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THE DOPAMINE-SENSITIVE ADENYLATE CYCLASE OF THE RAT CAUDATE NUCLEUS—3. THE EFFECT OF APORPHINES AND PROTOBERBERINES

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Abstract—A series of aporphines and protoberberines were tested for activity with DA and *beta*-type adenylate cyclases. Loss of DA agonist but not antagonist activity of apomorphine was associated with the removal or methylation of the hydroxyls or the S-configuration. The effects of other alterations in structure were not as clear-cut and alterations in antagonist activity were not similar in the two enzyme systems. Surprisingly the protoberberines were fairly potent as inhibitors of the DA-cyclase with little effects on the beta system. In most situations the S-isomer was much more potent than the R-antipode. None of the compounds studied possessed *beta*-agonist activity.

It is generally accepted that most of the behavioral and motor effects of L-DOPA can be mimicked by apomorphine. This parallelism has been extended to dopamine's action on the adenylate cyclase of the caudate nucleus and the retina [1, 2]. Several investigators have proposed that aporphines and even tetrahydroprotoberberines could be produced *in vivo* from the dopamine formed from L-DOPA during its use for treating Parkinson's disease [3, 4]. These agents could then act as agonists (i.e. false neurotransmitters) or antagonists (i.e. neuroleptics). Both bulbocapnine and nuciferine have been shown to possess dopamine antagonist activity [5–8].

These considerations suggested the need to examine a variety of aporphines and tetrahydroprotoberberines for their ability to act as agonists or antagonists of the DA sensitive adenylate cyclase of the rat caudate nucleus. Preliminary evaluation of a few selected aporphines have already been made [9–12]. In order to evaluate the specificity of their actions, these compounds were also tested for their effects on the beta sensitive adenylate cyclase of the rat erythrocyte. The results of these studies were evaluated from a structural standpoint in order to provide some concepts concerning the nature of the interactions of these compounds with the receptors of the adenylate cyclases.

MATERIALS AND METHODS

The dopamine sensitive cyclase of the rat caudate nucleus and the *beta* sensitive cyclase of the rat erythrocyte are prepared as described previously [13]. The cyclic AMP generated in 5 min at 37° from exogenously added unlabeled ATP is determined by the protein-binding assay described by Brown *et al.* [14].

The antagonist activity is recorded as an IC_{50} which refers to that concentration which reduces by 50 per cent the stimulation due to 10 μM *N*-methyldopamine. This agonist is used because the EC_{50} values were closer than for dopamine in both cyclase systems: 4 μM with the dopamine cyclase and the 20μM in the beta system. All IC_{50} values were obtained from complete dose response curves with at least two concentrations covering the linear portion.

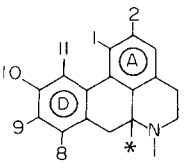
The source of the chemicals was as follows: ATP and EGTA [ethylene glycol-bis (β-aminoethyl-ether)-*N*,*N*'-tetraacetic acid], Sigma Chemical Corporation; Tris base [2-amino-2(hydroxymethyl)-1,3-propanediol], Schwartz/Mann: maleic acid, Matheson, Coleman and Bell; charcoal (Norite SG Extra), J. T. Baker; all test reagents, Hoffmann-La Roche Inc.

RESULTS

The effect of a variety of aporphine analogues can be seen in Table 1. The agents with IC_{50} values of 10μM or less are in order of decreasing potency at the DA receptor, isoboldine, nuciferine, glaucine, compound 1, bulbocapnine, nuciferoline, compound 2 and (S) apomorphine. While the presence of the S-configuration alone is sufficient to produce an antagonist, as in (S) apomorphine, compounds with the R configuration alone, as nuciferine or compound 1, can also be reasonably potent inhibitors. None of these are as potent as chlorpromazine (IC_{50} of 0.15 μM) in this system but do compare with pimozide (IC_{50} of 2 μM). The remaining compounds are much less potent with IC_{50} values reaching 200 μM.

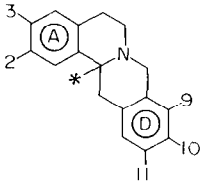
An analysis of the structural requirements for inhibitory activity at the DA receptor suggests that liberation of phenolic functions in ring A by *O*-demethylation reduces the activity (nuciferine vs nornuciferine vs compound 4). It is possible that the S-isomer is the more potent form since racemic compound 2 is more potent than its R-isomer (compound 4). It also appears that the methylenedioxy derivative is weaker than its dimethoxy analogue

Table 1. Inhibition of dopamine and beta-sensitive adenylate cyclases by aporphine derivatives



Compound	*	1	2	8	9	10	11	N	IC_{50} DA	(μM) *Beta*
Isoboldine	S	OH	OCH_3	H	OH	OCH_3	H	CH_3	1	85
Nuciferine	R	OCH_3	OCH_3	H	H	H	H	CH_3	4	1
Glaucine	S	OCH_3	OCH_3	H	OCH_3	OCH_3	H	CH_3	4	7
1	R	OH	OH	H	OH	OH	H	CH_3	5	200
Bulbocapnine	S	O—O		H	H	OCH_3	OH	CH_3	5	> 1000
Nuciferoline	R,S	OCH_3	OCH_3	H	H	H	OH	CH_3	7	30
2	R,S	OH	OH	H	H	H	H	CH_3	9	100
Isoapomorphine	S	H	H	H	H	OH	OH	CH_3	10	45
Nornuciferine	R	OCH_3	OH	H	H	H	H	CH_3	20	7
Laurepukine	R	O—O		H	H	H	OH	CH_3O^-	20	500
3	S	OH	OH	H	H	OCH_3	OCH_3	CH_3	20	70
Pukateine	R	O—O		H	H	H	OH	CH_3	25	80
4	R	OH	OH	H	H	H	H	CH_3	30	50
Roemerine	R	O—O		H	H	H	H	CH_3	40	20
Anonaine	R	O—O		H	H	H	H	H	40	100
Apocodeine	R	H	H	H	H	OCH_3	OH	CH_3	60	50
Morphothebaine	R	H	OH	H	H	OCH_3	OH	CH_3	200	100
5	R,S	H	H	OH	H	OH	OH	CH_3	200	> 1000
6	R,S	H	H	OAc	H	OAc	OAc	CH_3	200	> 1000

Table 2. Inhibition of dopamine and beta-sensitive adenylate cyclases of tetrahydroprotoberberine derivatives

Compound	*	2	3	9	10	11	IC_{50} DA	(μM) *Beta*
								
7 (R)	R	OH	OH	OH	OH	H	6.0	> 1000
7 (S)	S	OH	OH	OH	OH	H	6.7	> 1000
8	R,S	OH	OH	H	OH	OH	0.8	90
9 (R)	R	OH	OH	OCH_3	OCH_3	H	15.0	> 1000
9 (S)	S	OH	OH	OCH_3	OCH_3	H	0.6	180
10	R,S	OH	OH	OCH_3	OCH_3	OCH_3	4.5	260
11	R,S	O—O		OCH_3	OCH_3	OCH_3	4.0	> 1000
12	R,S	OH	OH	H	H	OH	2.0	60
Canadine (R)	R	O—O		OCH_3	OCH_3	H	6.0	> 1000
Canadine (S)	S	O—O		OCH_3	OCH_3	H	0.3	250
13 (R)	R	H	H	H	OH	OH	> 1000	> 1000
13 (S)	S	H	H	H	OH	OH	1.5	> 1000
14 (R)	R	H	H	OH	OH	H	> 1000	> 1000
14 (S)	S	H	H	OH	OH	H	2.0	> 1000
15 (R)	R	H	H	OCH_3	OCH_3	H	50.0	> 1000
15 (S)	S	H	H	OCH_3	OCH_3	H	8.0	> 1000

(nuciferine vs roemerine). It would seem that *N*-demethylation has little effect (roemerine vs anonaine) in this series. The data in Table 1 suggests that a phenolic function in ring A may lower activity (apocodeine vs morphothebaine). The introduction of a third hydroxyl group in position 8 of ring *D* (compound 5) eliminates DA agonist activity and reduces the antagonist potency. Acetylation of the three phenolic functions did not alter the potency. Forming an *N*-oxide did not seem to alter the activity of the R-isomeric pair (laurepukeine vs pukateine). The introduction of an hydroxyl at position 2 may have increased the activity of the R-isomer slightly (anonaine vs pukateine).

There does not seem to be any correlation bebetween inhibitory potency at the *beta*-receptor and that seen at the DA receptor. Bulbocapnine is uniquely inactive at the *beta*-receptor despite reasonably potent action at the DA receptor. The reason for this is not at all apparent. Two (R) isomers, nuciferine, nornuciferine, as well as one S-isomer, glaucine, appear to be the most potent agonists of the *beta* system.

The inhibitory action of the tetrahydroprotoberberine analogues is seen in Table 2. In this series the stereochemical effects are readily seen. With the exception of compounds 7 (R) and 7 (S), the S-isomer is much more potent than the R-antipode at the DA receptor. All but five of these agents lacked inhibitory activity at the *beta*-receptor. Compounds 8, 9 (S), 10, 12 and (S)-canadine possessed weak antagonist activity at the *beta*-receptor and all but (S)-canadine showed relatively weak agonist activity as well (data not shown).

DISCUSSION

It has previously been shown that apomorphine is a good agonist of the adenylate cyclase of the DA type but not the *beta* type [1, 15, 16]. At higher concentrations, however, antagonist activity can be seen with both types of adenylate cyclases [1, 13, 14]. It would be expected, therefore, that changes in the apomorphine molecule which eliminated agonist activity could permit antagonism to remain. Thus, alkylation of one or both of the critical hydroxyls of positions 10 and 11 eliminates agonist but not antagonist activity ([11, 12], Table 1). Likewise, the S-isomer of apomorphine retains only the antagonistic activity of the R-antipode.

The role played by other substituents, alone or combined, is more difficult to evaluate. There is some suggestion that methoxy rather than methylenedioxy groups at positions 1 and 2 increase inhibitory potency especially at the *beta*-receptor. It would also appear that when no 10,11-dihydroxy function is present, the R-form can be as inhibitory as the S-conformer. The introduction of an hydroxy and possibly an acetoxy group at position 8 seems to reduce markedly the potency at both receptors. Despite all of these indications it is apparent that many more variations in structure would have to be tested before a clear picture could emerge concerning the structural requirements for optimum inhibition of either or both adenylate cyclases.

In contrast to the results found here, bulbocapnine was found to be somewhat more potent than nuciferine which in turn was about equiactive with 1,2-dihydroxy-aporphine and isoapomorphine [10, 11]. It should be noted that both of the earlier studies used single concentrations of the antagonists in the presence of dopamine, while the results reported here are based on dose–response curves using *N*-methyldopamine as the agonist.

The situation with the tetrahydroprotoberberines is even more complicated. An examination of their structures shows them to possess a tetrahydroisoquinoline (THI) nucleus with the heterocyclic nitrogen lying gauche to either of the two aromatic rings (Table 2). It was shown previously that 1-benzyl substituted 6,7-dihydroxy-THI compounds possess agonist activity at the *beta*-receptor with no effects on the DA sensitive cyclase [17]. Thus, one would expect the tetrahydroprotoberberines to be antagonists at the *beta* not the DA receptor. That the reverse is generally found is surprising.

Reexamination of the aporphine molecule demonstrates that it too contains a THI nucleus which includes ring A (Table 1). Since ring D is important for agonist activity, it is possible that ring A is responsible for the antagonism of the DA response. In support of this notion is the observation that methylation of either phenolic group of dopamine also eliminates agonist activity but doesn't lead to the formation of an antagonist [13]. Thus, some portions of the apomorphine not required for agonist activity and possibly involving the THI portion of the molecule will be responsible for the antagonism.

The determination of the structural requirements for a good antagonist is made even more difficult when one compares the structures alluded to here with those of the structurally different phenothiazines and butyrophenones. Each class of antagonist need not necessarily overlap the same critical region of the receptor binding site and thus could be considered a separate problem from the standpoint of structure–activity relationship study. However, it remains possible that there is an identity of overlap which is of great importance for antagonist activity. It is important, therefore, that some effort be made to find this overlap and understand its relevance to antagonist activity.

The events at the *beta*-receptor may be even more complicating. It had previously been demonstrated that many S-isomers of 1-benzylated tetrahydroisoquinolines were good inhibitors of the beta adenylate cyclase even if they possessed agonist activity as well [17]. In many cases, however, they were even better inhibitors of the DA receptor. This preference for the DA receptor is even more prominent with the tetrahydroprotoberberines. Those tetrahydroprotoberberines which show inhibition at the *beta* receptor also possess some *beta* agonist activity (data not shown).

Most of the aporphines possessed antagonist activity at the *beta* receptor but this does not appear to correlate positively or negatively with DA antagonism. At the moment, there do not appear to be any good structural reasons why even the most active compounds are so potent. Two of them (nuciferine and nornuciferine) are R-isomers while the third (glaucine) has the S-configuration.

It is apparent that many more compounds which are structurally similar and different need to be examined before a good picture of the receptor binding sites critical for both agonist and antagonist activity can emerge.

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