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The Dopamine-Sensitive Adenylate Cyclase of the Rat Caudate Nucleus

II. A Comparison with the Isoproterenol-Sensitive (*Beta*) Adenylate Cyclase of the Rat Erythrocyte for Inhibition or Stimulation by Tetrahydroisoquinolines

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SUMMARY

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Tetrahydroisoquinoline (THI) derivatives were tested for agonist and antagonist activity with dopamine and *beta* adenylate cyclase systems. Agonist activity, seen only with the *beta* cyclase, was associated with hydroxyl groups at the positions 6 and 7 of the THI moiety, provided that the nitrogen was not methylated and a benzyl group was at position 1 in the *S* conformation. Methyl, phenyl, or phenethyl substituents at position 1 yielded inactive compounds. The most active derivative was (*S*)-1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, with an EC_{50} of 0.045 μM. Antagonist activity was detected with both systems and was greater with the *S* isomer, regardless of the presence of hydroxyl groups on the THI moiety. Methylation of the nitrogen increased the inhibitory potency of the *R* isomer much more than that of the *S* conformer. The antagonist activity seen with the *beta* cyclase was probably responsible for the reduced intrinsic activity of the agonists. It was proposed that agonists of both the phenethylamine and THI types were positioned at the receptor through an interaction of the catecholic ring. While the THI nitrogen was located too far from its binding site, interaction was permitted after the 1-benzyl moiety helped to move the nitrogen binding site closer. The nitrogen of the phenethylamine type of agonist would be free to seek out and contact its binding site. Movement of the receptor would begin, and when the nitrogen was *gauche* to the catecholic function, as in the THI molecule, activation would occur.

INTRODUCTION

Several derivatives of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline with substituents in position 1 possess *beta* agonist activity, as evidenced by their bronchodilator (1), lipolytic (2, 3), and cardiotonic (4) effects. One of these, trimetoquinol, contains a 3,4,5-trimethoxybenzyl group at

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position 1 and was more potent than isoproterenol (1, 5). Recently the isoproterenol-sensitive (*beta*) adenylate cyclases of the frog (6) and rat erythrocyte (7) were shown to be stimulated by trimetoquinol and tetrahydropapaveroline, respectively, while the dopamine-sensitive cyclase was inhibited by tetrahydropapaveroline (7).

THP[2] and salsolinol, substituted at position 1 with a 3,4-dihydroxybenzyl and a methyl group, respectively, have been suggested as possible condensation products of dopamine. Dopamine was viewed as reacting with the aldehydic metabolic products of dopamine and ethanol to yield THP (8) and salsolinol (9, 10), respectively. It has been suggested that these compounds were responsible for some of the undesirable effects (10, 11) resulting from L-dopa administration (12) and ethanol consumption (9, 10).

Compounds of the tetrahydroisoquinoline type have the nitrogen atom in a position which is fixed relative to the catechol function. By examining many of these compounds for agonist and antagonist activity with the dopamine and *beta* cyclases, some inferences could be drawn about the conformational requirements at both receptor sites.

MATERIALS AND METHODS

The dopamine cyclase of the rat caudate nucleus and the *beta* cyclase of the rat erythrocyte were prepared as described previously (13). The cyclic AMP generated in 5 min at 37° from exogenously added, unlabeled ATP was determined by the protein-binding assay described by Brown *et al.* (14). Maximal stimulation averaged 2 and 27 times basal levels (40–60 and 0.7–1.5 pmoles/5-min incubation) for the enzymes of the caudate nucleus and erythrocyte, respectively.

Agonist activity, recorded as an EC_{50}, refers to that concentration which elevated cyclic AMP to 50% of the maximum increase over basal level obtained with the test agent. Antagonist activity, recorded as IC_{50}, refers to that concentration which reduced by 50% the stimulation due to 10 μM *N*-methyldopamine. This agonist was used because the EC_{50} values were closer than for dopamine in both cyclase systems: 4 μM with the dopamine cyclase and 20 μM with the *beta* system. All EC_{50} and IC_{50} values were obtained from complete dose-response curves, with at least two concentrations covering the linear portions.

ATP and ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid were obtained from Sigma Chemical Company; Tris base, from Schwarz/Mann; maleic acid, from Matheson, Coleman & Bell; charcoal (Norit SG extra), from J. T. Baker; all test reagents, from Hoffman–La Roche, Inc.; and trimetoquinol isomers, from Tanabe Seiyaku Company, Ltd., Japan.

RESULTS

As expected, many of these THI compounds were agonists of the *beta* cyclase of the rat erythrocyte (Table 1). As reported previously, the 1-(3,4-dihydroxybenzyl) derivatives were quite active, with the *S* isomer (I) being somewhat more potent than the *R* antipode (12). Methylation of the catechol of the 1-benzyl moiety had little effect on activity, while methylation of the 6,7-hydroxyls (IV) yielded an essentially inactive compound, with an EC_{50} of more than 1000 μM. Replacing the dimethoxybenzyl substituent with a *p*-chlorobenzyl (IX) or *p*-hydroxybenzyl (VIII) did not alter the activity significantly.

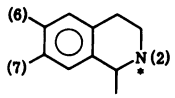
Introduction of the 3,4,5-trimethoxybenzyl group (VII) increased the activity quite markedly. In this case the *S* isomer was approximately 1000 times more potent than the *R* antipode (VIIa). The *S* isomer had a smaller EC_{50} (0.045 μM) than *R*-isoproterenol (0.14 μM), but its maximal stimulation, like that of all of the isoquinolines tested to date, was 50% or less. Surprisingly, the 3,4,5-trihydroxybenzyl derivative (V) was inactive. As reported previously (1, 7), methylation of the secondary nitrogen in the THI ring (III) also destroyed activity. The activity of all the agonists was inhibited by 1 μM propranolol.

It was important that a single methyl-

[2] The abbreviations used are: THP, tetrahydropapaveroline; THI, tetrahydroisoquinoline; TMQ, trimetoquinol.

TABLE 1

Effect of derivatives of 6,7-dihydroxytetrahydroisoquinolines on dopamine and beta adenylate cyclases



(1)

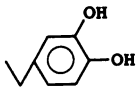
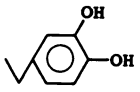
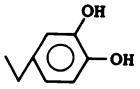
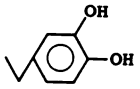
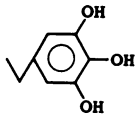
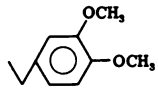
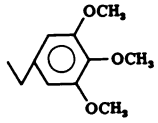
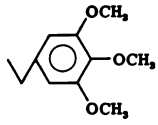
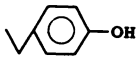
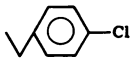
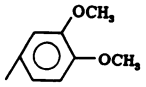
Compound	Substituent				Isomer	Cyclase	
	1	2	6	7		Dopamine: IC_{50}	*Beta*: EC_{50}
						μM	*μM*
I		H	OH	OH	*S*	25	1.0
II		H	OH	OH	*R*	150	7.0
III		CH_3	OH	OH	*S*	30	>1000
IV		H	OCH_3	OCH_3	*R,S*	8	>1000
V		H	OH	OH	*R,S*	4	>1000
VI		H	OH	OH	*R,S*	150	1.2
VII		H	OH	OH	*S*	70	0.045
VIIa		H	OH	OH	*R*	>1000	45.0
VIII		H	OH	OH	*R,S*	7	5.5
IX		H	OH	OH	*R,S*	30	2.0
X	CH_3	H	OH	OH	*R* or *S*	>1000	>1000

TABLE 1 Continued

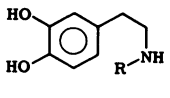
Compound	Substituent				Isomer	Cyclase	
	1	2	6	7		Dopamine: IC_{50}	*Beta*: EC_{50}
XI		H	OH	OH	*R,S*	>1000	>1000
XII		H	OH	OH	*R,S*	120	>1000

ene group separated the THI moiety from the aromatic ring linked to position 1, since substitution with a 3,4-dimethoxyphenyl group (XI) or a *p*-chlorophenethyl group (XII) yielded inactive compounds. Salsolinol (X), which has only a methyl group in position 1, was also relatively inactive.

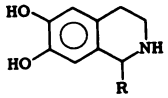
A comparison was made (Table 2) of the relative potencies of a few THI derivatives with the corresponding "open" phenethylamines. When the aromatic group had a *p*-hydroxy function, the potencies of both types were identical. If, however, the aromatic rings had *p*-chloro- or 3,4-dimethoxy functions, the THI compound was much more active. On the other extreme was the finding that the 1-methyl derivative (X)

TABLE 2

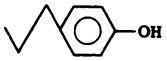
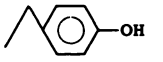
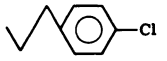
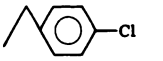
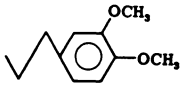
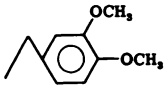
Relative potencies of open- and closed-ring analogues on beta adenylate cyclase



PEA[a]



THI[b]

Compound	Structure	R	EC_{50}
			μM
XIII	PEA		5.5
VIII	THI		5.5
XIV	PEA		12.0
IX	THI		2.0
XV	PEA		15.0
VI	THI		1.2
N-Ethyldopamine	PEA	$-CH_2CH_3$	40.0
X	THI	CH_3	>1000

[a] Phenethylamine.
[b] Tetrahydroisoquinoline.

was inactive while *N*-ethyldopamine had modest activity.

As stated earlier, the THI derivatives with *beta* agonist activity stimulated the cyclase to 50% or less of the maxima obtained with isoproterenol or *N*-methyldopamine. To clarify the reason for this lower intrinsic activity, the effect of a phenethylamine compound was studied in the presence of a THI substance. In order to minimize problems of interpretation associated with compounds of different affinities, the equipotent substances VIII and XIII were chosen for study. Dose-response curves for both compounds (Fig. 1) clearly illustrated the typically reduced maxima seen with all THI compounds. When 10 μM XIII was added to an equal amount of VIII, the total cyclic AMP produced was greater than with VIII alone but less than that seen with XIII alone. At 100 μM, the response was no greater than that obtained with VIII alone. It was apparent, therefore, that the THI compounds possessed antagonist as well as agonist activities.

None of the THI compounds possessed significant activity as stimulators of the dopamine-sensitive cyclase. The catecholic THIs in Table 1 and the non-catecholic THIs in Table 3, however, possessed dopamine antagonist activity. The *S* isomers were more potent antagonists than the *R* antipodes. An *N*-methyl group increased the potency of the *R* isomers but raised the potency of the *S* antipodes only slightly or not at all. Of the catecholic THI compounds in Table 1, all but the 1-methyl (X), 1-(3,4-dimethoxyphenyl) (XI), and *R*-trimethoxybenzyl (VIIa) derivatives possessed some antagonistic activity. Conversion of the 6,7-diphenols in compound I to their *O*-methyl ethers (IV) increased potency. In contrast, methylation of the phenolic groups of the 1-benzyl derivatives (I vs. VI and V vs. VII and VIIa) decreased their potency. None of the compounds in Tables 1 and 3 was an unusually potent inhibitor of the dopamine cyclase, and all were much weaker than chloropromazine (IC_{50}, 0.15 μM) or haloperidol (IC_{50}, 0.25 μM).

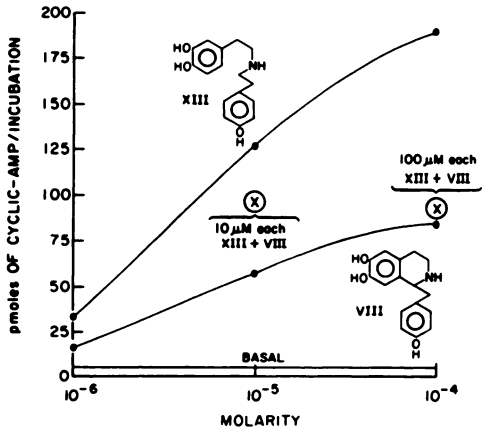


FIG. 1. *Effect of a tetrahydroisoquinoline compound (VIII) and its phenethylamine analogue (XIII) on rat erythrocyte adenylate cyclase when used alone (●——●) and in combination (⊗)*

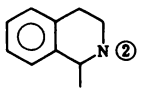
DISCUSSION

It is apparent that the various 6,7-dihydroxytetrahydroisoquinolines described as bronchodilators (1) and tested here exert their effects by directly stimulating the *beta* adenylate cyclase. However, they all also possess weak to modest antagonist activity at the dopamine receptor. There does not seem to be any direct correlation between potencies in the two systems, except that the *S* isomers were more potent than their *R* antipodes. Among the THI compounds lacking 6,7-diphenols, methylation of the nitrogen increased antagonist potency in the dopamine system but decreased it in the *beta* system.

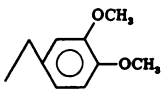
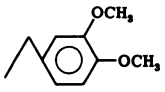
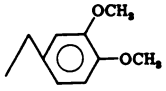
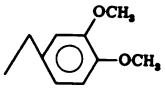
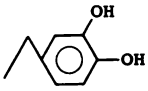
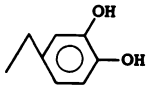
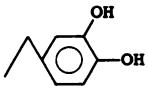
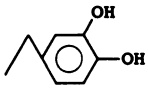
It was previously suggested that an agonist of the dopamine-sensitive cyclase must have the nitrogen *trans* to the aromatic ring (13, 15, 16). It might be anticipated, therefore, that a good antagonist should have a similar conformation. The nitrogen of the 1-benzyl derivatives of THI, however, is fixed in a *gauche* configuration relative to the THI aromatic ring, while it is rotatable and could lie *trans* to the benzyl aromatic ring (Fig. 2). This latter arrangement is of the phenethylamine type and may be necessary for antagonist activity. This concept is supported by the findings that 3-methyl and 3-phenyl substituents at position 1 of THI yielded inactive compounds while catecholic groups on ring C led to more potent inhibitors.

TABLE 3

Efficacy of tetrahydroisoquinolines lacking a catechol function as inhibitors of dopamine and beta adenylate cyclases



①

Compound	Substituent		Conformer	IC_{50}	
	1	2		Dopamine cyclase	*Beta* cyclase
				μM	*μM*
XVI		H	*R*	>1000	150
XVII		H	*S*	10	1.8
XVIII		CH_3	*R*	20	>1000
XIX		CH_3	*S*	3	90
XX		H	*R*	>1000	>1000
XXI		H	*S*	20	60
XXII		CH_3	*R*	50	>1000
XXIII		CH_3	*S*	15	>1000

Why, then, are not the 1-dihydroxybenzyl derivatives of THI (IV and XXI) agonists of either cyclase system? Although the bulk on the nitrogen would tend to reduce agonist activity at the dopamine receptor (13), it might be expected to increase such activity at the *beta* receptor. Perhaps these compounds are unable to effect the appropriate conformational change for activation of the catalytic unit, despite the fact that they inhibit and thus probably bind to or near the active site.

The relative *beta* agonist potencies of many of the THI derivatives examined here not only agree with reports for bronchodilator activity (1), cardiotonic activity (4), and lipolysis in isolated fat cells (3) but extend previous reports of activation of the *beta* cyclase by TMQ (6), salsolinol, and THP (7). A consideration of the structure-

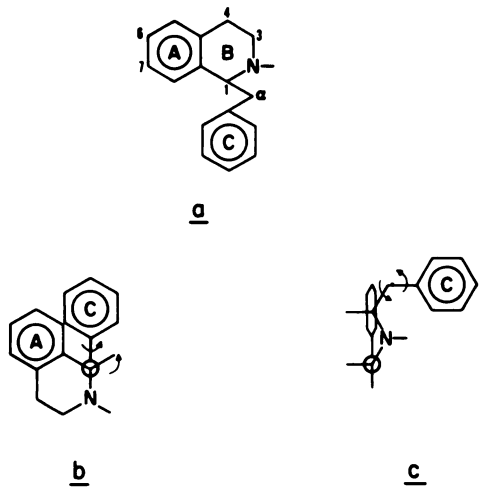


FIG. 2. *Molecular presentations of 1-benzylated tetrahydroisoquinolines in the planar (a) and Newman projections, viewed along carbon-carbon bonds 1 – α (b) and 3 – 4 (c)*

activity relationship for activation of the *beta* cyclase by the THI derivatives yields the same conclusions as those reached by Iwasawa and Kiyomoto (1) for bronchodilator activity, which are summarized as follows. (a) The catecholic function is required on ring A but not on ring C of the THI molecule. (b) Methylation of the nitrogen eliminates the activity. (c) A benzyl substituent is required at postion 1; thus derivatives with a methyl, phenyl, or phenethyl group at position 1 are inactive. (d) The *S* isomer is preferred. While the *S* isomer of THP is only 7 times more potent than the *R* antipode, the *S* isomer of TMQ is 1000 times more potent. Similar relationships were reported for the lipolytic action of these enantiomers (3).

Beta antagonist activity appears to be an intrinsic property of the THI structure, and its association with agonist activity reported here agrees with that reported for TMQ with the frog erythrocyte cyclase (6) and for THP with the rat erythrocyte cyclase (7). While the antagonist activity may be associated with the phenethylamine structure involving the benzyl aromatic ring, *beta* agonist activity is clearly a function of the THI aromatic ring. As shown in Fig. 2c, the nitrogen lies *gauche* to the aromatic moiety (ring A) and projects from its plane at an angle of almost 45° and away from the *m*-hydroxyl function. The potency of compounds like *S*-TMQ indicates that this position of the nitrogen is either essential or very acceptable for *beta* agonist activity. It is concluded, therefore, that this configuration approximates the conformation assumed by a phenethylamine agonist at the receptor site at the time of activation.

The relationship described above differs from that projected for the conformation of the agonists at the dopamine receptor. Using a polycyclic compound such as apomorphine as a model, it was suggested that a phenethylamine compound has the nitrogen in the *trans* conformation, in a plane almost 45° from that of the catecholic ring and leaning in the direction of the *m*-phenolic function. Based on the rigid molecules apomorphine and THI, the distance of the nitrogen to the 3-phenolic oxygen of a phenethylamine molecule must be 6.48 A at both the dopamine (17) and *beta* receptors. The distance between the nitrogen and the 4-phenolic function would be 7.8 A for the conformation of the dopamine agonist (17) and 6.1 A for that of the *beta* agonist. The distance separating the nitrogen from the center of the aromatic ring also differs, being approximately 5.2 A for the dopamine agonist and 3.7 A for the *beta* agonist.

Given that the conformation of the nitrogen in the THI compound is preferred or acceptable at the *beta* receptor, one must rationalize the relative inactivity of simple THI derivatives such as salsolinol. Either it cannot bind well, or, having bound, it is not able to effect the conformational change in the receptor required for activation of the adenylate cyclase. Regardless of the details of the conformational change, two regions of the receptor must be brought closer together or farther apart. In either case, when the catecholic portion binds to the receptor, it is possible that the fixed nitrogen of salsolinol is located too far from its binding site. The flexibility of the side chain of the phenethylamine analogue, however, permits the nitrogen to find its binding site on the receptor while its aromatic ring remains bound. These

interactions permit movement to occur in the agonist binding region of the receptor. When the movement reaches the point where the THI conformation can be accommodated, the catalytic unit is signaled to cyclize ATP. Once that happens, new interactions could occur which would reduce the binding of the agonist and effect its dissociation. The system would then be free to become recharged with ATP and ready to be signaled once again by the agonist. With this concept the 1-benzyl group is viewed as interacting with a hydrophobic region near the nitrogen binding site. The flexibility of this benzyl moiety would help to direct the movement of this area of the receptor so that the nitrogen binding site is brought close enough to the nitrogen of the THI to allow them to interact. The 1-phenyl-substituted THI would be inactive, because the aromatic ring would project almost at right angles to the plane of the catecholic moiety and would not interact with the suggested hydrophobic region accessible to a benzyl group.

It should be pointed out that none of the THI compounds has a hydroxyl group in position 3, a position analogous to the β-carbon of phenethylamine compounds. This group, when present, may be involved only in stabilizing the binding or promoting the establishment of the more *gauche* conformation in the phenethylamine compounds.

The role of the dopamine condensation products, salsolinol and THP, deserves some comment. Inhibition of the dopamine receptor reported here for these compounds was weaker than observed earlier (7). It must be emphasized, however, that the present data were derived from dose-response curves vs. *N*-methyldopamine while the earlier report was based on one concentration vs. dopamine. The current data, derived from dose-response curves, undoubtedly have greater validity.

It is still not certain that these substances are produced in humans during excessive alcohol or L-dopa consumption, despite their detection in urine (18). Even when special conditions were used to increase the production of these dopamine condensation products in animals, less than 1% of the dopamine content could be accounted for as salsolinol (19) or THP (20). Unless these weakly active compounds accumulate with time, it seems unlikely that the "on-off" response to L-dopa (11) or the side effects of alcohol (9, 10) could be due to THP or salsolinol, respectively. In this regard it was reported that salsolinol was readily taken up and stored in nerve endings (21).

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