopamine response. Selective *O*-methylation of the THPB molecule markedly altered the potency of the resultant compounds as antagonists depending on the position of the *O*-methyl substitution. Overall, these data are consistent with the idea that the orientation of the nitrogen atom in a fixed (*cis*; gauche) position 2 carbon atoms from a catechol nucleus renders antagonist properties to these compounds which interact with the dopamine receptor.

Proposals of endogenous formation of isoquinoline [tetrahydroisoquinolines, benzyltetrahydroisoquinolines and tetrahydroprotoberberines (THPB)] alkaloids as a consequence of L-3, 4-dihydroxyphenylalanine (L-DOPA) and/or chronic ethanol intake have been advanced [1–3]. Since the isoquinoline alkaloids can be derived by the condensation of dopamine with various aldehyde compounds [4], there is a possibility that these agents and their more complex metabolites may interact with catecholamine systems. Accordingly, various aspects of the pharmacologic properties of these alkaloids have been studied and reviewed [5]. In addition, selected neuroamine derived alkaloids have been shown to be substrate preferred inhibitors of brain monoamine oxidase [6], to competitively inhibit uptake and retention of dopamine and norepinephrine in synaptosomes [7], and to inhibit cation-dependent ATP phosphohydrolases in the central nervous system [8]. Furthermore, the precise *O*-methylation products of the optical isomers of tetrahydropapaveroline (THP) and THPB have been delineated [9].

It is well established that many antipsychotic drugs which produce an extrapyramidal syndrome indistinguishable from Parkinson's disease also block dopamine-stimulated adenylate cyclase in various dopaminergic areas of the central nervous system [10–12]. A close correlation exists between the abilities of neuroleptic drugs, such as the phenothiazines, to precipitate extrapyramidal side effects and to antagonize dopamine-stimulated adenylate cyclase activity, but the pharmacological actions of other antipsychotic agents, such as the butyrophenones, are thought to involve receptors other than those linked to adenylate cyclase. Like the phenothiazines, recent studies have shown that selected benzyltetrahydroisoquinolines, tetrahydroprotoberberines and derivatives of simple tetrahydroisoquinolines also antagonize dopamine-sensitive adenylate cyclase activity [13–15].

Several investigators have hypothesized that dopamine interacts with its receptor in a conformation with the nitrogen in the *trans* (extended) position 2 carbon atoms removed from the catechol nucleus [15–18]. Support for this suggestion is based on the *trans* conformation of rigid analogues, such as 2-amino-6,7-dihydroxy-1, 2, 3, 4-tetrahydronapthalene (ADTN), and the aporphine derivatives, apomorphine and *N-n*-propylnorapomorphine, all of which activate several dopamine-mediated responses [13, 16]. Paradoxically, apomorphine and *N-n*-propylnorapomorphine have been shown to antagonize certain dopamine responses [16]. The location of the nitrogen atom in a fixed (*cis;* gauche) position 2 carbon atoms removed from the catechol, as in tetrahydroisoquinoline, which is a part of the apomorphine and *N-n*-propylnorapomorphine structure, could account for the antagonist effect of these compounds on the dopamine response.

The nitrogen atom of protoberberine-related alkaloids is in a fixed position (*cis*) to the catechol nucleus,

and these alkaloids inherently possess two tetrahydroisoquinoline moieties within their molecular geometry. The recent availability of several positional and optical isomers (of the parent tetracyclic protoberberines in various states of phenolic methylation and nitrogen quaternarization) made it possible to further characterize the geometric, stereospecific and topographic requirements for antagonists of the dopamine receptor.

MATERIALS AND METHODS

Enzyme assay. Male Sprague-Dawley rats, weighing 150–200 g were decapitated. The caudate nuclei were removed as described previously [19], pooled and homogenized in 50 vol. (w/v) of 2 mM Tris (hydroxymethyl) aminomethanemaleate buffer-2 mM EGTA* (pH 7.4). The standard assay mixture (final volume 0.5 ml) for measurement of adenylate cyclase activity contained (in mmoles/l): Tris (hydroxymethyl) aminomethanemaleate, 80.2, pH 7.4; ATP, 0.5; $MgSO_4$, 2.0; theophylline, 10; EGTA, 0.6; 0.05 ml of tissue homogenate;.plus test substances as indicated. The enzyme was preincubated with all components of the standard assay system, except ATP, for 20 min at 0° according to the method of Clement-Cormier *et al.* [20]. The reaction was initiated by the addition of ATP and carried out for 2.5 min at 30°. The reaction was terminated by boiling, and cyclic AMP was measured using the method of Brown *et al.* [21]. Adenylate cyclase activity is expressed as pmol cyclic AMP formed/mg protein/min and represents mean values for three replicate samples.

Reagents. ATP, cyclic AMP, berberine sulfate and EGTA were purchased from the Sigma Chemical Co. (St. Louis, MO); 3-hydroxytyramine (dopamine) was from CalBiochem-Behring (San Diego, CA); (+)- and (−)-2,3,9,10-tetramethoxyberbine (tetrahydropalmatine) were obtained from the Aldrich Library of Rare Chemicals, Milwaukee, WI. Inorganic salts were all reagent grade. All other alkaloids (racemates or optical isomers) used in this study were synthesized in our laboratory. Alkaloid purity and structure were evaluated by combined gas chromatography–mass spectrometry, high pressure liquid chromatography, i.r. spectroscopy and elemental analysis [22, 23]. The optical rotation of asymmetric compounds was determined with a Zeiss manual polarimeter. All alkaloid solutions were prepared in 1 mN HCl to maximize stability.

RESULTS

The optical isomers of 2, 3, 10, 11-THPB were tested for their ability to antagonize dopamine-stimulated adenylate cyclase activity. The results shown in Table 1 indicate that (±)-2, 3, 10, 11-THPB inhibited the ability of 100 μM dopamine to elevate adenylate cyclase in a homogenate of the rat caudate nucleus. The concentration of this alkaloid required for 50 per cent inhibition of the dopamine response, IC_{50}, was observed to be 6 μM. The *S*-(−)- isomer of 2, 3, 10, 11-THPB was a more potent antagonist of dopamine-stimulated adenylate cyclase activity than the *R*-(+)-isomer of 2, 3, 10, 11-THPB. The data in Table 1 show that the positional isomer *S*-(−)-2, 3, 9, 10-THPB was as potent an antagonist of dopamine-stimulated adenylate cyclase activity as *S*(−)-2, 3, 10, 11-THPB.

The effect of selected concentrations of (±)-2, 3, 10, 11-THPB on the dopamine-stimulated adenylate cyclase activity in homogenates of the caudate nucleus is shown in Fig. 1. Dopamine at a concentration of 5 μM caused a half-maximal increase in enzyme activity; in the presence of 0.5 μM (±)-2, 3, 10, 11-THPB, approximately 100 μM dopamine was required for half-maximal stimulation of the enzyme. Although the maximum stimulation or the enzyme activity was the same in either the presence of the absence of THPB, higher concentrations of dopamine were required when THPB was present. Because the inhibition of enzyme activity by THPB was reversed by higher concentrations of dopamine, the enzyme interaction can be described as competitive with respect to dopamine. Based on competitive inhibitory kinetics, the inhibition constant, K_i, for (±)-2, 3, 10, 11-THPB for the dopamine-sensitive adenylate cyclase was calculated to be 0.2 μM,

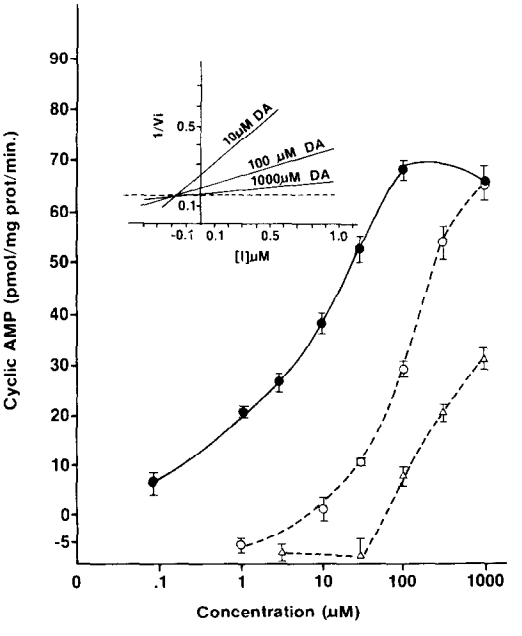
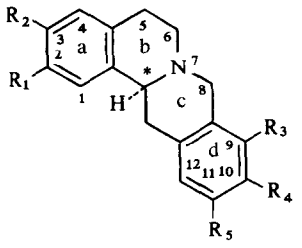
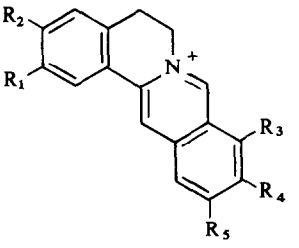
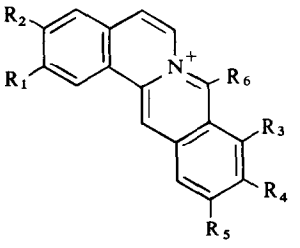


Fig. 1. THPB inhibition of dopamine-sensitive adenylate cyclase. Effects of various concentrations of dopamine, alone (●) or in combination with 0.5 μM (±)-2, 3, 10, 11-THPB (0) or 3.0 μM 2, 3, 10, 11-THPB (△), on adenylate cyclase activity in a homogenate of the rat caudate nucleus. Standard conditions as described in Materials and Methods for the measurement of adenylate cyclase activity. In the absence of added dopamine and THPB, 100 ± 10 pmoles of cyclic AMP were formed. THPB in the absence of dopamine had no effect on basal activity. The data give mean values and ranges for duplicate determinations on samples assayed in triplicate from a representative experiment. Inset: Dixon analysis of dopamine-stimulated adenylate cyclase inhibition by 2, 3, 10, 11-THPB. Alkaloid concentration is shown on the abscissa of the graph. Reciprocals of *V*, which represents cAMP (pmoles/mg of protein/min), are shown on the ordinate. Each point is the mean of data from a minimum of three experiments run in duplicate. The broken straight line indicates a large excess of substrate.

* EGTA = (Ethylene glycol-bis-(β-aminoethylether)-*N*, *N*′-tetraacetic acid).

Table 1. Substitution pattern of protoberberine alkaloids and IC_{50} determinations

Compound	Class	R_1	R_2	R_3	R_4	R_5	R_6	IC_{50}† (μM)
(+)-2, 3, 10, 11-Tetrahydroxyberbine	THPB‡	OH	OH	H	OH	OH		50.0
(±)-2, 3, 10, 11-Tetrahydroxyberbine	THPB	OH	OH	H	OH	OH		6.0
(−)-2, 3, 10, 11-Tetrahydroxyberbine	THPB	OH	OH	H	OH	OH		1.0
(−)-2, 3, 9, 10-Tetrahydroxyberbine	THPB	OH	OH	H	OH	H		3.0
(+)-2, 3, 9, 10-Tetramethoxyberbine	THPB	OCH_3	OCH_3	OCH_3	OCH_3	H		> 100
(−)-2, 3, 9, 10-Tetramethoxyberbine	THPB	OCH_3	OCH_3	OCH_3	OCH_3	H		55
(+)-2, 3, 10, 11-Tetramethoxyberbine	THPB	OCH_3	OCH_3	H	OCH_3	OCH_3		> 100
(−)-2, 3, 10, 11-Tetramethoxyberine	THPB	OCH_3	OCH_3	H	OCH_3	OCH_3		> 100
(±)-Tetrahydroprotoberberine (canadine)	THPB	O—CH_2—O		OCH_3	OCH_3	H		10.0
(±)-2-Methoxy-3, 10, 11-Trihydroxyberbine	THPB	OCH_3	OH	H	OH	OH		15.0
(±)-3-Methoxy-2, 10, 11-Trihydroxyberbine	THPB	OH	OCH_3	H	OH	OH		0.5
(±)-10-Methoxy-2, 3, 11-Trihydroxyberbine	THPB	OH	OH	H	OCH_3	OH		50.0
(±)-11-Methoxy-2, 3, 10-Trihydroxyberbine	THPB	OH	OH	H	OH	OCH_3		3.5
Berberine	Quat. PB§	O—CH_2—O		OCH_3	OCH_3	H		> 100
Pseudopalmatine	Quat. PB	OCH_3	OCH_3	H	OCH_3	OCH_3		> 100
Coralyne	Dehy. Quat. PB‖	OCH_3	OCH_3	H	OCH_3	OCH_3	CH_3	> 100
5, 6-Dehydropseudopalmatine	Dehy. Quat. PB	OCH_3	OCH_3	H	OCH_3	OCH_3	H	> 100
2, 3, 10, 11-Tetrahydroxy-dibenzo(a, g)-quinolinium	Dehy. Quat. PB	OH	OH	H	OH	OH	H	100

* Denotes chiral center.
† Concentration required for 50 per cent inhibition of adenylate cyclase activity in the presence of 100 μM dopamine.
‡ THPB represents the isoquinoline class of tetrahydroproterberine.
§ Quat. PB represents the isoquinoline class of quaternary protoberberine salts.
‖ Dehy. Quat. PB represents the isoquinoline class of quaternary dehydroprotoberberine salts.

as shown in the Dixon plot inset (Fig. 1). The dose–response curve for dopamine was shifted further to the right in the presence of 3 μM (±)-2, 3, 10, 11-THPB and the competitive nature of this interaction is illustrated in the Dixon plot inset.

Exhaustive *O*-methylation of THPB at all four hydroxyl positions, i.e. the 2, 3-positions of the "a" ring and the 10,11-positions of the "d" ring as in xylopinine or the 2, 3-positions of the "a" ring and the 9, 10-positions of the "d" ring as in tetrahydropalmatine, markedly decreased the potency of the compounds to antagonize the dopamine response (Table 1).

A study of selective mono-*O*-methylated derivatives of THPB which antagonize the ability of 100 μM dopamine to stimulate adenylate cyclase activity is presented in Table 1. The most potent of the mono-*O*-methylated compounds was (±)-3-*O*-methyl-2, 10, 11-trihydroxyberine, with an IC_{50} of 0.5 μM. The 3-*O*-methyl-2, 10, 11-trihydroxyberbine was even more potent than the 2, 3, 9, 10-tetrahydroxyberbines or the 2, 3, 10, 11-tetrahydroxyberbines. However, *O*-methylation at the 2-position, as in 2-*O*-methyl-3, 10, 11-trihydroxyberbine, markedly decreased the potency of this compound to antagonize the dopamine response. Selective *O*-methylation of the "d" ring at the 10- or 11-positions of THPB resulted in a weaker antagonist than the 3-*O*-methylated compound. Interestingly, there was a 100-fold difference between the ability of the antagonists, 3-*O*-methyl-2, 10, 11-trihydroxyberbine and 10-*O*-methyl-2, 3, 11-trihydroxyberbine, to inhibit cyclic AMP formation in the presence of dopamine.

The conversion of the hydroxyl groups on the "a" ring of THPB to a methylenedioxy function and *O*-methyl substitutions at the 10, 11-positions of the "d" ring, as in canadine (Table 1), did not alter the ability of this compound to antagonize dopamine-sensitive adenylate cyclase activity. Quaternarization of the THPB nitrogen to form the quaternary protoberberine salt, berberine, rendered the compound completely inactive as a dopamine antagonist (Table 1). Further aromatization of the quaternary protoberberine salt to form a quaternary dehydroprotoberberine salt, as in coralyne or 5, 6-dehydropseudopalmatine, also resulted in a loss of dopamine antagonist activity. However, 2, 3, 10, 11-tetrahydroxydibenzo-(a,g)-quinolinum did show some antagonist properties. A 50 per cent decrease in enzyme activity was observed in the presence of 100 μM of this compound. It is noteworthy that none of the tetrahydroprotoberberine compounds or their quaternary salts tested in this study exhibited any

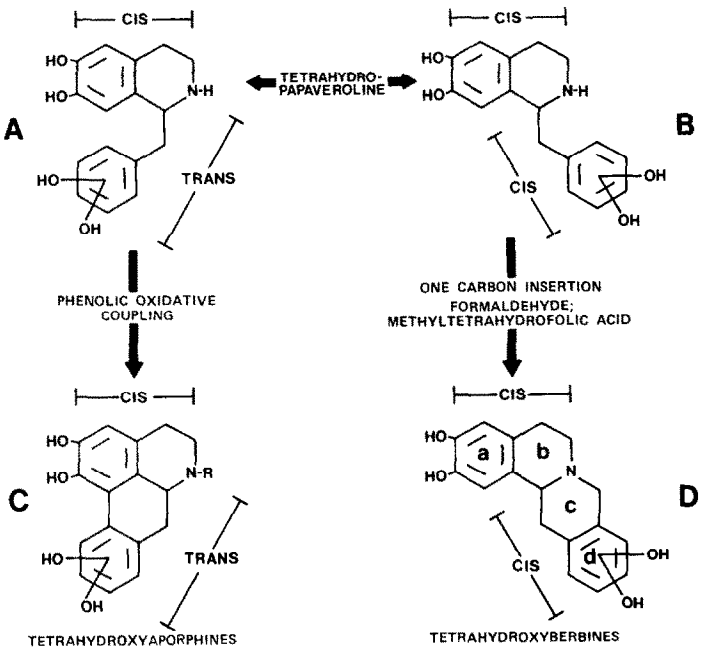


Fig. 2. Tetrahydroxyaporphine and tetrahydroxyberbine formation from tetrahydropapaveroline.

dopamine agonist activity.

The alternate conformational states of THP are shown in Fig. 2 (A and B). In conformation A, the nitrogen is *trans* to the benzylic catechol but *cis* to the isoquinoline catechol. The derivatives of tetrahydroxyaporphine, Fig. 2 (C), are rigid analogues of tetrahydropapaveroline. In configuration B, the nitrogen is *cis* to both the benzylic and isoquinoline catechols. These compounds in a fixed conformation are tetrahydroxyberbines.

The abilities of various dopamine-related compounds to stimulate cyclic AMP production in striatal homogenates are depicted in Fig. 3. In panel A, the classical dopamine receptor agonists, dopamine, (±)-ADTN and epinine, are shown to stimulate cyclic AMP production in a dose-responsive manner with respective EC_{50} values of 5 μM, 7 μM and 6 μM. Unlike dopamine and its analogs, the aporphine alkaloid, (−)-apomorphine, was able to stimulate cyclic AMP formation at low concentrations (1–10 μM), but at concentrations near 100 μM (−)-apomorphine did not appear to stimulate cyclic AMP formation in striatal homogenates (panel B). In fact, in the presence of 100 μM dopamine, apomorphine was found to inhibit the formation of cyclic AMP with a calculated IC_{50} of 15 μM (data not shown). Whereas, in the presence of dopamine, (±)-THP has been shown to antagonize the formation of cyclic AMP in striatal homogenates [16, 18], in the absence of any other test substance (±)- and (+)-THP stimulated cyclic AMP production at concentrations of 10–100 μM (panel C), while the (−)-isomer was inactive (data not shown). It is noteworthy that the maximal stimulation of adenylate cyclase activity produced by (−)-apomorphine, (±)- and (*R*)-(+)-THP was approximately 50 per cent of that observed with a maximal concentration of dopamine. Additionally, the dose–response curves for (−)-apomorphine and (±)-THP were biphasic in nature with respective EC_{50} values of 1.5 and 30 μM.

DISCUSSION

Biochemical evaluations can, in part, describe the potential for, and the efficacy of, the interaction of a class of compounds with a receptor system. For example, extensive structure–activity relationship studies

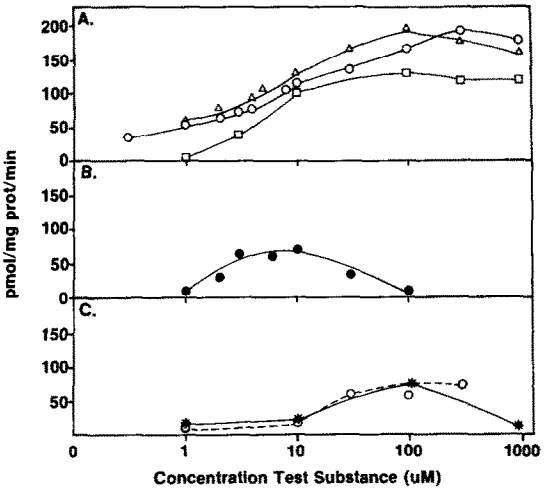


Fig. 3. Effects of various test substances on adenylate cyclase activity in homogenates of the rat striatum. In the absence of added test substance, 104 ± 6 pmoles/mg of protein/min (mean ± S.E.M. for three experiments) of cyclic AMP were formed. The increase in cyclic AMP above this basal level is plotted as a function of test substance concentration. Panel A: (△) dopamine; (0) (±)-ADTN; and (□) epinine; Panel B: (●) apomorphine; Panel C: (○) (+)-THP and (*) (±)-THP.

have been published on the stimulation of cyclic AMP production in response to dopamine and various dopamine agonists [11–16, 19, 24, 25]. To further complement such biochemical studies, agonist or antagonist relationships have been performed on receptor stimulation or blockade of amphetamine-induced stereotypy, rotational behavior following neurotoxic lesioning, neuroendocrine effects, microiontophoretic applications, locomotor activity and peripheral vasodilation (see Refs. 26 and 27 for review).

Ideally, specific antagonism of a homogeneous receptor should be the same for all tissues considered to possess the same receptor. However, the case of the dopamine receptor presents an enigma, due to its reported heterogeneity and multiplicity [28–32]. A number of important discrepancies in the actions of antagonists of the dopamine receptor exist. The fluorinated phenothiazines, such as fluphenazine and trifluoperazine, are more active than the butyrophenone, haloperidol, in inhibiting the effects of dopamine on striatal adenylate cyclase [10, 13], but these compounds are only 50 per cent as active as haloperidol in antagonizing peripheral dopamine receptors [33]. A more striking discrepancy involves the effects of sulpiride, a sulfamoylbenzamide derivative, which is active as an antagonist of the dopamine response in the kidney and anterior pituitary [33–36], but does not inhibit dopamine-induced stimulation of striatal adenylate cyclase activity [37, 38]. These data support the existence of at least two types of dopamine receptors, one coupled to adenylate cyclase, D-1, and the other uncoupled to this enzyme system, D-2 [39]. It has been suggested that the ergot alkaloids are agonists at D-1 receptors and antagonists at D-2. Studies of compounds bearing molecular similarities to dopamine can provide useful information for characterizing the topography of the different classes of dopamine receptors.

Molecular geometry is an important factor in receptor recognition and in drug design; thus, minor conformational alterations can markedly affect pharmacologic response, particularly at receptor sites where ligand archicture is critical. The case of isoquinoline alkaloids is an example of this situation. THP, a benzyltetrahydroisoquinoline (Fig. 2), possesses two freely rotating carbon–carbon bonds on the benzylic portion of the molecule. Conformation A is the orientation of THP required for oxidative coupling to produce the tetrahydroxyaporphines, Fig. 2 (C), and rotation through one of the carbon–carbon benzylic bonds of THP yields conformation B. This conformation is necessary for tetracyclic ring coupling with a carbonyl-1-carbon donor to form the tetrahydroxyberbine nucleus, Fig. 2 (D). Our findings, which demonstrate that THP is a partial agonist-antagonist, are consistent with the idea that the orientation of conformation A may render agonist properties to this compound since the nitrogen is *trans* to the benzylic catechol nucleus. Similarly, these data suggest that the antagonist properties of the compound may be due to the conformation B where the nitrogen is *cis* to the catechol groupings in the isoquinoline and benzylic portions of the molecule.

Evaluations of the fixed isoquinoline derivatives in conformation D of Fig. 2 reveal that tetrahydroprotoberberines were effective antagonists of the dopamine-sensitive adenylate cyclase. The data reveal that position 2 in the "a" ring and position 10 in the "d" ring of the THPB molecule play an important role in conferring antagonist characteristics. Support for this suggestion derives from the finding that 2-*O*-methyl-3, 10, 11-trihydroxyberbine and 10-*O*-methyl-2, 3, 11-trihydroxyberbine were less effective antagonists of the dopamine response than 3-*O*-methylated or 11-*O*-methylated trihydroxyberbine. It is noteworthy that position 2 on the "a" ring and position 10 on the "d"

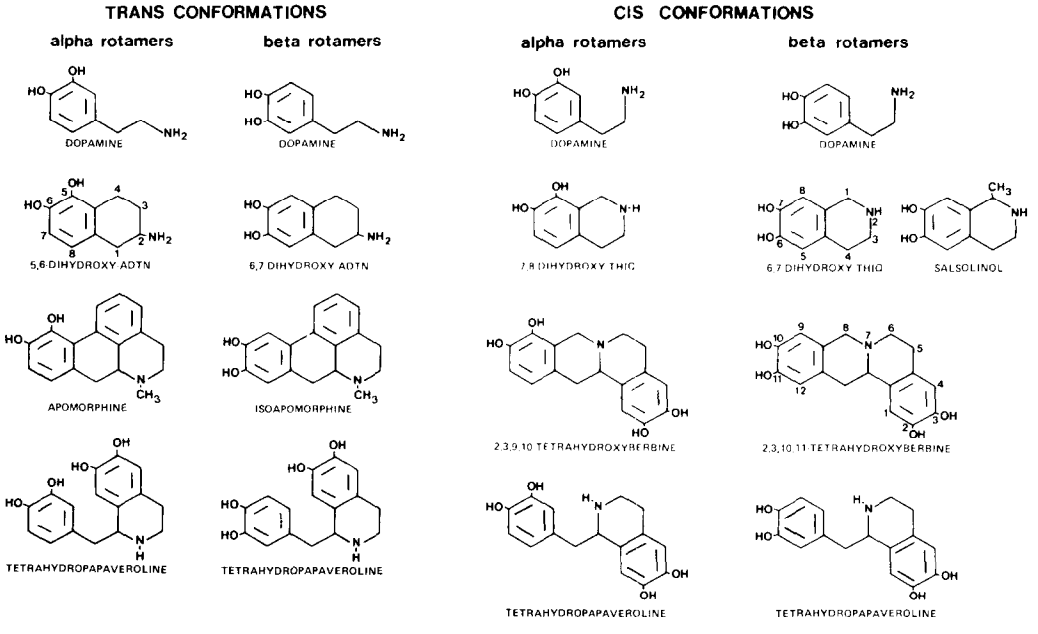


Fig. 4. Representation of dopamine receptor agonists and antogonists in *cis–trans* geometric and alpha–beta rotameric conformations.

ring are spatially equivalent positions in the two tetrahydroisoquinoline moieties of the THPB molecule. These two positions (2 or 10) also represent a shorter tertiary nitrogen to hydroxyl distance than the distance from the hydroxyl at position 3 or 11 to the tertiary nitrogen of the THPB molecule (Table 1).

The data also show that the presence of hydroxyl groups on the "a" ring was not an absolute requirement for classification of these compounds as antagonists of the dopamine response. The conversion of the hydroxyl groups of THPB to a methylenedioxy function, as in canadine, did not alter the antagonist properties of this compound. Similar observations were made by Sheppard *et al.* [14] and Sheppard and Burghardt [25] using the optical isomers of canadine. Thus, we feel that these results suggest that the orientation of the nitrogen is critical in determining the classification of a compound as an antogonist or agonist, whereas the hydroxylation pattern, nitrogen substitution or quaternarization will determine relative potency of the agonist or antagonist effects of these compounds.

Use of the alpha or beta rotameric ligand conformation (Fig. 4) as criteria for delineating the receptor topographic characteristics of the dopamine adenylate cyclase appears inappropriate because dopamine in the *trans* conformation can exist as the alpha on beta rotamer due to free rotation at the catechol β carbon–carbon bond. Furthermore, the benzyltetrahydroisoquinoline, tetrahydropapaveroline, is similar to dopamine in that the methylene benzylic bond is also freely rotating (Fig. 3). Therefore, the most preferential rotameric form for agonist–receptor interaction is not determinable. The tetralin compounds with hydroxyl functions at either the 5, 6-positions (5, 6-dihydroxy-ADTN)-alpha rotamer, or the 6, 7-positions (6, 7-dihydroxy-ADTN)-beta rotamer, have their hydroxyl groups and nitrogen conformation (*trans*) in a fixed position, thus representing the rigid analogs of the alpha and beta rotameric forms of dopamine in the *trans* (extended) conformation (Fig. 3). The results presented, showing 6, 7-dihydroxy-ADTN as a pure dopamine adenylate cyclase agonist, are in agreement with previously published studies [13]. To date, 5, 6-dihydroxy-ADTN has not yet been examined for agonist properties on striatal adenylate cyclase. Hydroxylated aporphines, like the tetralins, provide a series of compounds with fixed hydroxy and nitrogen configurations which may exist in alpha (apomorphine) or beta (isoapomorphine) rotameric forms (Fig. 3). Apomorphine was shown to be a partial agonist of the dopamine adenylate cyclase whereas isoapomorphine exhibited no agonist effect [16].

The results presented here demonstrate that both THP and apomorphine are mixed agonists/antagonists. The agonist properties are most likely related to the existence of a nitrogen in the *trans* conformation which is present in both molecules, while the antagonist properties probably result from a *cis* (gauche) nitrogen (tetrahydroisoquinoline moiety) which is also present in both molecules. The data are also consistent with the idea that dopamine adenylate cyclase agonist properties are dependent on a ligand structure with an extended nitrogen (*trans*) 2 carbon units removed from the catechol nucleus, whereas antagonist properties appear to require the nitrogen fixed *cis* (gauche) to the catechol ring. Our results support the hypothesis that the amino tetralin (*trans* nitrogen) portion of apomorphine is the most likely candidate for agonist properties of this molecule.

These results, moreover, may reconcile the failure of some specific agonists for dopaminergic receptors in the striatum and pituitary to stimulate adenylate cyclase activity. Lergotrile, an ergoline, and the ergopeptine, bromocriptine, are potent *in vivo* agonists of the regulation of prolactin release from the anterior pituitary [40–43]. Unilateral lesioning studies in rats show that bromocriptine and lergotrile, like dopamine or apomorphine, induce rotation toward the intact side [44, 45]. However, these compounds do not increase the formation of cyclic AMP by activating adenylate cyclase in homogenates of the pituitary or striatum, in fact, the ergots are antogonists of the dopamine-stimulated adenylate cyclase in the striatum [38, 46]. Bromocriptine has within its structure an extended nitrogen 2 carbon atoms removed from a benzene ring, which could account for the agonist properties of these compounds. However, within the pharmacopore of the ergot derivatives there is a nitrogen in the *cis* position 2 carbon atoms removed from the benzene ring. The data presented here suggest that the lack of appropriate hydroxyl functions on the benzene ring, plus the presence of a *cis* nitrogen, could account for some of the antagonist properties of these compounds with respect to dopamine-sensitive adenylate cyclase activity.

In conclusion, the dopamine receptor–cyclase complex has a very precise and predictable criteria for the definition of a compound as an agonist and/or antagonist and, therefore, it would seem most reasonable to describe receptor topographic requirements in terms of nitrogen conformation (i.e. *cis*:isoquinoline and *trans*: tetralin), ligand stereochemistry, hydroxyl modifications (i.e. methoxylation, methylenedioxy functions) and nitrogen quaternarization, rather than an alpha or beta rotameric classification.

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