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Dopamine- and Serotonin-Sensitive Adenylate Cyclase in the Gill of *Aplysia californica*

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SUMMARY

Stimulation of adenylate cyclase by dopamine and serotonin was examined in a particulate fraction of *Aplysia* gill homogenates. Dopamine augmented activity 3- to 5-fold (EC_{50}, 10 μM), whereas serotonin increased activity 15- to 20-fold (EC_{50}, 1 μM). Lysergic acid diethylamide and several ergot alkaloids were full or partial agonists for enzyme stimulation. Structure-activity relationships of dopaminergic and serotonergic stimulation and the blocking action of several antagonists suggest that structural similarities exist between the receptors that mediate dopamine and serotonin stimulation of adenylate cyclase.

INTRODUCTION

Stimulation of adenylate cyclase by DA[2] (1, 2) and 5-HT (3, 4) as well as the blocking action of antipsychotic drugs on DA receptors (5), on DA-stimulated adenylate cyclase (6), and on 5-HT receptors (7) has been extensively studied in the mammalian central nervous system. Smooth muscle excitation has been shown to involve DA and 5-HT as neurotransmitters whose action may be mediated by cyclic AMP (8). In the marine mollusk *Aplysia*, histochemical studies have provided evidence for DA- and 5-HT-containing neurons innervating the gill smooth muscle (9), and the presence of receptors for both amines has been demonstrated (10). Both neurohormones increase cyclic AMP synthesis (11, 12), although their effects on gill movement are quite dissimilar (9, 10). From their studies on DA- and 5-HT-stimulated cyclic AMP accumulation, Kebabian *et al.* (12) suggested that distinct receptors for each neurohormone exist in this tissue. However, a variety of DA and 5-HT receptor antagonists were unable to block either receptor selectively. In this paper we describe hormonal stimulation of adenylate cyclase in particulate preparations of *Aplysia* gill. Activation of the enzyme by DA, 5-HT, and a variety of psychotomimetic agents and the blocking action of several DA and 5-HT antagonists are examined.

MATERIALS AND METHODS

[2,8-^{3}H]adenosine 3′,5′-cyclic phosphate (30 Ci/mmole) and [α-^{32}P]ATP (20–30 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.) Aqueous counting scintillant (ACS) was obtained from Amersham Searle (Arlington Heights, Ill.). ATP, GTP, cyclic AMP, creatine phosphokinase (Type I, rabbit muscle), creatine phosphate, dithiothreitol, 3-isobutyl-1-methylxanthine, bovine serum albumin, imidazole, ergotamine tartrate, apomorphine HCl, 3-hydroxy-4-methoxyphenethylamine HCl (4-methyldopamine), D,L-α-methyl-*p*-tyrosine methyl ester HCl, 3-hydroxytyramine HCl, 2-bromo-α-ergocryptine methanesulfonate, *N*-acetyldopamine, *N*-methyldopamine, α-ergocryptine, ergonovine maleate, 5-methoxy-*N*,*N*-dimethyltryptamine, tryptamine HCl, 6-methoxytryptamine, 5-hydroxytryptamine, 5-methoxytryptamine, acetylcholine chloride, and D,L-isoproterenol HCl were purchased from Sigma Chemical Company (St. Louis, Mo.). *N*-Acetylserotonin, tyramine HCl, L-(−)-dihydroxyphenylalanine (dopa), 6-hydroxydopamine HBr, 4-methoxydopamine, 5-hydroxydopamine HBr, 5-hydroxytryptophan, octopamine HCl, α-ethyltryptamine acetate, 5-methoxytryptophol, 5-hydroxytryptophol, and 5,7-dihydroxytryptamine were obtained from Regis Chemical Company (Morton Grove, Ill.). Other agents were obtained from the following sources: Gpp(NH)p, ICN (Plainview, N. Y.); dopamine HCl and serotonin creatine sulfate, Nutritional Biochemicals Corporation (Cleveland, Ohio); D,L-epinephrine HCl, K & K Laboratories (Plainview, N. Y.); histamine dihydrochloride, Fisher Scientific Company (Toronto, Ont.); LSD-25, *N*,*N*-dimethyltryptamine, *N*,*N*-diethyltryptamine, and 2-bromo-LSD, Sandoz (Basle, Switzerland); cyproheptadine HCl, Merck and Company (Rahway, N. J.). Fluphenazine dihydrochloride and haloperidol were gifts from Dr. P. Seeman, University of Toronto (Toronto, Ont.); methergoline was a gift from Dr. J. W. Phillis, University of Saskatchewan, and chlorpromazine HCl was obtained from Dr. S. Roth, University of Calgary (Calgary, Alta.). *Aplysia* gills were obtained from

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[2] The abbreviations used are: DA, dopamine; 5-HT, serotonin (5-hydroxytryptamine); Gpp(NH)p, 5′-guanylylimidodiphosphate; LSD-25, lysergic acid-diethylamide.

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Dr. K. Lukowiak, Department of Physiology, University of Calgary, and were kept at −80° until used.

Preparation of gill particulate fraction. Frozen tissue was cut into pieces; suspended in 10 volumes of 0.25 M sucrose, 20 mM Tris-HCl, and 1 mM dithiothreitol (pH 7.5); and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle for 100 passes. The homogenate was passed through a 100-μm nylon filter under light suction to remove connective tissue and was centrifuged at 38,000 × *g* for 20 min. The pellet was washed twice by suspending it in buffer followed by centrifugation; it was finally suspended in 10 volumes of buffer (based on initial tissue weight) to a protein concentration of 3–4 mg/ml. The preparation was divided into aliquots sufficient for a single experiment which were stored at −80° for not longer than 1 week before use.

Adenylate cyclase assay. The method of Salomon *et al.* (13) was used. Reactions were initiated by addition of gill fraction (90–120 μg of protein) to reaction mixtures containing 40 mM Tris-HCl (pH 7.5), 8 mM $MgSO_4$, 5.5 mM KCl, 1 mM 3-isobutyl-1-methylxanthine, 10 mM creatine phosphate, 1 mM cyclic AMP, creatine phosphokinase (530 μg/ml), 100 μM GTP, 0.5 mM [α-^{32}P]ATP (40 dpm/pmole), and test substances in a final volume of 150 μl. Incubations were carried out at 25° for 10 min in a shaking bath. Reactions were terminated by the addition of 0.1 ml of "stop" solution, and ^{32}P-labeled cyclic AMP was isolated and quantitated (13) by scintillation spectrometry using ACS cocktail. All values reported are the average of duplicate determinations, which agreed within 5%; each experiment was performed at least twice using different preparations. Protein was determined by the method of Lowry *et al.* (14).

RESULTS

In preliminary experiments, basal and hormone-stimulated activity was examined in the presence of varying concentrations of Mg^{2+} and Mn^{2+}. Basal activity was saturated with 8 mM Mg^{2+} and 0.5 mM Mn^{2+}; V_{max} was 7 times greater with the latter cation (Table 1). Gpp(NH)p augmented activity 21-fold in the presence of 8 mM Mg^{2+}. GTP at 100 μM had no effect on enzyme activity in the presence of 0.5 mM Mn^{2+} but increased activity 3-fold with 8 mM Mg^{2+}. In the presence of 8 mM Mg^{2+} (and 100 μM GTP), DA and 5-HT stimulated basal activity 5-fold and 20-fold, respectively. In the presence of 0.5 mM Mn^{2+}, stimulations by DA and 5-HT were 1.3- and 3.2-fold respectively. Throughout the study Mg^{2+} (8 mM) was the divalent cation employed for expression of hormonal stimulation.

TABLE 1

Effects of Mn^{2+} and Mg^{2+} on expression of hormonal stimulation of adenylate cyclase

All values are means ± standard errors of three separate experiments with different preparations.

Assay	8.0 mM Mg^{2+}		0.5 mM Mn^{2+}	
	Specific activity	-Fold stimulation[a]	Specific activity	-Fold stimulation[a]
	pmoles/min/mg		*pmoles/min/mg*	
Basal	1.4 ± 0.4	—	9.7 ± 0.5	—
Gpp (NH)p (10 μM)	29.8 ± 2.2	21.0	39.9 ± 1.8	4.1
GTP (100 μM)	4.2 ± 1.0	3.0	9.3 ± 0.5	1.0
GTP (100 μM) + DA (100 μM)	7.3 ± 0.1	5.1	12.7 ± 1.5	1.3
GTP (100 μM) + 5-HT (100 μM)	24.0 ± 1.1	16.9	31.0 ± 1.0	3.2

[a] Over basal activity without GTP.

Stimulation of adenylate cyclase by various agents. In addition to DA and 5-HT, several ergot compounds augmented enzyme activity (Fig. 1). Half-maximal activation (EC_{50}) by 5-HT occurred at 1 μM. DA was much less potent (EC_{50}, 10 μM) and had an efficacy relative to 5-HT of 0.33. Of the ergot alkaloids tested, ergotamine was equieffective with 5-HT and was 10 times more potent; α-ergocryptine was as potent as 5-HT but not as effective (relative efficacy 0.55). LSD-25 was an effective agonist, less potent than 5-HT (EC_{50}, 2 μM), but 5 times more potent than DA and slightly more effective than the latter. Ergonovine was equipotent with DA and marginally more effective.

Experiments were performed to investigate whether the effects of DA and 5-HT were additive. In Fig. 2, dose-response curves for DA and 5-HT stimulation of the enzyme in the presence and absence of a subsaturating concentration of the other agonist are shown. At subsaturating concentrations of 5-HT, the presence of 5 μM DA further augmented activity; i.e., the response was additive. At concentrations of 5-HT approaching saturation, 5 μM DA caused only a slight additional increase in activity. Similarly, when varying concentrations of DA were examined in the presence of 5 μM 5-HT, the effects were additive only at subsaturating concentrations of the former. In additional experiments a supersaturating concentration of DA (1 mM) was tested in the presence of 100 μM 5-HT; stimulation of enzyme activity was equiv-

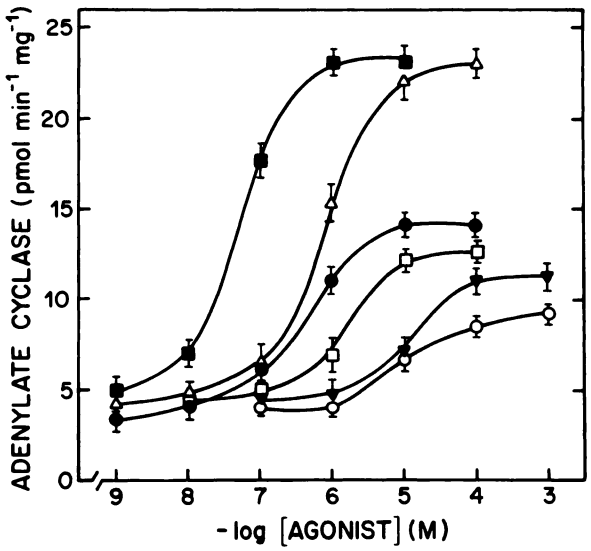


FIG. 1. *Activation of adenylate cyclase by various agents*

Activation by agents was determined under standard assay conditions as described under Materials and Methods. Data presented are averages of three separate experiments with different preparations: ergotamine (■), 5-HT (△), α-ergocryptine (●), LSD-25 (□), ergonovine (▼), DA (○). *Vertical bars* represent standard error of the mean.

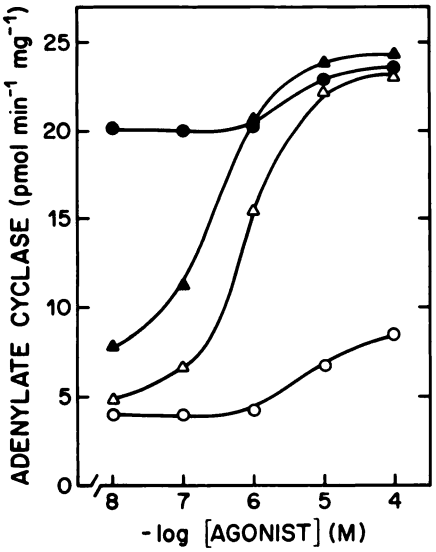


FIG. 2. *Combined effects of DA and 5-HT on adenylate cyclase activity*

The dose-response curves for DA activation were obtained in the absence (○) and presence (●) of 5 μM 5-HT. The dose-response curves for 5-HT activation were obtained in the absence (△) and presence (▲) of 5 μM DA. All points are averages of two experiments using different preparations.

alent to that achieved with 5-HT alone (Table 2). Similarly, stimulation produced by 1 mM DA and 10 μM ergotamine was equivalent to that produced by the latter agent alone.

TABLE 2

Combined effects of saturating concentrations of several agonists on adenylate cyclase activity

All values are means ± standard errors of three separate experiments with different preparations.

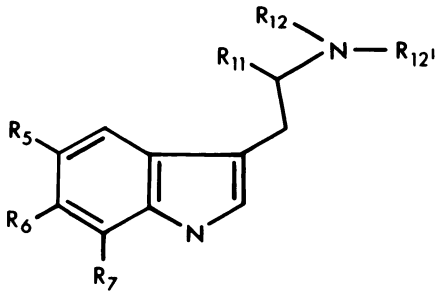
Agonist	Specific activity
	pmoles/min/mg
Control	5.3 ± 1.1
DA (1 mM)	12.1 ± 1.3
5-HT (100 μM)	24.5 ± 1.1
Ergotamine (10 μM)	22.1 ± 2.1
DA (1 mM) + 5-HT (100 μM)	22.2 ± 0.8
DA (1 mM) + ergotamine (10 μM)	21.9 ± 1.0
5-HT (100 μM) + ergotamine (10 μM)	23.9 ± 1.7

To examine structural requirements for agonist action, a variety of DA and 5-HT analogues was examined. Data for 5-HT analogues are presented in Table 3. Methylation of the 5-OH resulted in a slight loss in potency; methylation of the 5-OH as well as the amino residue (5-methoxy-*N*,*N*-dimethyltryptamine) caused only a slight loss in potency and a small decline in efficacy. A significant reduction in both potency and efficacy resulted from removal of the 5-OH [tryptamine, *N*,*N*-dimethyl- and *N*,*N*-diethyltryptamine and by the presence of a methoxy group in position 6 as compared with position 5 (6-methoxytryptamine)]. The presence of two hydroxyls in the indole ring in positions 5 and 7 destroyed agonist activity. Modification of the ethylamine side-chain, for example, by replacement of the amino group with a hydroxyl

TABLE 3

Structure-activity relationship of adenylate cyclase activation by various 5-HT analogues

Values are calculated from dose-response curves obtained over the concentration range 0.1 μM–1 mM. All values are means of two separate experiments with different preparations.



Analogue	R_5	R_6	R_7	R_{11}	R_{12}	$R_{12'}$	EC_{50}	Relative efficacy[a]
							μM	
5-Hydroxytrptamine	OH	H	H	H	H	H	1.0	1.00
5-Methoxytryptamine	OCH_3	H	H	H	H	H	3.2	1.00
5-Methoxy-N,N-dimethyltryptamine	OCH_3	H	H	H	CH_3	CH_3	3.2	0.93
Tryptamine	H	H	H	H	H	H	12.6	0.77
N,*N*-Dimethyltryptamine	H	H	H	H	CH_3	CH_3	7.5	0.80
N,*N*-Diethyltryptamine	H	H	H	H	CH_2CH_3	CH_2CH_3	16.0	0.48
6-Methoxytryptamine	H	OCH_3	H	H	H	H	23.8	0.36
5,7-Dihydroxytryptamine	OH	H	OH	H	H	H	—[b]	0.00
5-Methoxytryptophol	OCH_3	H	H	OH	—	—	50	0.21
5-Hydroxytryptophol	OH	H	H	OH	—	—	100	0.35
N-Acetyl-5-hydroxytryptamine	OH	H	H	H	H	$COCH_3$	—[b]	0.00
5-Hydroxytryptophan	OH	H	H	COOH	H	H	—[b]	0.00
α-Ethyltryptamine	H	H	H	CH_2CH_3	H	H	—[b]	0.00

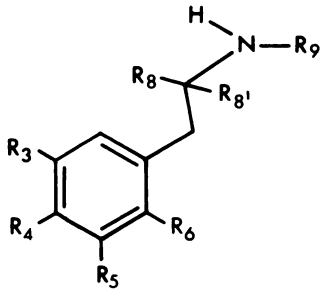
[a] Fraction of maximal 5-HT activation.

[b] Displayed no agonist activity.

TABLE 4

Structure-activity relationship of adenylate cyclase activation by various DA analogues

Values are calculated from dose-response curves obtained over the concentration range 0.1 μM–1 mM. All values are means of two separate experiments with different preparations.



Analogue	R_3	R_4	R_5	R_6	R_8	$R_{8'}$	R_9	EC_{50}	Relative efficacy[a]
								μM	
Dopamine	OH	OH	H	H	H	H	H	10	1.00
N-methyldopamine	OH	OH	H	H	H	H	CH_3	2	1.00
Tyramine	H	OH	H	H	H	H	H	50	0.80
4-Methoxydopamine	OH	OCH_3	H	H	H	H	H	200	0.80
α-Methyltyrosine	H	OH	H	H	CH_3	COOH	H	316	0.20
5-Hydroxydopamine	OH	OH	OH	H	H	H	H	—[b]	0.00
6-Hydroxydopamine	OH	OH	H	OH	H	H	H	—[b]	0.00
Dopa	OH	OH	H	H	COOH	H	H	—[b]	0.00
N-Acetyldopamine	OH	OH	H	H	H	H	$COCH_3$	—[b]	0.00

[a] Fraction of maximal DA activation.

[b] Displayed no agonist potency.

(tryptophol derivatives), by acetylation of the amino group (*N*-acetyl-5-HT), or by insertion of a carboxyl (5-hydroxytryptophan) destroyed all activity. It appears that positions 5, 6, and 7 of the indole ring as well as the ethylamine side-chain are crucial for agonist activity. In additional experiments, the inactive congeners were tested over the concentration range 0.1 μM–100 μM in the presence of 5 μM 5-HT; none displayed antagonist activity.

The effect of several dopamine analogues on adenylate cyclase is summarized in Table 4. Monomethylation of the amino group produced increased potency. Removal of the 3-OH (tyramine) resulted in a loss of both potency and efficacy; potency was greatly reduced by methylation of the 4-OH. The presence of an additional OH in position 5 or 6 destroyed agonist activity, as did modification of the ethylamine side chain (dopa and *N*-acetyldopamine). Thus, as with 5-HT, the position and integrity of the ring hydroxyls as well as the ethylamine side-chain seem to be crucial for agonist activity. Apomorphine and bromo-α-ergocryptine, agonists at DA receptors that are not linked to adenylate cyclase (15), were totally ineffective. Each of the inactive congeners was examined for antagonist activity. At concentrations up to 1 mM none antagonized the action of 0.1 mM DA.

A variety of other putative neurohormones was examined for agonist action over the concentration range 1 nm–1 mM (in the presence of 100 μM GTP): epinephrine, norepinephrine, isoproterenol, octopamine, adenosine, histamine, and acetylcholine were inactive.

Effects of various agents on agonist-stimulated adenylate cyclase activity. To investigate further the nature of the receptors linked to adenylate cyclase, a variety of DA and 5-HT receptor blockers was examined. Stimulation by DA, 5-HT, and ergotamine was antagonized in a dose-dependent manner by chlorpromazine, a DA receptor blocker (5, 6); by methergoline, a 5-HT antagonist (16); and by 2-bromo-LSD, a derivative of LSD capable of interaction with both DA and 5-HT receptors (7) (Fig. 3). Inhibitory constants (K_i) of the above-mentioned agents as well as for the DA blocker haloperidol and the 5-HT antagonist cyproheptadine are given in Table 5. All five agents tested were able to block effectively stimulation by 5-HT, DA, LSD-25, and ergotamine. Chlorpromazine and haloperidol were somewhat more specific as DA antagonists, whereas methergoline and cyproheptadine were more specific for 5-HT. Stimulation of enzyme activity by 5-HT and ergotamine was examined further in the absence and presence of constant concentrations of chlorpromazine or methergoline. Inhibition of agonist-stimulated activity by both agents was competitive as indicated by a rightward shift of the dose-response curves (Fig. 4). In the presence of 5 and 50 μM methergoline as well as 50 μM chlorpromazine, ergotamine displayed a two-component dose-response curve. One component contained 70–75% of total activity and saturated at 10 μM; the second contained 25–30% of total activity and saturated at 1 mM.

DISCUSSION

Previous studies (10, 17) have demonstrated the presence of receptors for DA and 5-HT in *Aplysia* gill. Both neurotransmitters increased cyclic AMP levels in gill slices (12), but their effect on adenylate cyclase was not examined. The present study establishes that adenylate cyclase in this tissue is stimulated by both neurohor-

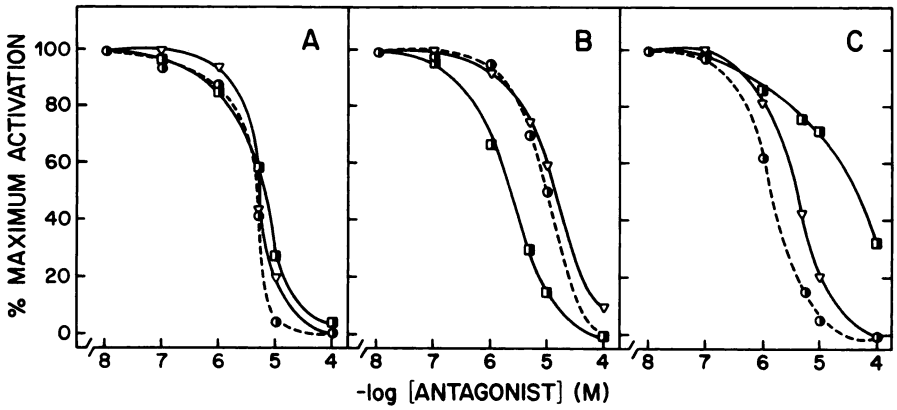


FIG. 3. *Effects of various agents on DA-, 5-HT-, and ergotamine-stimulated adenylate cyclase activity*

Inhibition of adenylate cyclase activity by chlorpromazine (◑), 2-bromo-LSD (▽), or methergoline (◧) was determined under standard assay conditions in the presence of: A, 50 μM DA; B, 5 μM 5-HT; and C, 0.5 μM ergotamine. All values are means of two experiments using different preparations. Activity is normalized to percentage maximal agonist activation.

mones, 5-HT being the more potent. Mg^{2+} afforded greater stimulation by both agonists than did Mn^{2+}; basal activity was significantly greater in the presence of the latter cation. The enzyme was stimulated partially or fully by several ergot alkaloids and LSD-25, in a manner analogous to the interaction of these agents with DA and 5-HT receptors in membrane preparations from nerve (18–20) and on smooth muscle (21). In *Aplysia* gill, ergotamine was as effective as 5-HT in stimulating adenylate cyclase but 10 times more potent, analogous to ergotamine-induced vasoconstriction through 5-HT receptors in vascular smooth muscle (22).

On the basis of their studies on cyclic AMP levels in *Aplysia* gill and heart slice preparations, Kebabian *et al.* (12) suggested that receptors for DA and 5-HT are distinct entities. This suggestion was based on their observation that in the gill maximal responses to DA and 5-HT were additive, and in the heart 5-HT augmented cyclic AMP levels but DA did not. With respect to adenylate cyclase activation, we found that at subsaturating concentrations the response to the two agonists was additive. However, at saturating concentrations of 5-HT or ergotamine, DA produced no further stimulation. Moreover, saturating concentrations of 5-HT and ergotamine together produced a response no greater than that of either agonist alone. These findings would elimi-

TABLE 5

Inhibition of agonist-stimulated adenylate cyclase activity by various agents

Gill particulate fractions were incubated with 5 μM 5-HT, 50 μM DA, or 50 μM LSD-25 under standard conditions together with five concentrations of each antagonist. The concentrations of drugs required to inhibit agonist-stimulated activity by 50% (IC_{50} values) were calculated and were converted to K_i values according to the equation $K_i = IC_{50}/(1 + c/EC_{50})$. EC_{50} is the concentration of agonist for half-maximal stimulation and c is the concentration of agonist in the assay for the K_i studies. Values given are means ± standard errors of three experiments performed in duplicate, with different preparations.

Agonist	Inhibition of agonist-stimulated activity (K_i)				
	2-Bromo-LSD	Chlorpromazine	Haloperidol	Methergoline	Cyproheptadine
			μM		
5-HT	2.08 ± 0.02	1.67 ± 0.43	2.63 ± 0.40	0.33 ± 0.13	2.10 ± 0.32
DA	0.58 ± 0.25	0.75 ± 0.25	1.67 ± 0.55	0.83 ± 0.30	4.70 ± 0.87
LSD-25	0.91 ± 0.31	1.00 ± 0.37	5.09 ± 1.19	0.51 ± 0.20	5.11 ± 0.65

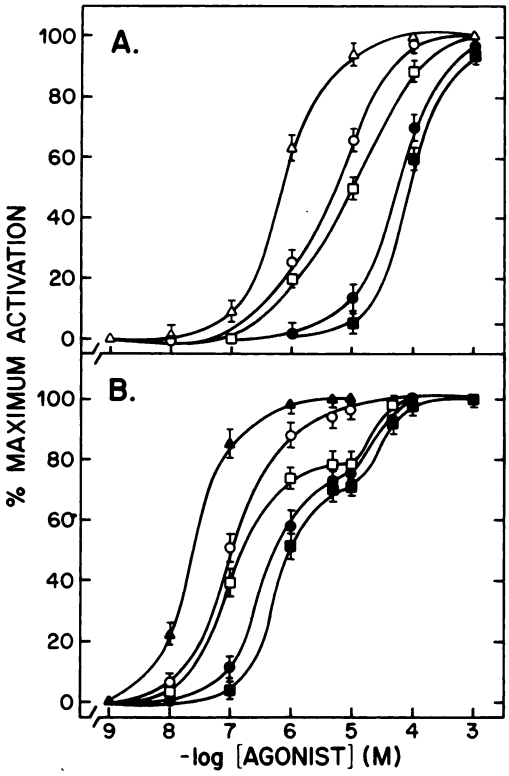


FIG. 4. *Effects of chlorpromazine and methergoline on dose-response curves for 5-HT- and ergotamine-stimulated adenylate cyclase activity*

Dose-response curves for adenylate cyclase stimulation were obtained under standard assay conditions. A, 5-HT alone (△), 5-HT in the presence of 5 μM (○) and 50 μM (●) chlorpromazine, and 5-HT in the presence of 5 μM (□) and 50 μM (■) methergoline. B, Ergotamine alone (▲), ergotamine in the presence of 5 μM (○) and 50 μM (●) chlorpromazine, and ergotamine in the presence of 5 μM (□) and 50 μM (■) methergoline. All *points* are averages of values from duplicate incubations in *three* separate experiments with different preparations. Activity was normalized to percentage maximal agonist activation. *Vertical bars* represent standard error of the mean.

nate the possibility of separate distinct cyclase species, each linked to a single receptor responsive to only one class of agonist. The structure-activity studies revealed similar structural requirements for both DA and 5-HT activation of the enzyme; that is, the ring hydroxyls and the ethylamine side-chain were crucial for activity in both. When the DA molecule was modified as in *N*-methyl DA, there was a 5-fold increase in potency with no loss in efficacy. Such modification could result in a "better fit" into the receptor. These findings would seem to be compatible with a single class of receptors (5-HT receptors) at which DA is a partial agonist and the ergots display varying efficacy and potency. However, in view of the observations of Kebabian *et al.* (12), such a receptor would seem unlikely. One would not expect that two peripheral organs in the same animal would have different 5-HT receptors. In another invertebrate, *Fasciola hepatica*, the 5-HT receptor is coupled to adenylate cyclase and in this system DA was inactive (23). However, other differences exist between the latter system and that in *Aplysia* gill. LSD-25 as a partial agonist was one-half as potent as 5-HT on the gill enzyme but 50 times more potent in the liver fluke system. In addition, two 5-HT analogues, 5-methoxy-*N*,*N*-dimethyltryptamine and *N*,*N*-dimethyltryptamine were potent agonists in *Aplysia* gill but were antagonists of 5-HT action on the liver fluke enzyme.

The studies with DA and 5-HT antagonists provide additional insight into the nature of the receptor(s) linked to adenylate cyclase in *Aplysia* gill. Chlorpromazine and haloperidol, compounds generally considered to be specific for DA receptors, and methergoline and cyproheptadine, generally considered specific for 5-HT receptors, were capable of blocking enzyme activation induced by either agonist and also by LSD-25. From examination of the K_i values for inhibition (Table 5) it appears that chlorpromazine and haloperidol were somewhat more effective in blocking DA stimulation, whereas methergoline and cyproheptadine displayed some specificity for 5-HT activation. This would suggest that there exists a measure of distinction between 5-HT and DA receptor sites. This possibility is supported by the effect of chlorpromazine and methergoline on ergotamine stimulation (Fig. 4B). In the presence of both of these agents the ergotamine dose-response curve appeared to contain two components. The saturation characteristics and relative efficacies suggested a population of receptors (possibly 5-HT receptors) that express 70–75% of total enzyme stimulation, and a second population (possibly DA receptors) which mediates 25–30% of total stimulation. These findings, which were reproducible in five separate experiments, are consistent with other studies in which ergotamine has been shown to interact with postsynaptic receptors for both 5-HT and DA (19) and with recent binding studies (17) which suggest that LSD-25 interacts with DA and 5-HT receptors in several *Aplysia* tissues. From the present study it seems most plausible to suggest that in *Aplysia* gill two separate classes of receptors exist, one for DA and one for 5-HT, and that these receptors have overlapping specificity so that 5-HT can fully activate both receptor classes. Ergotamine would also activate at least the 5-HT receptors producing maximal enzyme activation. Perhaps binding studies will be useful in providing a decisive answer as to the nature of 5-HT and DA interaction in this tissue.

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