MOLECULAR PHARMACOLOGY, 13, 512-520

Octopamine-Sensitive Adenylate Cyclase in Cockroach Brain: Effects of Agonists, Antagonists, and Guanylyl Nucleotides

A. J. HARMAR[1] AND A. S. HORN[2]

Medical Research Council Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, University of Cambridge, Cambridge CB2 2QD, England

(Received November 1, 1976)

(Accepted December 16, 1976)

SUMMARY

HARMAR, A. J. & HORN, A. S. (1977) Octopamine-sensitive adenylate cyclase in cockroach brain: effects of agonists, antagonists, and guanylyl nucleotides. *Mol. Pharmacol.*, 13, 512-420.

The pharmacological properties of a cell-free, octopamine-sensitive adenylate cyclase present in homogenates of the brain of the cockroach, *Periplaneta americana*, have been examined. In accordance with previous reports, octopamine elicited small increases in adenylate cyclase activity in homogenates of both brain and thoracic ganglia. Guanosine 5'-triphosphate, which was routinely included in the assay system, greatly enhanced responses to octopamine, while 5'-guanylylimidodiphosphate greatly increased both basal and octopamine-sensitive adenylate cyclase activities. A variety of phenylethylamines were tested for stimulatory effects upon adenylate cyclase activity in this system: the most potent agonists were found to be octopamine and *p*-fluorophenylethanolamine. The naturally occurring D(−) isomer of octopamine was over 200 times as potent as the L(+) isomer. A variety of drugs were tested as possible antagonists of the octopamine-sensitive adenylate cyclase; the most potent antagonists were the *alpha* adrenoceptor antagonist phentolamine and the histamine and 5-hydroxytryptamine antagonist cyproheptadine. A dopamine-sensitive adenylate cyclase was also observed in homogenates of cockroach brain, and was similar to dopamine-sensitive adenylate cyclases in other tissues in its responses to epinine and to the rigid dopamine analogue 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, and in the stereoselective blockade of responses to dopamine by the potent neuroleptic agent α-flupenthixol. Adenylate cyclase responses to dopamine and octopamine were additive. The structural characteristics necessary for stimulation of octopamine-sensitive adenylate cyclase appear to differ markedly from those required for stimulation of dopamine or *beta* adrenoceptor-linked adenylate cyclase systems.

INTRODUCTION

Evidence is accumulating to suggest that octopamine is a neurotransmitter in a number of invertebrates, including molluscs (1–4), crustacea (5–7), and insects (8–20). It seems likely that many of the postsynaptic actions of octopamine (15–22), as of some other neurotransmitters (23), may be mediated by cyclic 3',5'-AMP. Robertson and Steele (15–17) showed that low concentrations of octopamine activated glycogen phosphorylase in the nerve cord of the cockroach, *Periplaneta americana*, a

[1] Recipient of an Internal Graduate Studentship from Trinity College, Cambridge. Present address, Friedrich Miescher-Institut, Postfach 273, CH-4002, Basel, Switzerland.

[2] Present address, Department of Pharmacy, Laboratory for Pharmaceutical Chemistry, University of Groningen, Groningen, The Netherlands.

ISSN 0026-895x

tissue which contains substantial amounts of octopamine (17). Nathanson and Greengard (18–20) demonstrated that incubation of *Periplaneta* thoracic ganglia with octopamine, dopamine, or 5-hydroxytryptamine resulted in increased cyclic AMP synthesis, and that adenylate cyclases sensitive to these three neurotransmitters were present in homogenates of the ganglia. Carlson (10–13) showed that octopamine and synephrine were the most potent of a number of phenylethylamines in stimulating luminescence in the perfused lantern of the firefly, *Photuris;* norepinephrine and dopamine were at least an order of magnitude less potent. Since the lantern contains substantial amounts of octopamine, but no synephrine (14), octopamine is probably the transmitter in this system. Little information is available concerning the structural requirements for agonist or antagonist activity at octopamine receptors; we have therefore tested a number of phenylethylamines and other drugs as possible agonists or antagonists of the octopamine-sensitive adenylate cyclase. Guanylyl nucleotides have been implicated as regulators of a number of hormone- (24) and neurotransmitter- (25, 26) sensitive adenylate cyclases; the effects of guanosine 5′-triphosphate and 5′-guanylylimidodiphosphate upon the octopamine-sensitive adenylate cyclase were also examined.

MATERIALS AND METHODS

Drugs and reagents. All common laboratory reagents were of analytical grade and were purchased from Fisons, Ltd. Other drugs and chemicals were obtained from the following sources: ATP, GTP, DL-octopamine HCl, DL-phenylethanolamine, tyramine HCl, dopamine HCl, 5-hydroxytryptamine creatinine sulfate, DL-normetanephrine HCl, DL-norepinephrine HCl, DL-epinephrine HCl, L-phenylephrine HCl, and yohimbine HCl, from Sigma; DL-synephrine tartrate and β-phenylethylamine HCl, from Koch-Light; 5′-guanylylimidodiphosphate, from Boehringer; α-methyl-DL-octopamine HCl, from Aldrich; DL-isoproterenol HCl, from Lilly; DL-*m*-octopamine, from Phase Separations; phentolamine methanesulfonate, from Ciba; piperoxan HCl and cyproheptadine HCl, from Merck Sharp & Dohme; dichloroisoproterenol HCl, practolol HCl, and DL-propranolol HCl, from ICI; promethazine HCl and chlorpromazine HCl, from May and Baker; H35/25, from Hässle; and α- and β-flupenthixol HCl, from Lundbeck.

We are especially grateful to Dr. P. N. Patil for gifts of the D and L isomers of octopamine, to Dr. R. Pinder for 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene HBr, and to Dr. R. W. Fuller (Lilly Research Laboratories) for the halogenated phenylethanolamines.

Octopamine- and dopamine-sensitive adenylate cyclases (cockroach brain). Adult male cockroaches, *Periplaneta americana,* were obtained from cultures maintained at the Department of Zoology, University of Cambridge. Insects were decapitated, and their brains were removed and placed in cold insect Ringer's solution (18) containing NaCl, 214 mM; $CaCl_2$, 9 mM; KCl, 3.1 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 0.4 mM; $NaHCO_3$, 25 mM; and D-glucose, 10 mM. This solution had a pH of 7.4 after equilibration with a mixture of 95% O_2 and 5% CO_2 at 0°. After brief washing in a similar Ringer's solution from which $CaCl_2$ had been omitted, tissues were homogenized (10 mg/ml) in 6 mM Tris-maleate buffer (pH 7.4) containing 2 mM EGTA,[3] using a glass homogenizer with a motor-driven Teflon pestle. Adenylate cyclase activity was measured in an assay system slightly modified from that of Nathanson and Greengard (18), containing Tris-maleate (pH 7.4), 80 mM; theophylline, 10 mM; $MgSO_4$, 2 mM; EGTA, 0.5 mM; GTP, 0.1 mM; ATP, 0.5 mM; and tissue homogenate (0.1 mg, wet weight) plus test substances as indicated, in a final volume of 80 μl. After preliminary incubation of the assay system in the absence of ATP for 5 min at 0°, ATP was added to start the reaction. After incubation at 30° for 3 min in a shaking water bath, the reaction was terminated by boiling for 1.5 min. Cyclic AMP formation was linear for incubation times of up to 5 min, and for tissue concen-

[3] The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

trations of up to 0.2 mg, wet weight, per assay tube. The contents of each assay tube were then centrifuged for 5 min in a Beckman Microfuge to remove denatured protein, and 10-μl aliquots of the supernatant were assayed for cyclic AMP by the method of Brown, Ekins, and Albano (27). When assay conditions differed from the above, they are described in the figure legends.

Dopamine-sensitive adenylate cyclase (rat striatum). Dopamine-sensitive adenylate cyclase in homogenates of rat striatum was measured by the method of Miller, Horn, and Iversen (28).

RESULTS

Effect of guanylyl nucleotides upon octopamine-sensitive adenylate cyclase. Only a small stimulation of adenylate cyclase activity was elicited by octopamine if GTP was omitted from the assay system. Results of a typical experiment are given in Table 1: in the absence of GTP, octopamine (0.3 mM) stimulated adenylate cyclase activity to 112% ± 8% (SEM) of basal activity in brain homogenates and to 138% ± 8% of basal levels in homogenates of thoracic ganglia. In the presence of 0.1 mM GTP, the stimulations were 270% ± 20% and 141% ± 7% of controls, respectively. The effects of various concentrations of GTP and of 5'-guanylylimidodiphosphate upon adenylate cyclase activity in cockroach brain homogenates are shown in Fig. 1. Both nucleotides stimulated octopamine-sensitive adenylate cyclase activity to as much as 4 times the level observed in the absence of added guanylyl nucleotides; maximal responses to both nucleotides were observed at 0.1 mM. Basal activities were stimulated up to 2-fold by GTP and up to 8.5-fold by 5'-guanylylimidodiphosphate. All subsequent adenylate cyclase assays were carried out in the presence of 0.1 mM GTP.

TABLE 1

Effect of GTP upon adenylate cyclase activity in homogenates of cockroach brain and thoracic ganglia

Cockroach brains and thoracic ganglia were assayed for adenylate cyclase activity as described in MATERIALS AND METHODS, in the presence and absence of octopamine (0.3 mM) and GTP (0.1 mM). Values represent the means ± standard errors of four separate incubations.

Additions	Adenylate cyclase activity	
	Thoracic ganglia	Brain
	pmoles cyclic AMP formed/min/mg tissue	
None	10.8 ± 0.4	18.3 ± 0.4
Octopamine	14.9 ± 0.9	20.5 ± 1.6
GTP	16.3 ± 2.3	25.7 ± 5.1
Octopamine + GTP	23.0 ± 1.2	69.3 ± 5.2

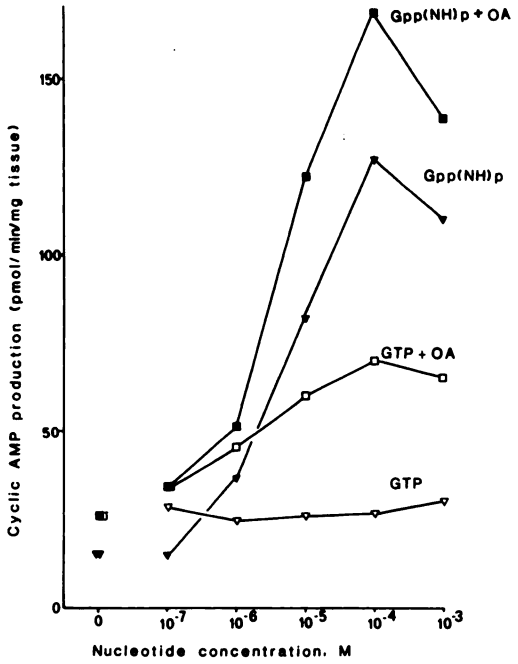


FIG. 1. *Effect of GTP and 5'-guanylylimidodiphosphate upon basal and octopamine-stimulated adenylate cyclase activity*

A homogenate of cockroach brain was assayed for adenylate cyclase activity in the presence and absence of 0.3 mM octopamine (OA) and of increasing concentrations of GTP or 5'-guanylylimidodiphosphate [Gpp(NH)p]. Points represent the means of four separate incubations; standard errors were less than 10% of the means.

Effects of dopamine, octopamine, and other phenylethylamines. Adenylate cyclase responses to octopamine and dopamine were quite variable. The maximal response to octopamine in the presence of GTP was associated with an increase in adenylate cyclase activity to between 200% and 400% of the activity in the absence of

added drugs (Fig. 2). In 26 experiments, the average maximal stimulation elicited by octopamine was 275% ± 13% of controls. Dopamine elicited a maximal response of between 150% and 300% of basal activity (Fig. 2); in six experiments, the average maximal stimulation was 208% ± 27% of controls. There was no significant stimulation by 5-hydroxytryptamine at concentrations of up to 1 mM. Although the effects of maximally stimulating concentrations of octopamine and dopamine upon the cockroach adenylate cyclase were essentially additive (Table 2), the addition of norepinephrine to assay systems containing either octopamine (0.1 mM) or dopamine (0.03 mM) did not evoke a further increase in adenylate cyclase activity, and the addition of norepinephrine to assay systems containing both octopamine and dopamine significantly reduced adenylate cyclase activity.

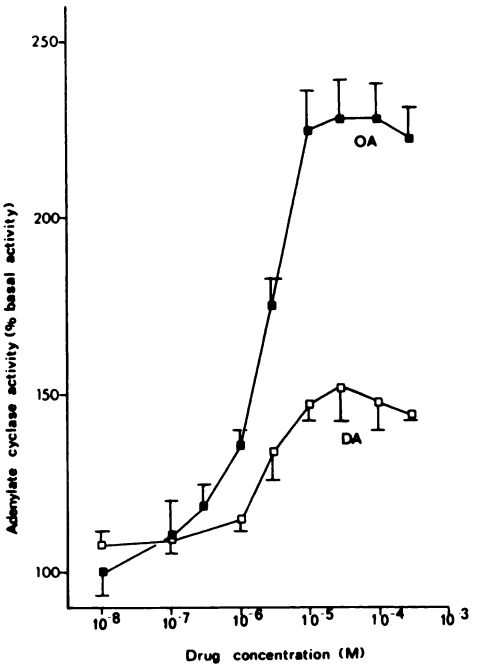


FIG. 2. *Effect of dopamine and octopamine upon adenylate cyclase activity in homogenates of cockroach brain*

Adenylate cyclase was measured as described in MATERIALS AND METHODS, in the presence of increasing concentrations of octopamine (OA) or dopamine (DA). Results are expressed as a percentage of the adenylate cyclase activity observed in the absence of added biogenic amines; in experiments with dopamine, this was 31.8 ± 1.6 pmoles of cyclic AMP formed per minute per milligram of tissue; in experiments with octopamine, the control adenylate cyclase activity was 24.1 ± 0.1 pmoles/min/mg of tissue. Points represent the means ± standard errors for four separate incubations.

TABLE 2

Additive effects of octopamine and dopamine upon adenylate cyclase activity in homogenates of cockroach brain

Adenylate cyclase activity was measured as described in MATERIALS AND METHODS, in the presence and absence of octopamine (0.1 mM), dopamine (0.03 mM), and norepinephrine (0.3 mM). These amine concentrations elicited maximal increases in adenylate cyclase activity when added alone. All values are means ± standard error for four separate incubations, and represent the absolute increases in adenylate cyclase activity above that (15.4 ± 0.4 pmoles/min/mg of tissue) observed in the absence of added amines.

Additions	Increase in adenylate cyclase activity
	pmoles/min/mg tissue
Octopamine	31.0 ± 0.4
Dopamine	14.2 ± 1.4
Norepinephrine	14.3 ± 1.3
Octopamine + dopamine	48.3 ± 2.2
Octopamine + norepinephrine	32.4 ± 0.8
Dopamine + norepinephrine	14.0 ± 0.7
Octopamine + dopamine + norepinephrine	30.5 ± 2.2

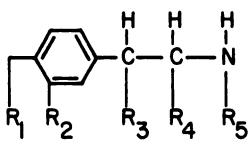
Responses of the insect adenylate cyclase system to octopamine and a variety of other phenylethylamines are summarized in Table 3. The most potent agonists were DL-octopamine, D(−)-octopamine, and *p*-fluoro-DL-phenylethanolamine. The L(+) isomer of octopamine was over 200 times less potent than the naturally occurring D(−) isomer in stimulating adenylate cyclase, whereas the potencies of DL-octopamine and D(−)-octopamine were not significantly different. Of the 14 phenylethylamines tested, only norepinephrine and epinephrine showed significant activity in stimulating the dopamine-sensitive adenylate cyclase from rat striatum.

The effects of dopamine and the potent dopamine agonists epinine and ADTN upon the insect adenylate cyclase system

TABLE 3

Effect of phenylethylamine derivatives upon adenylate cyclase activity in homogenates of cockroach brain and rat striatum

Adenylate cyclase activity was measured as described in MATERIALS AND METHODS. Most of the compounds tested were inactive at 0.1 mM in stimulating the dopamine-sensitive adenylate cyclase in rat striatum; they are indicated by minus signs. Norepinephrine and epinephrine were weak dopamine agonists: at 0.1 mM they elicited 69% ± 16% and 44% ± 16% of the stimulation due to 0.03 mM dopamine, respectively, as indicated by plus signs. The EC_{50} values for DL-octopamine and D(−)-octopamine as agonists of the octopamine-sensitive adenylate cyclase are given as means ± standard errors for three different experiments; they are not significantly different ($p > 0.2$ by Student's t-test).

Compound						Maximal response relative to DL-octopamine	EC_{50}	Effect on dopamine-sensitive adenylate cyclase (rat striatum)
	R_1	R_2	R_3	R_4	R_5	%	μM	
D-(−)-Octopamine	OH	H	OH	H	H	100	3.5 ± 0.2	−
DL-Octopamine	OH	H	OH	H	H	100	2.7 ± 1.3	−
p-Fluoro-DL-phenylethanolamine	F	H	OH	H	H	100	4.4	−
DL-Synephrine	OH	H	OH	H	CH_3	100	11	−
DL-Phenylethanolamine	H	H	OH	H	H	100	32	−
DL-Normetanephrine	OH	OCH_3	OH	H	H	100	66	−
DL-Metanephrine	OH	OCH_3	OH	H	CH_3	100	160	−
α-Methyl-DL-octopamine	OH	H	OH	CH_3	H	100	200	−
DL-Norepinephrine	OH	OH	OH	H	H	54 ± 11	240	+
Tyramine	OH	H	H	H	H	61 ± 10	290	−
L(+)-Octopamine	OH	H	OH	H	H	81 ± 12	720	−
DL-Epinephrine	OH	OH	OH	H	CH_3	41 ± 5		+
L-Phenylephrine	H	OH	OH	H	CH_3	30 ± 24		−
DL-Isoproterenol	OH	OH	OH	H	$CH(CH_3)_2$	18 ± 4		−
β-Phenylethylamine	H	H	H	H	H	12 ± 13		−
DL-*m*-Octopamine	H	OH	OH	H	H	13 ± 8		−

are summarized in Table 4.

As in the rat striatal adenylate cyclase system (28), both epinine and ADTN were comparable in potency to dopamine, and elicited similar maximal responses.

Antagonists of responses to octopamine and dopamine. Although norepinephrine stimulated adenylate cyclase in homogenates of cockroach brain (Tables 2 and 3), it also antagonized adenylate cyclase responses to octopamine (Fig. 3), with an IC_{50} of about 90 μM.

A number of *alpha* and *beta* adrenoceptor-blocking agents, cyproheptadine, and several thioxanthene and phenothiazine derivatives were tested for their activities as antagonists of the octopamine-sensitive adenylate cyclase (Tables 5 and 6). The most potent antagonists were cyproheptadine and phentolamine. A double-reciprocal plot of the effect of octopamine upon adenylate cyclase activity in the presence and absence of cyproheptadine (0.1 μM) is shown in Fig. 4. Cyproheptadine increased the apparent K_a for octopamine without affecting V_{max}; the K_a for octopamine was

TABLE 4

Comparison of effects of dopamine agonists upon dopamine-sensitive adenylate cyclase activity in homogenates of cockroach brain and of rat striatum

Drug	EC_{50}	
	Cockroach brain	Rat striatum[a]
	μM	μM
Dopamine	1.7	2.0
Epinine	6.8	1.5
ADTN	8.0	4.0

[a] Data from ref. 29.

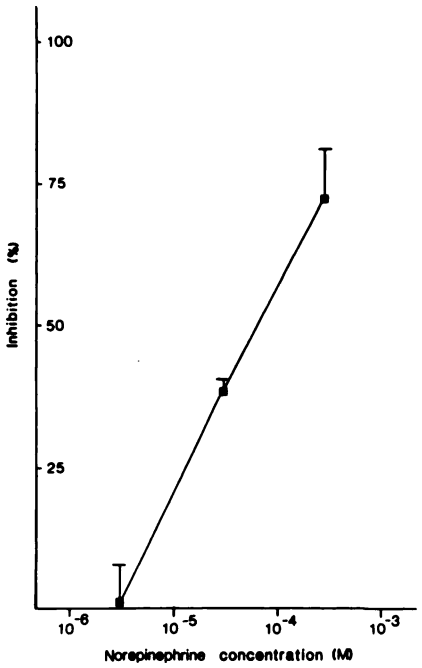


FIG. 3. *Effect of* DL-*norepinephrine upon octopamine-stimulated adenylate cyclase*

Adenylate cyclase was measured in homogenates of cockroach brain as described in MATERIALS AND METHODS, in the presence and absence of octopamine (3 μM) and increasing concentrations of DL-norepinephrine. For each concentration of norepinephrine, results are expressed as 100 × {1 - [(stimulation in the presence of octopamine + norepinephrine) - (stimulation in the presence of norepinephrine alone)]/ (stimulation in the presence of octopamine alone)}. In the absence of norepinephrine, basal adenylate cyclase activity was 18.7 ± 3.0 pmoles of cyclic AMP formed per minute per milligram of tissue, and octopamine-stimulated activity was 37.5 ± 1.9 pmoles/min/mg of tissue. Each point is the mean ± standard error of four replicate determinations.

estimated at 4.2 μM, and the K_i for cyproheptadine, at 0.07 μM. Although cyproheptadine was the most potent antagonist studied, its effects were not specific for octopamine-sensitive cyclase; it was also a potent antagonist of dopamine-sensitive adenylate cyclase both in cockroach brain and in rat striatum (Table 6).

The α-isomer of the thioxanthene neuroleptic flupenthixol was slightly more potent as an antagonist of octopamine-sensitive adenylate cyclase than the β-isomer (Table 6). However, α-flupenthixol was a more potent antagonist of dopamine-sensitive adenylate cyclase, and the blockade of responses to dopamine was more stereoselective than that of responses to octopamine.

Absence of octopamine-sensitive adenylate cyclase activity from homogenates of rat brain regions. Regions of rat brain were dissected out by the method of Glowinski and Iversen (30), homogenized (50 mg/ml) in 6 mM Tris-maleate buffer (pH 7.4) containing 2 mM EGTA, and assayed for octopamine-sensitive adenylate cyclase as described in MATERIALS AND METHODS. There was no significant stimulation of adenylate cyclase by 0.3 mM octopamine in any of the brain regions examined (hypothalamus, striatum, pons-medulla, cerebellum, cortex, and cervical spinal cord).

DISCUSSION

The octopamine-sensitive adenylate cyclase described here is similar to that described in cockroach thoracic ganglia by Nathanson and Greengard (18–20) in its sensitivity to antagonists, its affinity for octopamine, and its association with an

TABLE 5

Inhibitors of octopamine-sensitive adenylate cyclase in homogenates of cockroach brain

Adenylate cyclase activity was determined as described in MATERIALS AND METHODS. IC_{50} values represent the concentrations of drugs causing 50% inhibition of the stimulation of adenylate cyclase activity by 3 μM DL-octopamine. Some drugs inhibited basal adenylate cyclase activity; such effects have been allowed for in the calculation of results. Practolol and H35/25 were inactive at 0.1 mM. 2-Chlorophenylethanolamine, 3,4-dichlorophenylethanolamine, 3-bromophenylethanolamine, and 2-fluorophenylethanolamine were inactive at 0.03 mM.

Drug	Maximal inhibition	IC_{50}
	%	μM
Cyproheptadine	100	0.13
Phentolamine	100	0.20
Promethazine	100	1.00
Chlorpromazine	100	2.40
Piperoxan	100	3.30
α-Flupenthixol	100	6.00
β-Flupenthixol	100	21
Propranolol	70 ± 15	75
Dichloroisoproterenol	100	75
Yohimbine	82 ± 5	180

TABLE 6

Effects of cyproheptadine and isomers of flupenthixol upon octopamine- and dopamine-stimulated adenylate cyclase in cockroach brain and rat striatum

K_i values were calculated, assuming competitive inhibition, from the relationship $IC_{50} = K_i(1 + S/K_m)$, where K_m is the concentration of dopamine or octopamine required for half-maximal stimulation of adenylate cyclase (3.5 μM octopamine and 1.7 μM dopamine in the cockroach brain system; 5 μM dopamine in the rat striatal system) and S is the concentration of agonist used (3 μM octopamine or 100 μM dopamine).

Drug	Octopamine-sensitive adenylate cyclase (cockroach brain)		Dopamine-sensitive adenylate cyclase (cockroach brain)		Dopamine-sensitive adenylate cyclase (rat striatum)	
	IC_{50}	K_i	IC_{50}	K_i	IC_{50}	K_i
	μM	μM	μM	μM	μM	μM
Cyproheptadine	0.13	0.07	3.6	0.06	1.5	0.071
α-Flupenthixol	6.0	3.2	1.2	0.02	0.022[a]	0.001[a]
β-Flupenthixol	21	11	21	0.35	>100[a]	>1[a]

[a] Data from ref. 30.

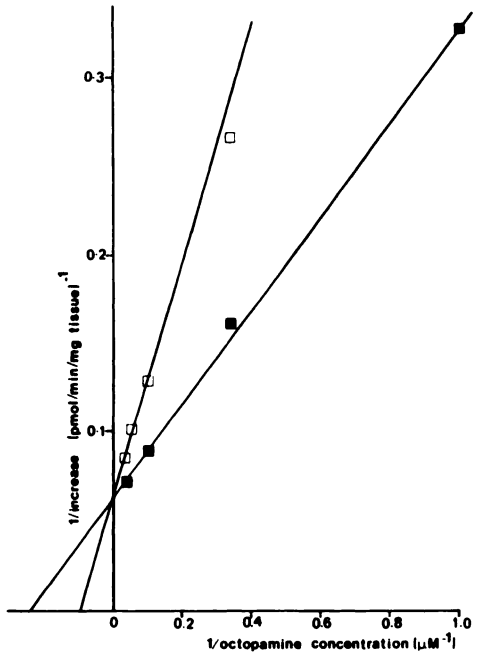


FIG. 4. *Effect of cyproheptadine upon octopamine-sensitive adenylate cyclase*

Double-reciprocal plot of the increase in adenylate cyclase activity elicited by octopamine, as a function of octopamine concentration, in the presence (□) and absence (■) of cyproheptadine, 0.1 μM. Adenylate cyclase activity was measured as described in MATERIALS AND METHODS, and all points represent the means of four separate incubations.

apparently distinct population of adenylate cyclase linked to dopamine receptors. The cockroach brain adenylate cyclase system differs from that of Nathanson and Greengard (18–20) in the apparent absence of 5-hydroxytryptamine sensitivity and in the absolute magnitude of enzyme activity associated with the basal and octopamine-stimulated states.

The inclusion of GTP in the assay system enhanced the relative magnitude of octopamine-stimulated activity over basal activity, transforming a small, poorly reproducible response into one which could be studied in detail. The effects of guanylyl nucleotides upon the insect adenylate cyclase were consistent with earlier reports (25, 26) that GTP may have an important role in the regulation of adenylate cyclase in nervous tissue.

The effects of dopamine and octopamine upon the adenylate cyclase system were additive, suggesting the presence of separate populations of adenylate cyclase linked to receptors for octopamine and dopamine (18, 20). As reported by Nathanson and Greengard (18, 20), responses to norepinephrine were not additive with responses to dopamine, and although norepinephrine stimulated adenylate cyclase, it also antagonized responses to octopamine. It seems likely that there are no receptors for norepinephrine in cockroach brain, but that norepinephrine had a dual action as an agonist of dopamine-sensitive adenylate cyclase (with a K_a of about 37 μM) and as an antagonist of octopamine-sensitive adenylate cyclase (with a K_i of about 44 μM).

The properties of the dopamine-sensi-

tive adenylate cyclase in homogenates of cockroach brain were similar to those observed in rat striatal homogenates (29): the dopamine agonists epinine and ADTN were comparable in potency to dopamine as stimulants of insect adenylate cyclase, and evoked similar maximal responses. The dopamine antagonist α-flupenthixol was more potent in antagonizing the adenylate cyclase response to dopamine than the response to octopamine.

A summary of the effects of a number of phenylethylamines upon cockroach brain adenylate cyclase is given in Table 3. Of the amines tested, only norepinephrine and epinephrine were active as agonists of the rat striatal dopamine-sensitive adenylate cyclase; the effects of the remaining amines upon insect adenylate cyclase were therefore almost certainly not due to actions at dopamine receptors. Since the most potent agonist of the adenylate cyclase response was octopamine, in particular its D(−) isomer, and since cockroach nervous tissue contains abundant octopamine (17), it is likely that the order of potency of the phenylethylamines in Table 3 reflects their relative affinities for an adenylate cyclase for which the endogenous ligand is D(−)-octopamine. Since norepinephrine and epinephrine were active as dopamine agonists, their potency as octopamine agonists may be rather less than that suggested by the data in Table 3.

A number of conclusions may be drawn concerning the structure-activity relationships for stimulation of octopamine-sensitive adenylate cyclase. (a) The response was stereoselective for the D(−) isomer of octopamine, which was over 200 times as potent as the L(+) isomer. (b) The presence of a β-hydroxyl group was essential for potent activity. (c) The absence of a *p*-hydroxyl group substantially reduced potency. *p*-Fluorophenylethanolamine, however, was a potent agonist, which shows that other groups can be substituted for the hydroxyl group without a great loss of potency. (d) The presence of a *m*-hydroxyl group drastically reduced potency. (e) Addition of a methoxy group in the *meta* position also decreased potency; however, *m*-methoxyphenylethylamines were more potent than the corresponding *m*-hydroxy compounds. (f) α-Methylation of octopamine resulted in about a 60-fold decrease in potency. (g) *N*-Methylation slightly reduced potency, and substitution of the still larger isopropyl group reduced potency substantially.

These relationships are strikingly different from those observed at adrenoceptors (31) and dopamine receptors (28), but closely resemble those observed by Carlson (12) in the firefly lantern. *N*-Methylated amines were more potent in stimulating the firefly lantern than their parent primary amines, whereas the reverse relationship was found in the octopamine-sensitive adenylate cyclase system. However, the apparent potency of drugs in eliciting luminescence in the lantern may reflect differences in the distribution and degradation of such drugs within the tissue, as well as in their activity as octopamine agonists.

There was no obvious correlation between the structures of the various drugs found to be potent antagonists of the octopamine-sensitive adenylate cyclase, and such drugs had widely differing properties as antagonists of other receptors. Thus the most potent octopamine antagonists were cyproheptadine (a potent histamine and 5-hydroxytryptamine antagonist), phentolamine (an imidazoline with potent *alpha* adrenoceptor-blocking activity), and promethazine (a phenothiazine with histamine-blocking activity). The *beta* adrenoceptor-blocking agents tested [dichloroisoproterenol, propranolol, the selective $beta_1$ antagonist practolol (32), and the $beta_2$ antagonist H35/25 (33)] all had little or no activity as octopamine antagonists. There was no obvious correlation between the potency of neuroleptic drugs (chlorpromazine, α-flupenthixol, and its inactive β-stereoisomer) and their activity as octopamine antagonists. Although α-flupenthixol was more potent than the β-isomer, the difference in potency of the two isomers was not as marked in the octopamine-sensitive adenylate cyclase system as in the dopamine-sensitive adenylate cyclase from rat striatum. The *alpha* adrenoceptor-blocking agents tested (phentolamine,

piperoxan, and yohimbine) differed widely in their potencies as octopamine antagonists. None of the drugs tested could be regarded as a specific octopamine antagonist.

The pharmacological properties of the octopamine-sensitive adenylate cyclase are strikingly similar to those of the firefly lantern, in which octopamine is almost certainly the transmitter. It is therefore likely that adenylate cyclase plays an important role in the mediation of octopaminergic neurotransmission. Confirmation of a neurotransmitter role for octopamine will require the development of specific octopamine antagonists, for which the octopamine-sensitive adenylate cyclase appears to be a good screening system.

ACKNOWLEDGMENTS

The authors are grateful to Dr. L. L. Iversen for helpful criticism of the manuscript, and to Miss Susan Gardiner for expert technical assistance.

REFERENCES

1. Saavedra, J. M., Brownstein, M. J., Carpenter, D. O. & Axelrod, J. (1974) *Science*, 185, 364–365.
2. Brownstein, M. J., Saavedra, J. M., Axelrod, J., Zeman, G. H. & Carpenter, D. O. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 4662–4665.
3. Walker, R. D., Ramage, A. G. & Woodruff, G. N. (1972) *Experientia*, 28, 1173–1174.
4. Carpenter, D. O. & Gaubatz, G. L. (1974) *Nature*, 252, 483–485.
5. Wallace, B. R., Talamo, B. R., Evans, P. D. & Kravitz, E. A. (1974) *Brain Res.*, 74, 349–355.
6. Evans, P. D., Talamo, B. R. & Kravitz, E. A. (1975) *Brain Res.*, 90, 340–347.
7. Kravitz, E. A., Evans, P. D., Talamo, B. R., Wallace, B. G. & Battelle, B. A. (1976) *Cold Spring Harbor Symp. Quant. Biol.*, 40, 127–133.
8. Hoyle, G. (1975) *J. Exp. Zool.*, 193, 425–431.
9. Hoyle, G. & Barker, D. L. (1975) *J. Exp. Zool.*, 193, 433–439.
10. Carlson, A. D. (1968) *J. Exp. Biol.*, 48, 381–387.
11. Carlson, A. D. (1968) *J. Exp. Biol.*, 49, 195–199.
12. Carlson, A. D. (1969) *Adv. Insect Physiol.*, 6, 51–96.
13. Carlson, A. D. (1972) *J. Exp. Biol.*, 57, 737–743.
14. Robertson, H. A. & Carlson, A. D. (1976) *J. Exp. Zool.*, 195, 159–164.
15. Robertson, H. A. & Steele, J. E. (1970) *Proc. Can. Fed. Biol. Soc.*, 13, 137.
16. Robertson, H. A. & Steele, J. E. (1972) *J. Neurochem.*, 19, 1603–1606.
17. Robertson, H. A. & Steele, J. E. (1973) *J. Physiol. (Lond.)*, 237, 34–35P.
18. Nathanson, J. A. & Greengard, P. (1973) *Science*, 180, 308–310.
19. Nathanson, J. A. & Greengard, P. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 797–801.
20. Nathanson, J. A. (1976) in *Trace Amines and the Brain* (Usdin, E. & Sandler, M., eds.), pp. 161–190, Dekker, New York.
21. Levitan, I. B., Madsen, C. J. & Barondes, S. H. (1974) *J. Neurobiol.*, 5, 511–525.
22. Levitan, I. B. & Barondes, S. H. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 716–721.
23. Beam, K. G. & Greengard, P. (1976) *Cold Spring Harbor Symp. Quant. Biol.*, 40, 157–168.
24. Rodbell, M., Lin, M. C., Salomon, Y., Londos, C., Harwood, J. P., Martin, B. R., Rendell, M. & Berman, M. (1975) *Adv. Cyclic Nucleotide Res.*, 5, 3–29.
25. Clement-Cormier, Y. C., Parrish, R. G., Petzold, G. L., Kebabian, J. W. & Greengard, P. (1975) *J. Neurochem.*, 25, 143–149.
26. Hegstrand, L. R., Kanof, P. D. & Greengard, P. (1976) *Nature*, 260, 163–165.
27. Brown, B. L., Ekins, R. & Albano, J. D. M. (1972) *Adv. Cyclic Nucleotide Res.*, 2, 25–40.
28. Miller, R. J., Horn, A. S. & Iversen, L. L. (1974) *Mol. Pharmacol.*, 10, 759–766.
29. Miller, R. J., Horn, A. S., Iversen, L. L. & Pinder, R. M. (1974) *Nature*, 250, 238–240.
30. Glowinski, J. & Iversen, L. L. (1966) *J. Neurochem.*, 13, 655–669.
31. Horn, A. S. & Phillipson, O. T. (1976) *Eur. J. Pharmacol.*, 37, 1–11.
32. Dunlop, D. & Shanks, R. G. (1968) *Br. J. Pharmacol.*, 32, 201–208.
33. Levy, B. & Wilkenfeld, B. E. (1969) *Eur. J. Pharmacol.*, 5, 227–234.