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The Dopamine-Sensitive Adenylate Cyclase of Rat Caudate Nucleus

I. Comparison with the Isoproterenol-Sensitive Adenylate Cyclase (Beta Receptor System) of Rat Erythrocytes in Responses to Dopamine Derivatives

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SUMMARY

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Various analogues of dopamine were examined for agonist activity with respect to the dopamine-sensitive adenylate cyclase of rat caudate nucleus and the isoproterenol-sensitive enzyme of rat erythrocytes. In both systems decreased activity was associated with 5-methyl, 6-methyl, 2-phenyl, or $(R,-)$-α-methyl substitution. With the dopamine-sensitive cyclase system N-alkyl, $(S,+)$-α-methyl, and $(R,-)$-β-hydroxy substitution resulted in decreased activity, in contrast to results with the isoproterenol-sensitive cyclase. The isomers of the tetrahydroisoquinolines, salsolinol and its N-methylated derivatives, were inactive, lending support to the concept that the nitrogen and catechol moieties were in a *trans* rather than a *gauche* conformation. It is suggested that the receptors for the dopamine- and isoproterenol-sensitive adenylate cyclases could have identical primary structures but that they are folded in the membrane in such a way that the binding sites for the active groups of the agonists differ in their relative positions.

INTRODUCTION

An adenylate cyclase which responded to dopamine has been found on rat erythrocyte ghosts, but proved to be of the *beta* adrenergic receptor type. In addition, this enzyme was not responsive to apomorphine (1). A more specific dopamine-sensitive adenylate cyclase was first demonstrated for the superior cervical ganglion by Kebabian and Greengard (2), then for the retina by Brown and Makman (3) and for the rat caudate nucleus by Kebabian *et al.* (4). These workers reported that apomorphine was a potent agonist in these systems (4, 5). Both groups demonstrated that dopamine was a more potent agonist than norepinephrine, that isoproterenol had little or no agonist activity, and that the response to dopamine was inhibited by *alpha* but not by *beta* adrenergic blocking agents. This pattern of response contrasts with the *beta* receptor system, where isoproterenol is the more potent agonist, whose effects are blocked more by *beta* rather than *alpha* blocking agents (1). The availability of a

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variety of dopamine derivatives made it possible to probe the structural requirements for agonist activity in the isoproterenol- and dopamine-sensitive adenylate cyclase systems. The studies reported here are concerned with the effects of substituents at various positions of the side chain and aromatic ring on the potency of dopamine as a stimulator of both cyclases. Some effort has been made to explain these effects in terms of the topography of the receptor and the conformation of the agonist.

METHODS

The removal of the rat caudate nucleus, homogenization, incubation for measurement of cyclase activity, and determination of cyclic 3′,5′-AMP were performed as described by Kababian *et al.* (4). Briefly, the caudate nuclei from 150–250-g adult male Charles River rats were homogenized with 25 volumes of 2 mM Tris-maleate buffer, pH 7.4, containing 2 mM EGTA.[1] The cyclase assay was performed in triplicate in 0.5 ml of medium containing 80 mM Tris-maleate, 0.5 mM ATP, 2 mM $MgSO_4$, 10 mM theophylline, 0.2 mM EGTA, 50 μl of cyclase preparation (equivalent to 2 mg, wet weight), and the test substance under study. After incubation for 5 min at 37° the reaction was terminated by boiling for 2 min and centrifugation to remove insoluble material. The cyclic AMP content of each sample was determined in duplicate by the charcoal exclusion method of Brown *et al.* (6). None of the compounds tested had any effect on this assay.

The erythrocytes were collected and washed as described by Sheppard and Burghardt (7). The hemolysates were prepared with the Tris-maleate-EGTA buffer described above. The ghosts, equivalent to 0.15 ml of packed erythrocytes, were incubated for 7.5 min in the same medium used for the caudate nucleus, and the cyclic AMP was analyzed as described above.

Several concentrations of each agonist, ranging from 1 to 100 μM, were studied in order to obtain dose-response curves where possible. For those compounds demonstrating little activity at 100 μM, a full curve was not developed. In all the cases the relative potency refers to the amount of dopamine divided by the amount of agonist required to give the same response in the same experiment. Where almost complete dose-response curves were obtained, the values utilized were near the ED_{50}.

It was necessary to have a dopamine or isoproterenol dose-response curve for each experiment because of the variability of response from preparation to preparation. The EC_{50} values were 1.7–7.0 μM for dopamine and 0.14 μM for isoproterenol with the dopamine- and isoproterenol-sensitive cyclases, respectively.

The major reagents and chemicals were obtained from the following sources: Tris-maleate, EGTA, and ATP, Sigma Chemical Company; *N*-ethyldopamine, metatyramine, and metaraminol, Sterling-Winthrop Corporation; (*S*,+)- and (*R*,−)-α-methyldopamine, Dr. C. Porter, Merck Sharp & Dohme, Inc.; all other dopamine derivatives, Hoffmann–La Roche, Inc.; charcoal (Norit SC "extra"), J. T. Baker Chemical Company. All of the dopamine derivatives were used as hydrohalide salts.

RESULTS

The previous demonstration that the (*S*,+) isomer of norepinephrine was as active as dopamine in the isoproterenol-sensitive adenylate cyclase system of rat erythrocytes (1) was not observed with the dopamine cyclase of rat caudate nucleus (Table 1). Dopamine was many times more potent than the (*S*,+) isomer of norepinephrine and slightly more potent than its (*R*,−) or natural isomer. Metatyramine was weakly active with the dopamine- but not the isoproterenol-sensitive cyclase, while the reverse was true for metaraminol.

The observed order of potency with respect to adenylate cyclase activation of the *beta* receptor system of rat erythrocytes was reported to be *N*-methyldopamine > *N*-isopropyldopamine > dopamine (1). It was of interest to determine the consequences of alkyl substitution of the nitrogen atom of dopamine on stimulation of the

[1] The abbreviation used: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

TABLE 1

Effects of 100 μM dopamine, norepinephrine isomers, and p-deoxy derivatives on stimulation of adenylate cyclases of rat caudate nucleus and erythrocytes

The basal cyclic AMP production (mean ± standard error) was 55.8 ± 3.3 and 5.7 ± 0.1 pmoles per incubation for the adenylate cyclases of caudate nucleus and erythrocytes, respectively.

Agonist	Increase over basal in cyclic AMP per incubation	
	Caudate nucleus	Erythrocytes
	-fold	*-fold*
Dopamine	2.3	2.0
(*R*,−)-Norepinephrine	1.9	5.9
(*S*,+)-Norepinephrine	1.4	1.7
Metatyramine	1.4	1.2[a]
Metaraminol	1.1[a]	2.2

[a] Not significantly increased over basal production. All other values are statistically significant at $p < 0.05$.

dopamine-sensitive adenylate cyclase of rat caudate nucleus. The addition of an alkyl group to the nitrogen atom of dopamine resulted in reductions of activity which were generally proportional to the size of the substituent (Table 2). Thus dopamine was not significantly more potent than its *N*-methyl derivative, although both were much more potent than *N*-isopropyldopamine, which was inactive at 100 μM. An additional methyl group on *N*-methyldopamine reduced the activity slightly, but the addition of a third methyl group to form the quaternized nitrogen almost abolished activity. The *N*-ethyl and *N*,*N*-diethyl derivatives possessed only about 7.5% of the activity of dopamine. The *N*-ethyl, *N*-propyl, and *N*-isopropyl-*N*-methyl derivatives also revealed only a small percentage of the dopamine activity, and all three yielded maximum responses which were about 50% of that seen with dopamine.

Derivatives of dopamine and *N*,*N*-dimethyldopamine with substituents on the ring and in the α-position of the side chain are listed in Table 3. Dopamine and its 2-methyl derivative were equipotent with respect to stimulation of the enzyme of the

TABLE 2

Ability of N-alkyl derivatives of dopamine to stimulate rat caudate nucleus adenylate cyclase

Relative activity refers to the ratio of dopamine to the test substance at equieffective concentrations.

Dopamine analogue	Relative activity
N-Methyl	0.900
N,*N*-Dimethyl	0.600
N,*N*,*N*-Trimethyl	—[a]
N-Ethyl	0.025
N,*N*-Diethyl	0.075
N-Propyl	0.025
N-Isopropyl	—[a]
N-Methyl-*N*-isopropyl	0.018

[a] No measurable activity at 100 μM.

caudate nucleus. In contrast, 2-phenyl substitution reduced the potency to about 5% of that of dopamine, and 6-methylation reduced it further, to 1%. The addition of a (±)α-methyl group reduced the potency of both dopamine and its 2-methyl derivative to about 1–2% of the original activity. With the other α-methylated derivatives no activity could be detected up to 100 μM. A similar pattern was noted with the erythrocyte enzyme, except that (±)α-methyl substitution increased the potency of dopamine and its analogues 3–4-fold, in sharp contrast to the activity seen with the caudate nucleus enzyme.

In the *N*,*N*-dimethyldopamine series, the 2-methyl group again had no effect, while the 2-phenyl and 6-methyl groups reduced the potency 99% or more with the dopamine-sensitive cyclase. When tested with the isoproterenol-sensitive cyclase of erythrocytes, the 6-methyl group reduced potency by only 61%. In the tertiary amine series the (±)α-methyl group again reduced the potency of dopamine with the dopamine-sensitive enzyme. With the isoproterenol-sensitive cyclase a modest reduction rather than an increase in potency was obtained with the 2-methyl and 6-methyl analogues.

The two isomers of α-methyldopamine were tested (Table 4). Only the (*R*,−) isomer was active with the enzyme of the

TABLE 3

Effects of various substituents on aromatic ring or side chain of dopamine and its N,N-dimethyl derivative on ability to activate adenylate cyclases of rat caudate nucleus or erythrocytes

Relative activity refers to the ratio of dopamine to the test substance at equieffective concentrations. A complete dopamine dose-response curve was obtained in each experiment.

Dopamine analogue	Relative activity	
	Caudate nucleus	Erythrocytes
	1.00	1.00
2-CH_3	1.09[a]	1.15[a]
2-C_6H_5	0.06	0.10
6-CH_3	0.01	0.10
DL-α-CH_3-2-CH_3	0.02	3.30
DL-α-CH_3-2-C_6H_5	—[b]	0.20
DL-α-CH_3-6-CH_3	—[b]	0.20
DL-α-CH_3	0.01	4.00
N,*N*-$DiCH_3$	0.60	0.88
2-CH_3-*N*,*N*-$DiCH_3$	0.58	0.76
2-C_6H_5-*N*,*N*-$DiCH_3$	—[b]	—[b]
5-CH_3-*N*,*N*-$DiCH_3$	—[b]	—[b]
6-CH_3-*N*,*N*-$DiCH_3$	0.01	0.34
DL-α-CH_3-2-CH_3-*N*,*N*-$DiCH_3$	0.01	0.48
DL-α-CH_3-2-C_6H_5-*N*,*N*-$DiCH_3$	—[b]	—[b]
DL-α-CH_3-5-CH_3-*N*,*N*-$DiCH_3$	—[b]	—[b]
DL-α-CH_3-6-CH_3-*N*,*N*-$DiCH_3$	—[b]	0.19

[a] Not significantly different from 1.0. All others have values of $p < 0.05$.

[b] No measurable activity at 100 μM.

caudate nucleus, and it had less than 10% of the activity of dopamine. With the erythrocyte enzymes the potency of the $(R,-)$ isomer was equivalent to dopamine and comparable to that obtained with the caudate nucleus enzyme. The $(S,+)$ isomer, on the other hand, was much more potent than the $(R,-)$ isomer of α-methyldopamine.

Other dopamine analogues were tested and found to be inactive at 100 μM. These included 3-*O*-methyl-, 4-*O*-methyl-, 6-hydroxy-, and 6-aminodopamine, tyramine, and amphetamine. With the dopamine-sensitive cyclase L-dopa appeared to have about 10% of the potency of dopamine. All these compounds were inactive with the erythrocyte enzyme. When tested as possible inhibitors or potentiators of the dopamine response, L-dopa and L-isoproterenol, both

TABLE 4

Ability of two isomers of α-methyldopamine to activate adenylate cyclases of rat caudate nucleus and erythrocytes

Results are means ± standard errors.

Dopamine analogue	Concentration	cAMP content	
		Caudate nucleus	Erythrocytes
	μM	*pmoles/incubation*	
None		41.4 ± 2.3	5.1 ± 0.2
Dopamine	10	80.3 ± 4.0	7.4 ± 0.2
α-Methyl $(R,-)$	10	43.0 ± 5.4	8.4 ± 0.2
	30	49.9 ± 6.5	13.7 ± 0.4
	100	61.3 ± 5.4	23.8 ± 1.2
α-Methyl $(S,+)$	10	41.6 ± 2.7	14.8 ± 0.6
	30	45.8 ± 6.2	30.0 ± 0.9
	100	44.8 ± 6.5	60.5 ± 2.5

excellent substrates for catechol *O*-methyltransferase, were inactive at 100 μM against the isoproterenol- and dopamine-sensitive cyclases, respectively. The role of oxidative enzymes and conditions was evaluated and found to be minimal, since dopamine stimulation was not enhanced by incubation in an N_2 atmosphere or in the presence of the monoamine oxidase substrates tyramine and serotonin.

Certain isoquinolines which have been shown to be formed from dopamine and acetaldehyde under conditions *in vitro* (8) were tested and found to have little or no activity in both cyclase systems up to 100 μM. These include the two stereoisomers of salsolinol (6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline) as well as their *N*-methylated derivatives.

DISCUSSION

The dopamine-sensitive cyclase (4, 5), in contrast to the isoproterenol-sensitive enzyme of rat erythrocytes (1), had been shown to be activated by apomorphine. The potency of this agent was found to be similar to that of dopamine (4), and therefore it seemed reasonable to consider the configuration of its catechol and nitrogen groups as a model for dopamine at its receptor site (9, 10). The Newman projec-

tions in Fig. 1 point to the probability that the nitrogen and catechol groups are in a *trans* relationship. Dopamine, of course, is free to rotate around certain C—C bonds, as indicated by the arrows in Fig. 1. Although the *trans* conformation predominates in crystals (11), solutions contain a significant and perhaps predominent (12) fraction of the molecules in a *gauche* conformation, which corresponds to that obtained by a 120° rotation of the carbon atom bound to the nitrogen. That this is not the conformation at the receptor site is indicated by the absence of activity of the tetrahydroisoquinoline salsolinol and its derivatives.

It is important to point out that the discussion which follows is based on confidence that the results truly reflect interactions with the receptors and are not affected by metabolic degradation of the agonists. The failure of isoproterenol to enhance the response to dopamine eliminates catechol *O*-methyltransferase as a factor. Likewise, monoamine oxidase and other oxidative mechanisms can be viewed as unimportant in 5-min incubations, since the dopamine response could not be increased by flushing the incubations with N_2 or incubation with substrates of monoamine oxidase such as tyramine or serotonin.

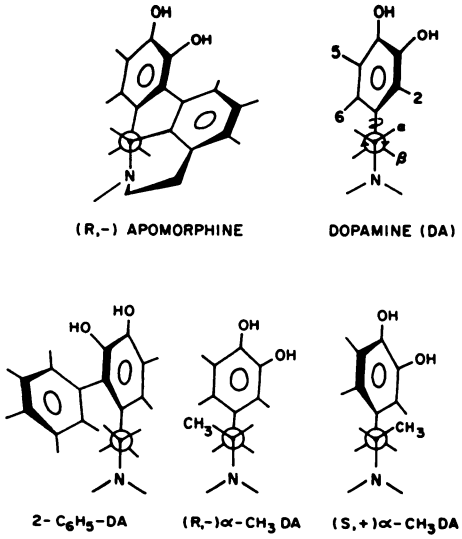

FIG. 1. *Newman projections of apomorphine, dopamine, 2-phenyldopamine (2-C_6H_5-DA), (R,−)-α-methyldopamine (α-CH_3DA), and (S,+)-α-methyldopamine (α-CH_3DA)*

The structure of the apomorphine molecule suggests that the activity of dopamine at the dopamine receptor might not have been greatly altered by a methyl or phenyl group at position 2 of the catechol moiety, a methyl group on the α-carbon with an (*S*,+) configuration, an alkyl group even as large as a propyl moiety on the nitrogen, or dialkylation to form a tertiary nitrogen. The data obtained in these studies, of course, prove several of these ideas false. While a methyl group at position 2 was without effect, a phenyl group was clearly very inhibitory. The (*S*,+)-α-methyl group was also very inhibitory, as were substituents on the nitrogen larger than a methyl group. The latter two findings correspond with observations made at more physiological levels of organization. Goldberg *et al.* (13) demonstrated that *N*-ethyl-, *N*-propyl-, and *N*-isopropyldopamine, as well as isoproterenol, failed to promote vasodilation through a dopamine receptor in the dog kidney, and clinical experience has shown that α-methyldopa, which yields (*S*,+)-α-methyldopamine after decarboxylation, is ineffective in the treatment of parkinsonism.

The marked reduction in potency seen with the 2-phenyl, (*R*,−)-α-methyl, and *N*-alkyl derivatives suggests that these substituents are unable to adopt the conformations seen in the apomorphine molecule because of interactions with other regions of the receptor. The presence of some activity suggests that these compounds probably do interact with the dopamine binding site, but that a hydrophobic region interacts with these substituents and distorts the molecule from its optimum conformation. While several such regions may exist, it is felt that these results can be accommodated to a model having a single hydrophobic region to the left of the molecule at the 9 o'clock position.[2] This region could bind the 2-phenyl group and effect a

[2] The Newman projections in Fig. 1 are viewed from the C—C bond of the side chain and give the relative positions of the catechol hydroxyl and the side chain nitrogen, regardless of which portion actually moves.

90° rotation of the catechol moiety as seen in Fig. 1. The binding of the (*S*, +)-α-methyl group could cause the nitrogen to move to the left, resulting in an increase in the *N* to 3-O distance. The (*R*, −)-α-methyl group would move the nitrogen with a lesser increase in *N* to 3-O distance and the retention of some activity. The *N*-alkyl substituents of 2 or more carbon atoms may also interact with the same hydrophobic region to effect changes in the conformation of the catecholamine. It is equally likely that they produce steric hindrance to attachment of the nitrogen to its binding site.

Similar reasoning may be applied to the *beta* adrenergic receptor, with the notable differences being that *N*-alkylation, β-hydroxylation, and (*S*, +)-α-methyl substitution increase the effects of dopamine. Perhaps the receptor molecules have the same primary structure in both systems but differ in their conformation in the membrane. Thus the *beta* receptor would be organized with the nitrogen binding group closer to the postulated hydrophobic region, and this would require a conformation of the dopamine molecule similar to that seen in Fig. 1 for the (*S*, +)-α-methyl compound.

It has been noted from pharmacological studies that the catechol and amine functions are important for the activity of catecholamines. In agreement with this concept are the observations that methylation of either of the catechol hydroxyls results in a loss of activity.

The failure of isoproterenol and L-dopa to inhibit the activation of the dopamine- and *beta*-sensitive cyclases, respectively, suggests that the catechol binding site is not fully exposed in both receptors. It has been suggested that sequential binding occurs at the *beta* receptor so that the initial interactions effect a partial unfolding of the receptor, making the rest of the binding site available (14). The initial interaction was envisioned as involving a folded catecholamine. Although this could apply to the *beta* receptor it would not permit the rigid extended conformation of apomorphine to act on the dopamine receptor. Thus a different interaction-unfolding mechanism would have to be applied to the dopamine receptor, and perhaps the *beta* receptor as well. It would be premature at this time to attempt a more detailed description of these mechanisms.

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