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The Effect of *Alpha, Beta,* and Dopamine Receptor-Blocking Agents on the Stimulation of Rat Erythrocyte Adenyl Cyclase by Dihydroxyphenethylamines and Their β-Hydroxylated Derivatives

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SUMMARY

A study was made of the activation of rat erythrocyte adenyl cyclase by dopamine, D(−)-norepinephrine, and their *N*-methyl and *N*-isopropyl derivatives. The concentrations required for 50% maximal stimulation (ED_{50}) for norepinephrine, epinephrine, and isoproterenol were 5, 0.69, and 0.24 μM, respectively. For dopamine, *N*-isopropyldopamine, and *N*-methyldopamine the ED_{50} values were 84, 8, and 6.8 μM. While the maximum stimulation achieved with norepinephrine and its *N*-alkylated derivatives was the same, that for dopamine and its derivatives varied with potency. It was postulated that the D(−)-configuration of the β-hydroxylated series, in contrast to the deoxy analogues, induced a more activating conformational change in the cyclase. This was supported by the finding that L(+)-isoproterenol was equipotent with *N*-isopropyldopamine. Propranolol, chlorpromazine, haloperidol, phentolamine, serotonin, and phenoxybenzamine inhibited to the same extent the stimulation of adenyl cyclase produced by *N*-methyldopamine and norepinephrine. At low concentrations of inhibitor the stimulation by norepinephrine, but not by dopamine, could be reduced. These observations, along with the inactivity of apomorphine as a stimulator of adenyl cyclase, negate the existence of a specific dopamine receptor associated with the rat erythrocyte adenyl cyclase.

INTRODUCTION

Attempts from various laboratories to demonstrate stimulation of adenyl cyclase by dopamine have consistently met with failure (1–4). Therefore, the finding that DA[1] was capable of activating the rat erythrocyte adenyl cyclase system (5) afforded the unique opportunity of studying the relative importance of the β-hydroxyl group of dihydroxyphenethylamines for this activation, as well as the possible presence of a specific DA receptor. Such a receptor has been described for the central nervous system (6) and the renal and mesenteric vasculature (7). These DA-specific responses could be mimicked by apomorphine and inhibited by haloperidol, but not by phenoxybenzamine, phentolamine, or propranolol (8).

The studies described below demonstrate that activation of erythrocyte adenyl cyclase has a preference but not an absolute requirement for the D(−)-β-hydroxyl group on the dihydroxyphenethylamine side chain. In addition, none of the responses to DA and its analogues conformed to the pharmacological profile established for a DA receptor.

[1] The abbreviations used are: DA, dopamine (3,4-dihydroxyphenethylamine); *N*-methyl-DA and *N*-isopropyl-DA, the *N*-methyl and *N*-isopropyl derivatives of dopamine; NE, norepinephrine.

METHODS

The preparation of rat erythrocyte ghosts and the assay for adenyl cyclase were carried out as described previously (9). In brief, 0.2 ml of ghosts was incubated in quadruplicate in 0.5 ml of 0.05 M Tris-HCl buffer (pH 7.4) in the presence of $MgCl_2$ (1 μmole), ATP-8-^{14}C (1 μCi/0.24 μmole), and adenosine cyclic 3′,5′-phosphate (1 μmole) for 30 min at 37°. Aliquots of 0.1 ml were chromatographed on paper with carrier ATP, ADP, AMP, and adenosine with a solvent composed of absolute ethanol—1 M ammonium acetate–water (5:1:1 by volume). The spots were located with ultraviolet light and cut out, and ^{14}C was determined in a liquid scintillation counter (Nuclear-Chicago, mark I).

The chemical compounds were obtained from the following sources: D(−)-norepinephrine bitartrate, dopamine HCl, and serotonin creatinine sulfate, Sigma Chemical Company; D(−)-epinephrine *l*-bitartrate, D(−)-isoproterenol *d*-bitartrate, and L(+)-isoproterenol *d*-bitartrate dihydrate, Winthrop Laboratories; haloperidol, McNeil Laboratories, Inc.; phentolamine HCl, Ciba Pharmaceutical Company; propranolol HCl, Ayerst Laboratories; phenoxybenzamine HCl, Smith Kline & French Laboratories; *N*-methyldopamine HBr, *N*-isopropyldopamine HBr, and chlorpromazine HCl, Hoffmann-La Roche, Inc., Basle, Switzerland; ATP-8-^{14}C, tetrasodium salt (35–50 mCi/mmole), Schwarz BioResearch; and ATP, disodium salt, P–L Biochemicals, Inc.

RESULTS

The responsiveness of erythrocyte adenyl cyclase to the catecholamines varied in intensity from experiment to experiment. In order to obtain valid data concerning relative potencies of various agents, it was necessary to compare compounds in the same experiment. The data in each figure and table were qualitatively reproducible in repetitive experiments.

Dose-response curves for the D(−)-β-hydroxylated catecholamines (Fig. 1) show the following order of potency: isoproterenol > epinephrine > norepinephrine. The maximum stimulation achieved with all three, however, was identical. Graphical estimations of the concentration resulting in 50% of maximum stimulation (ED_{50}) gave values for D(−)-isoproterenol, D(−)-epinephrine, and D(−)-norepinephrine of 0.24, 0.69, and 5 μM, respectively.

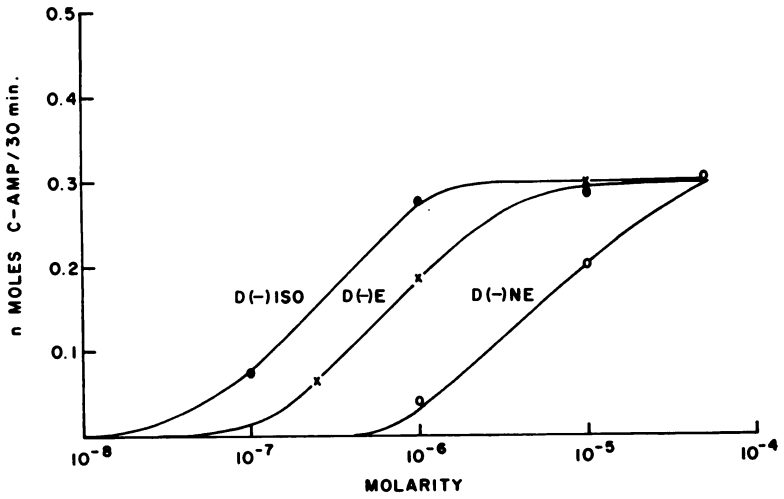

FIG. 1. *Stimulation of rat erythrocyte adenyl cyclase by various concentrations of* D(−)*-isomers of isoproterenol (ISO), epinephrine (E), and norepinephrine (NE)*

Each incubation contained the equivalent of 0.2 ml of packed cells and was run in quadruplicate. All points represent the mean production of adenosine cyclic 3′,5′-phosphate (C-AMP) minus the basal values.

The ability of the non-β-hydroxylated catecholamines, DA and *N*-methyl-DA, to activate the rat erythrocyte adenyl cyclase is shown in Fig. 2. Dopamine, with an ED_{50} of 84 μM, is much weaker than NE, with an ED_{50} in this experiment of 4 μM. In addition, the maximal stimulation of the enzyme with NE was greater than with DA. The addition of a methyl group to the nitrogen of DA (*N*-methyl-DA) increased the potency (ED_{50}, 7.2 μM) and maximal stimulation such that this compound was only slightly weaker than NE. It is interesting that, after maximum stimulation was achieved, a further increase in concentration of the three agonists effected a decrease in the generation of cyclic 3′,5′-AMP. It would appear from Fig. 3 that for isoproterenol and epinephrine inhibition does not occur immediately after maximum stimulatory concentrations are reached. Instead, stimulation remains at a high level until the concentration exceeds 1 mM, and inhibition proceeds equally for isoproterenol, epinephrine, and norepinephrine. Thus, potency as an activator is not related to potency as an inhibitor of adenyl cyclase.

Consideration of the relative importance of the β-hydroxyl group in the activation of the adenyl cyclase led to a comparison of D(−)-isoproterenol, L(+)-isoproterenol, and *N*-isopropyl-DA (Fig. 4). As with DA, activation by *N*-isopropyl-DA reached a maximum value which was lower than that achieved by the D(−)-β-hydroxylated analogue. *N*-Isopropyl-DA (ED_{50}, 8 μM) is appreciably more potent than DA (ED_{50}, 84 μM) and slightly less potent than *N*-methyl-DA (ED_{50}, 6.8 μM). It was extremely interesting to observe that L(+)-isoproterenol yielded a dose-response curve almost identical with that of *N*-isopropyl-DA. No point on the L(+)-isoproterenol curve was significantly different from the comparable point on the *N*-isopropyl-DA curve. The data of Figs. 2 and 3 suggest that not only the potency but the maximum stimulation achievable with these three agents followed the order *N*-methyl-DA > *N*-isopropyl-DA > DA. This was substantiated by a direct

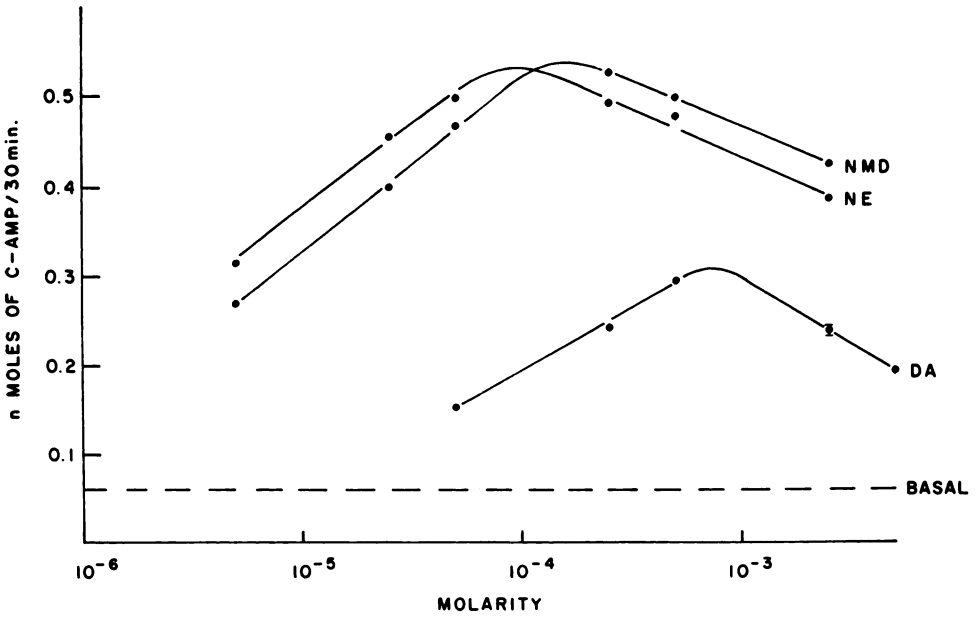

FIG. 2. *Activation of rat erythrocyte adenyl cyclase by D(−)-norepinephrine (NE), dopamine (DA), and N-methyldopamine (NMD)*

The dashed line represents the basal production of adenosine cyclic 3′,5′-phosphate (C-AMP). Incubations were carried out as described in Fig. 1 and METHODS. At any given concentration, the values in the presence of *N*-methyldopamine and norepinephrine were not significantly different.

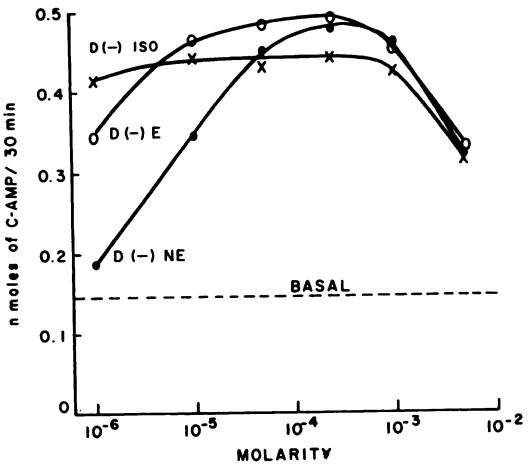

FIG. 3. *Stimulation of rat erythrocyte adenyl cyclase by high concentrations of* D(−)*-isomers of isoproterenol (ISO), epinephrine (E), and norepinephrine (NE)*

Incubations were carried out as described in Fig. 1 and METHODS. At 1 μM, the values for isoproterenol, epinephrine, and norepinephrine were significantly different ($p < 0.05$). At 10 μM, only the value for norepinephrine differs significantly ($p < 0.05$). No significant differences were noted at 100 μM and above.

comparison of the three compounds in a single experiment. The net amounts of cyclic 3′,5′-AMP produced in 30 min in the presence of 1 mM *N*-methyl and *N*-isopropyl derivatives and dopamine itself were 260 ± 9, 195 ± 5, and 129 ± 3 pmoles (± S.E.M.), respectively. Each value was significantly different from any of the others, with values of $p < 0.05$.

The specific peripheral vascular response to DA, in contrast with that to NE, has been observed in the presence of *alpha* and *beta* blockade with phenoxybenzamine and propranolol, respectively. The increased renal and mesenteric blood flow which was obtained could then be blocked with haloperidol (3). It was necessary, therefore, to determine whether the stimulation of adenyl cyclase by DA and NE would be affected differently by these inhibitors. Since *N*-methyl-DA has been shown to act like DA in these vascular beds (6), and since it is found to be almost as potent as NE as an activator of adenyl cyclase, it was used to study the effects of various pharmacological inhibitors.

It can be seen in Table 1 that serotonin (250 μM), chlorpromazine (50 μM), haloperidol (50 μM), phentolamine (500 μM), and

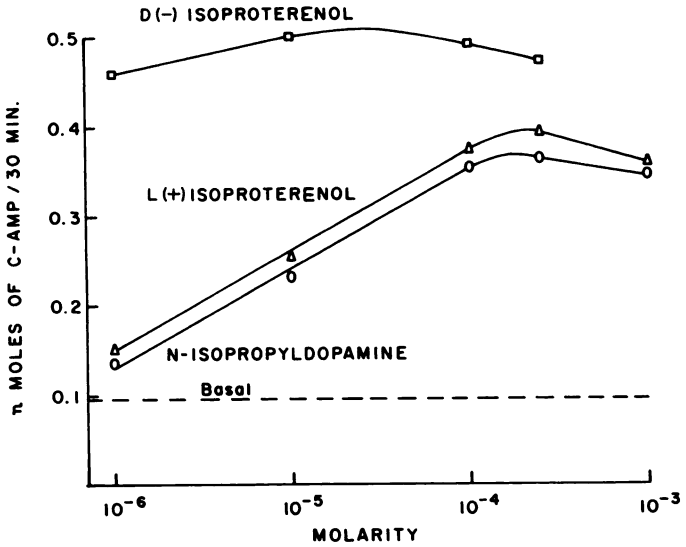

FIG. 4. *Activation of rat erythrocyte adenyl cyclase by N-isopropyldopamine and* D(−)*- and* L(+)*-isomers of isoproterenol*

Incubations were carried out as described in Fig. 1 and METHODS. At any given concentration, the values in the presence of L(+)-isoproterenol and *N*-isopropyldopamine were not significantly different.

TABLE 1

Effect of various inhibitors on stimulation of rat erythrocyte adenyl cyclase by D(−)-norepinephrine, D(−)-epinephrine, and N-methyldopamine

Net production of cyclic 3′,5′-AMP refers to the increase over basal values obtained by the addition of the catecholamine. p values are relative to basal cyclic 3′,5′-AMP production. All other values were significantly different from basal production, with $p < 0.05$. p values for all results relative to the stimulations obtained with no additions were all highly significant (<0.001).

Addition	Net cyclic 3′,5′-AMP production		
	N-Methyl-DA (10 μM)	NE (10 μM)	Epinephrine (1 μM)
	pmoles/30 min		
None	306	315	304
Serotonin (250 μM)	66	98	91
Chlorpromazine (50 μM)	−35	−15[a]	−16[a]
Haloperidol (50 μM)	85	97	98
Phentolamine (500 μM)	62	54	68
Propranolol (0.1 μM)	7[a]	3[a]	8[a]

[a] $p > 0.05$.

propranolol (0.1 μM) inhibited the activation of adenyl cyclase by norepinephrine, epinephrine, and *N*-methyl-DA to the same extent.

The inhibition by propranolol or haloperidol of the increased blood flow obtained with NE or DA, respectively, was observed in the presence of concentrations of phenoxybenzamine which did not affect the *beta* responses to catecholamines (7). Since the erythrocyte adenyl cyclase behaves as a *beta* receptor, it was decided to test whether the presence of phenoxybenzamine at a concentration which did not affect the catecholamine-induced stimulation would alter the inhibitory action of haloperidol and propranolol. It can be seen in Table 2 that 10 μM phenoxybenzamine did not affect the stimulation of adenyl cyclase by NE or *N*-methyl-DA or the inhibition obtained with haloperidol or propranolol.

When only 50 μM serotonin, 5 μM haloperidol, and 50 μM phentolamine were tested, the response to NE (10 μM), but not to DA (100 μM), was significantly inhibited (Table 3).

Attempts to demonstrate activation of adenyl cyclase by apomorphine failed at concentrations below 0.1 mM. Above that concentration only inhibition of the NE

TABLE 2

Effect of combinations of inhibitors on stimulation of rat erythrocyte adenyl cyclase by D(−)-norepinephrine and N-methyldopamine

Net production of cyclic 3′,5′-AMP is described in Table 1. p values are relative to stimulation obtained without the addition of inhibitors.

Inhibitor	Net cyclic 3′,5′-AMP production	
	NE (10 μM)	*N*-Methyl-DA (10 μM)
	pmoles/30 min	
None	336	208
Phenoxybenzamine (10 μM)	315	194
Haloperidol (50 μM)	125[a]	64[a]
Propranolol (0.1 μM)	21[a]	10[a]
Haloperidol (50 μM) + phenoxybenzamine (10 μM)	117[a]	71[a]
Propranolol (0.1 μM) + phenoxybenzamine (10 μM)	12[a]	3[a]

[a] $p < 0.001$.

TABLE 3

Effect of low concentrations of several inhibitors on stimulation of rat erythrocyte adenyl cyclase by dopamine and D(−)-norepinephrine

Net production of cyclic 3′,5′-AMP is described in Table 1. p values are relative to stimulation obtained without the addition of inhibitors. All values were significantly different from basal production, with p values < 0.05.

Addition	Net cyclic 3′,5′-AMP production	
	DA (100 μM)	NE (10 μM)
	pmoles/30 min	
None	74	270
Serotonin (50 μM)	69	146[a]
Haloperidol (5 μM)	72	179[a]
Phentolamine (50 μM)	59	179[a]

[a] $p < 0.01$.

response could be obtained; it was related to the formation of a precipitate with some components of the buffer.

DISCUSSION

The results of these experiments indicate that rat erythrocyte adenyl cyclase possesses a classical *beta* type of receptor, in which D(−)-isoproterenol, D(−)-epinephrine, and D(−)-norepinephrine displayed decreasing potencies and the maximum responses (intrinsic activities) were the same. In the non-β-hydroxylated series, *N*-methyl-DA exceeded *N*-isopropyl-DA in potency and intrinsic activity and the *N*-isopropyl derivative was superior to dopamine. The responses to both classes of catecholamines are inhibited most strongly by propranolol, supporting the idea that the non-β-hydroxylated compounds also act through a *beta* receptor system. Though one may postulate separate receptors for these two groups of catecholamines, one can rationalize the results on the basis of a single receptor. Current thought on the nature of adenyl cyclase system leans toward the model of Robison *et al.* (10), which describes the receptor as a separate macromolecule associated with the adenyl cyclase in such a fashion that the formation of the agonist-receptor complex causes activation of the cyclase. This could be envisioned as occurring through conformational changes in both receptor and cyclase. The absence of the D(−)-β-hydroxyl group would induce a less favorable conformational change in the receptor, resulting in less activation of adenyl cyclase. This concept is supported by the observation that L(+)-isoproterenol has the same activity as *N*-isopropyldopamine. One may conclude that when the β-hydroxyl group does not project in the correct plane it, like the non-β-hydroxylated analogue, produces a less favorable conformation.

The relationships among the D(−)-, L(+)-, and deoxy analogues of catecholamines, demonstrated here for the activation of the enzyme adenyl cyclase, have previously been discussed for both *alpha* and *beta* responses of isolated tissues and whole animals (11, 12). The *alpha* receptor has recently been incorporated into the cyclase model (10) as an entity separate from the *beta* receptor but in some associative state with the enzyme. One is, however, forced to consider the previously expressed ideas (13, 14) that *alpha* and *beta* receptors may involve the same macromolecule. The nature of the response would then be determined by how the macromolecule is organized in a membrane, in much the same way as the performance of a machine may be determined by the arrangement of the gears. The ultimate characterization of the catecholamine receptor of adenyl cyclase must await the results of studies aimed at solubilizing the various components in stable forms so that recombination will restore activity.

The failure of other investigators to demonstrate activation of adenyl cyclase is not understood, but may be related to the relatively small stimulation (1–2-fold) achieved with NE over basal levels of cyclic 3′,5′-AMP production. Our own experience has shown that a DA response was difficult to obtain if stimulation with NE was 2-fold or less. In the experiments reported, NE stimulation ranged from 4 to 8 times the basal production.

It is concluded that the adenyl cyclase system examined in these studies does not possess the pharmacological properties described for the dopamine receptors of the renal and mesenteric vascular systems (7) and the nervous system (6). This conclusion is based on the lack of response to apomorphine, the absence of special sensitivity to inhibition by haloperidol or chlorpromazine, and the greater potency of *N*-isopropyl-DA as compared to DA. It is quite possible that adenyl cyclase is not involved in any of these specific dopamine responses, but until this enzyme system is put to test in dopamine-receptive tissues, one may not dismiss its role as unimportant.

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