Archives of Insect Biochemistry and Physiology 6:191–201 (1987)

Pharmacology of [^{3}H]Mianserin Binding in the Nerve Cord of the American Cockroach, *Periplaneta americana*

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The binding of [^{3}H]mianserin to homogenates of cockroach nerve cord was investigated, using a centrifugal binding assay. Nonspecific binding was defined as binding in the presence of 0.1 μM phentolamine. Specific binding was saturable and indicated a high affinity site (K_D = 39.6 nM and B_{max} = 0.8 fmol μg^{-1}) and a low affinity site (K_D = 648.9 nM and B_{max} = 5.9 fmol μg^{-1}). The binding was reduced or abolished by heat, detergents, trypsin and $HgCl_2$. Incorporation of $CaCl_2$ or NaCl into the medium reduced binding in a dose-dependent fashion, whereas $MgCl_2$ and NaF inhibited binding in a biphasic manner. The stable GTP analog, 5′-guanylylimidodiphosphate, reduced the high affinity binding at 10 μM. Phentolamine, D,L-octopamine, demethylchlordimeform (DCDM), clonidine, and D,L-synephrine are effective displacers of [^{3}H]mianserin-binding, whereas dopamine, acetylcholine, serotonin (5-HT), and histamine show poor or no displacement. The pharmacological properties of octopamine-sensitive adenylate cyclase stimulation in cockroach nerve cord are similar to those of [^{3}H]mianserin-binding.

Key words: [N-methyl-^{3}H]mianserin, cockroach nerve cord, octopamine receptor, cyclic AMP

INTRODUCTION

Octopamine functions in insects as a neuromodulator [1,2], neurotransmitter [3,4], and neurohormone [5,6]. The pharmacological properties of a putative octopamine receptor have been studied in a variety of insect preparations [7], principally by monitoring the effects of potential agonists and

Acknowledgments: This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada and American Cyanamid. The support and counsel of Drs. Alison Chalmers and Kent Jennings are gratefully acknowledged.

Received April 7, 1987; accepted July 27, 1987.

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antagonists on octopamine-mediated electrophysiological responses [8,9] or octopamine-mediated activation of adenylate cyclase [10–12]. These studies indicate that the putative octopamine receptor shows some pharmacological similarities to the α-adrenergic receptor of vertebrates; however, mianserin, cyproheptadine, and gramine, which are antagonists of 5-hydroxytryptamine and histamine [13], are also effective blockers of octopamine-mediated processes [9,14]. The general trends reported in these studies have been confirmed by preliminary investigations involving direct binding of radiolabeled octopamine to head preparations of *Drosophila* [15] and CNS* preparations of *Locusta* [16] and *Musca* [17]. However, detailed binding studies have not yet been reported, and no attempts have been made to correlate binding studies and biochemical responses in the same preparation.

Mianserin is a potent inhibitor of octopamine-mediated activation of adenylate cyclase in the nerve cord of *Periplaneta americana* [11], and, in the present study, [N-methyl-^{3}H]mianserin has been used as a ligand to characterize the pharmacological properties of a putative octopamine receptor in cockroach nerve cord. The results of the binding studies are compared with those obtained for octopamine-sensitive adenylate cyclase in the same preparation.

MATERIALS AND METHODS

Insects

Adult male cockroaches, between 1 and 3 months after the final molt, were taken from a colony of *P. americana* maintained under standard conditions in this laboratory [18].

Chemicals

[N-Methyl-^{3}H]mianserin hydrochloride (50 Ci/mmol) was obtained from New England Nuclear (Boston, MA). The following drugs were from Sigma Chemical Co. (St. Louis, MO): acetylcholine hydrochloride, clonidine hydrochloride, D,L-dithiothreitol, dopamine hydrochloride, 5-hydroxytryptamine creatinine sulfate, D,L-synephrine, taurine, trypsin, and tyramine. Chlordimeform hydrochloride and demethylchlordimeform hydrochloride were generously donated by American Cyanamid (Princeton, NJ). Sodium dodecyl sulfate was purchased from Bio-Rad Laboratories (Richmond, CA), L-epinephrine bitartrate from Calbiochem Behring Corp. (La Jolla, CA), phentolamine from Ciba-Geigy (Dorval, Quebec), L-glutamic acid from Fisher Scientific Co. (Fair Lawn, NJ), N-1-heptanesulfonic acid from Helix Associates, Inc. (Newark, DE), disodium EDTA dihydrate from J.T. Baker Chemical

*Abbreviations: ATP = adenosine triphosphate; B_{max} = apparent number of receptor sites; CDM = chlordimeform; CNS = central nervous system; DCDM = demethylchlordimeform; EDTA = ethylenedioxybis(ethylenenitrilo)tetra(acetic acid); GABA = gamma aminobutyric acid; Gpp(NH)p = 5′-guanylylinidodiphosphate; GTP = guanosine triphosphate; 5-HT = 5-hydroxytryptamine; K_D = apparent dissociation constant; K_i = inhibition constant; PMSF = phenylmethylsulfonyl fluoride; SDS = sodium dodecyl sulfate; SH = sulphydryl.

Co. (Phillipsburg, NJ), gamma amino butyric acid from Nutritional Biochemical Corp. (Cleveland, OH), and (+)-butaclamol from Research Biochemicals Inc. (Wayland, MA). All other chemicals were of analytical grade and were purchased from Sigma Chemical Co.

Protein Assay

Total protein was assayed using the Bio-Rad protein assay kit [19], with bovine serum albumin used as a standard.

Binding Assay

Tissue preparation. Nerve cords were dissected free of adhering tissues and placed on ice in 50 nM Tris-HCl buffer (containing 5 mM $MgCl_2$), pH 7.4. The nerve cords were homogenized on ice in a glass-Teflon homogenizer, using 30 strokes at 600 rpm. The homogenate was then centrifuged at 30,000*g* for 30 min. The supernatant was discarded, and the pellet was resuspended in 1 ml ice-cold buffer, frozen in liquid N_2, and stored at −70°C until required. The homogenate was thawed and homogenized for 50 strokes at 600 rpm.

[^{3}H]Mianserin binding assay. Aliquots of homogenate, containing 20–40 μg ml^{-1} protein, were placed in 3-ml polypropylene tubes and incubated in Tris-HCl buffer at 26°C, in the presence or absence of the appropriate non-labeled ligand. The reaction was started with the addition of 2 mM [^{3}H]mianserin. Nonspecific binding was determined in the presence of 0.1 mM phentolamine. All samples were run in triplicate, with the total assay volume being 1.0 or 1.5 ml. The reaction was terminated after 40 min by centrifugation (7,000*g* for 30 min at 4°C). The supernatant was decanted, and the pellets, containing bound ligand, were resuspended in 1.0 ml ice-cold buffer and immediately centrifuged at 7,000*g* for 30 min at 4°C. The supernatant was aspirated, and the pellets were resuspended in 100 μml of buffer and vortexed for 1 min at 200 rpm. GF/B filters (Brandel, Gaitherburg, MD) were used to absorb the mixture and were then placed overnight in 3 ml of scintillation fluid prior to counting in a β-counter.

Adenylate Cylase Assay

Incubation of nerve cord homogenates. Nerve cords were dissected free of adhering tissues, rinsed in ice-cold glucose-trehalose saline [20], and homogenized in a solution containing 2 M EGTA and 6 mM Tris-acetate buffer, pH 7.4. Enzyme activity was measured following incubation of 100 μl homogenate in a medium containing 75 mM Tris, 0.1 mM 3-isobutyl-1-ethylxanthine [3-isobutyl-1-ethylpurine-2(3H),6(1H)-dione], 10 mM magnesium acetate, 50 μM GTP, and 0.5 mM ATP in a final volume of 200 μl. The reaction was started by the addition of ATP and the incubation proceeded at 30°C in a shaking water-bath.

Estimation of cyclic AMP. The reaction was terminated by the addition of 1.0 ml of 0.4 M perchloric acid and adjusted to pH 6.2 with 2.5 M potassium hydrogen carbonate. Following centrifugation at 2,000*g* for 5 min, the cyclic AMP content of the supernatant phase was determined by radioimmunoas-

say, using the "RIANEN" kit (New England Nuclear). Cyclic AMP production was linear for 7 min, with protein concentrations between 5.7–91.2 μg protein. The samples were monitored within these parameters. The assay of cyclic AMP demonstrated both linearity and parallellism, indicating lack of interfering compounds.

Estimation of K_i Values

The K_i values were calculated according to the formula: $K_i = IC_{50}/(1 + S/K_a)$, where IC_{50} is the concentration of the antagonist at which the agonistic response is inhibited by 50%; S is the concentration of the agonist; and K_a is the concentration of agonist required to effect 50% of the maximal response [21].

RESULTS

[N-Methyl-^{3}H]Mianserin Binding

Preliminary experiments indicated that the binding of 2 nM [^{3}H]mianserin to nerve cord homogenates is linearly proportional to the protein content of the homogenate, between 3–100 μg protein per ml, and that nonspecific binding, defined as the binding observed in the presence of 0.1 mM phentolamine, comprises 59% of total binding.

Specific binding of [^{3}H]mianserin, defined as total binding minus nonspecific binding, is saturable (Fig. 1A). The curvilinear nature of the Scatchard plot (Fig. 1B) over the range tested (0.2–18.0 nM), suggests the existence of both a high-affinity site (K_D = 39.6 nM and B_{max} = 0.8 fmol μg^{-1}) and a low-affinity site (K_D = 648.9 nM and B_{max} = 5.9 fmol μg^{-1}). The Hill plot (Fig. 1C) yields a slope of 0.6, suggesting negative cooperativity between the binding sites. The stable guanyl nucleotide, Gpp(NH)p, at 10 μM, markedly reduces the binding of [^{3}H]mianserin (Table 1). Scatchard analysis, in the presence of 10 μM Gpp(NH)p, indicates that under these conditions there is a single binding site with a K_D = 696.2 nM and B_{max} = 2.0 fmol μmg^{-1} protein (Fig. 1B).

At 26°C, in the presence of 2 nM [^{3}H]mianserin, maximal binding is achieved after about 40 min (Fig. 2). Specific mianserin binding, as reported in Figure 3, was very low at pH 2 but increased to a maximum between pH 7.0–7.4, beyond which it rapidly declined.

[^{3}H]Mianserin-binding is almost entirely abolished (91.4%) by heat treatment, and incubation with trypsin also produces a marked decrease (90.6%) in binding (Table 1). Treatment with the two detergents, SDS and heptanesulfonic acid, reduced binding by 89.5% and 79.3%, respectively. PMSF and $HgCl_2$ caused a reduction in binding of 22.5% and 65.8%, respectively. Binding is not significantly altered by the incorporation of 1 mM EDTA or 0.1% ascorbate in the incubation medium, but is greatly reduced (77.8%) by 5 mM EDTA. No significant decrease in activity is observed after storage of tissue at −70°C for 4–5 weeks.

[^{3}H]Mianserin binding is markedly affected by the ionic composition of the incubation medium (Fig. 4). Low concentrations (less than 0.1 mM) of

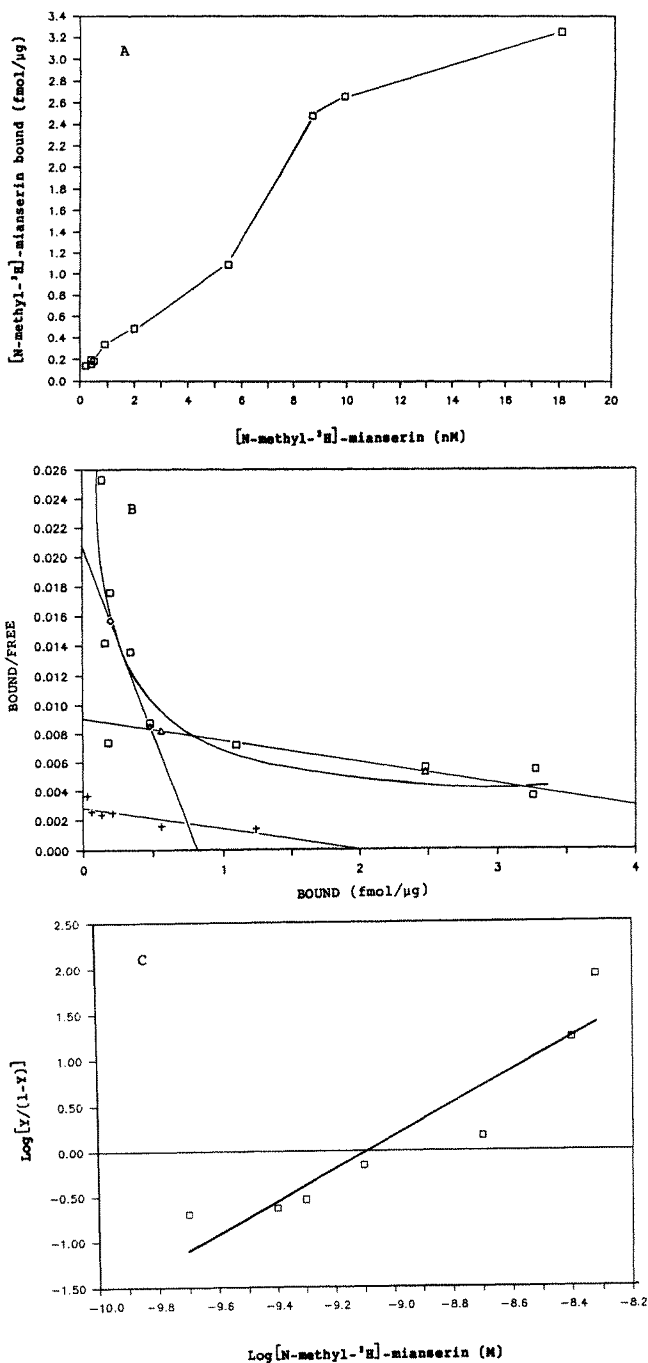

Fig. 1. Binding of [N-methyl-^{3}H]mianserin to nerve cord homogenates of *P. americana*. Each point represents the mean of four experiments performed in triplicate with standard errors of less than 15%. **A)** Saturation of specific binding. **B)** Scatchard analysis. K_D and B_{max} were determined by least-squares linear regression analysis. □, Scatchard; +, Gpp(NH)p; △, lower affinity; ◇, high affinity. **C)** Hill plot. The Hill coefficient was determined by least-squares linear regression analysis.

TABLE 1. Effect of Various Treatments on [^{3}H]Mianserin-Binding in Nerve Cord Homogenates of *P. americana*

Treatment	[^{3}H]Mianserin-specific binding (% of control)[a]
10 min at 100°C	8.6 ± 0.1 (6)
Trypsin (0.1 mg ml^{-1}, 10 min)	9.4 ± 0.1 (6)
Heptanesulfonic acid (0.01%)	20.7 ± 0.6 (4)
SDS (0.01%)	10.5 ± 0.2 (5)
$HgCl_2$ (10 μM)	35.2 ± 1.5 (4)
PMSF (0.1 μM)	78.5 ± 4.8 (4)
Ascorbate (0.1%)	97.6 ± 5.5 (4)
EDTA (1 mM)	106.1 ± 4.4 (4)
EDTA (5 mM)	23.2 ± 0.3 (4)
DTT (0.1 mM)	69.2 ± 2.5 (4)
Gpp(NH)p (10 μM)	35.5 ± 2.1 (4)

[a]Values indicate mean ± standard error of the mean for the number of determinations shown in parentheses.

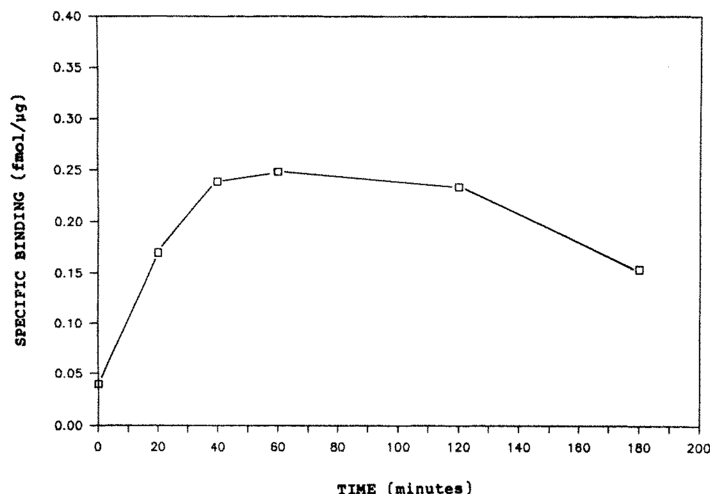

Fig. 2. Binding of [N-methyl-^{3}H]mianserin (2 nM) to *P. americana* nerve cord homogenate as a function of time. Data points are means of four experiments done in triplicate with standard errors of less than 10%.

$CaCl_2$ and NaCl reduce binding slightly, whereas at higher concentrations (> 1 mM), binding is effectively inhibited. Both $MgCl_2$ and NaF enhance binding at lower concentrations (less than 1 mM) but greatly reduce it at higher levels (> 10 mM). Optimal specific binding (150% of control) is observed with 5 mM $MgCl_2$.

A pharmacological profile of [^{3}H]mianserin was produced (Table 2) by testing the ability of various compounds to displace [^{3}H]mianserin from binding sites. Thus potent displacement is displayed by octopamine, the formamidine, DCDM, and ligands that exhibit high affinity for α-adrenergic receptors in vertebrate systems (i.e., phentolamine, clonidine, and naphazoline). The β-adrenergic ligand, propanolol, shows poor displacement activity

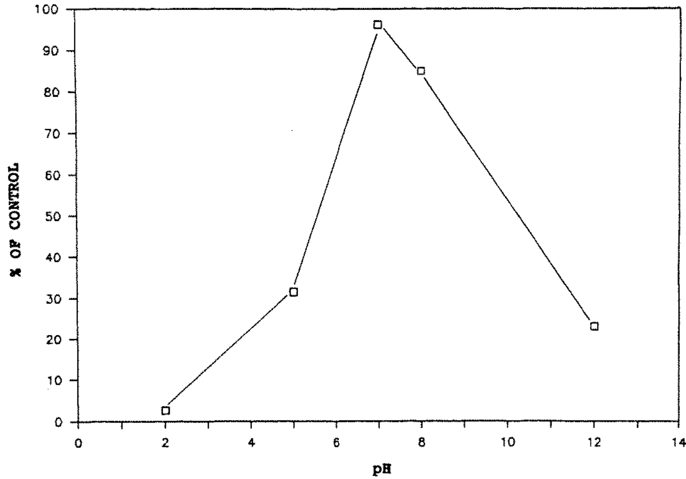

Fig. 3. Influence of buffer pH on [^{3}H]mianserin-specific binding. Values represent means of three experiments done in triplicate with standard errors of less than 10%. Control was incubation with 50 M Tris-HCl, pH 7.4.

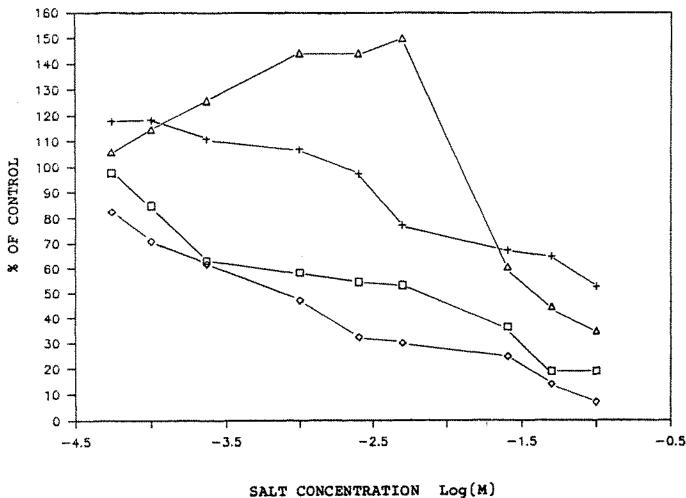

Fig. 4. The effect of the ionic composition of the reaction medium on the specific binding of [N-methyl-^{3}H]mianserin to *P. americana* nerve cord homogenates. Values are presented as percent specific binding relative to control (50 mM Tris-Cl, pH 7.4). Data points are the means of four experiments performed in triplicate, with standard errors of less than 15%. +, NaF; △, $MgCl_2$; □, Nacl; ◇, $CaCl_2$.

(Table 2), as do ligands that interact with dopamine, 5-HT, norepinephrine, histamine, GABA, and acetylcholine receptors.

Octopamine-Sensitive Adenylate Cyclase

Some compounds used in the [^{3}H]mianserin-binding experiments were tested for their ability to stimulate or inhibit octopamine-sensitive adenylate cyclase (Table 3). On the basis of their K_a values, DCDM is the most effective

TABLE 2. Inhibition of [^{3}H]Mianserin-Binding to *P. americana* Nerve Cord Homogenate by Various Ligands

Ligand	No.	IC_{50} (M)[a]	K_i (M)[b]
Phentolamine	5	2.82×10^{-8}	2.67×10^{-8}
D,L-Octopamine	5	1.78×10^{-7}	1.69×10^{-7}
DCDM	3	2.51×10^{-7}	2.39×10^{-7}
Naphazoline	3	5.75×10^{-7}	5.49×10^{-7}
Clonidine	3	6.31×10^{-7}	6.01×10^{-7}
D,L-Synephrine	4	1.41×10^{-6}	1.34×10^{-6}
Tyramine	4	5.01×10^{-6}	4.74×10^{-6}
Chlordimeform	3	5.01×10^{-6}	4.77×10^{-6}
Cis-flupentixol	3	6.31×10^{-6}	5.97×10^{-6}
(+)-Butaclamol	3	6.31×10^{-6}	5.97×10^{-6}
D,L-Propranolol	4	2.00×10^{-5}	1.89×10^{-5}
Dopamine	4	$> 10^{-4}$	ND
5-HT	4	$> 10^{-4}$	ND
(±)-Norepinephrine	4	$> 10^{-4}$	ND
Acetylcholine	4	$> 10^{-4}$	ND
GABA	4	$> 10^{-4}$	ND
Taurine	4	$> 10^{-4}$	ND
Histamine	4	$> 10^{-4}$	ND
(−) Epinephrine	4	$> 10^{-4}$	ND
Glutamate	4	$> 10^{-4}$	ND

[a]IC_{50} values (that concentration of ligand that inhibited the binding of [^{3}H]mianserin by 50%) were calculated from displacement curves.
[b]K_i values were calculated using the formula $K_i = IC_{50}/(1 + C/K_D)$. ND, not determined.

TABLE 3. Effect of Potential Agonists and Antagonists on Basal and Octopamine-Mediated Cyclic AMP Production in Nerve Cord Homogenates of *Periplaneta americana*

Treatment	K_a/K_i (μM)	Maximal stimulation (% of control)
DCDM[a,b]	0.42	220
Naphazoline[b]	1.85	510.5
Synephrine[b]	2.51	516.5
Octopamine[a,b]	5.00	491
Clonidine	13.60	208.5
Tyramine[b]	18.50	496.4
Mianserin[a,c]	0.021	NS[d]
Phentolanine[a,c]	0.051	NS
Cis-flupentixol[a,c]	0.890	NS
(+)-Butaclamol[a,c]	14.00	NS
Propranolol[a,c]	659.5	NS

[a]Previously published [15].
[b]Agonist.
[c]Antagonist.
[d]NS, not stimulating.

agonist, followed by naphazoline, synephrine, octopamine, clonidine, and tyramine. Additivity studies indicate that these compounds are activating an octopamine-sensitive site that is coupled to adenylate cyclase (unreported data). DCDM and clonidine, partial octopamine agonists [22,23], elicit maximal cyclic AMP productions of 220% and 208% of control, respectively. The antagonists tested against the octopamine-sensitive adenylate cyclase give the following order of effectiveness: mianserin > phentolamine > cis-flupentixol > (+)-butaclamol > propanolol.

DISCUSSION

Mianserin, a potent antagonist of octopamine-stimulated adenylate cyclase in cockroach nerve cord [11] and *Manducca* brain [15], has been used as a potential ligand to study octopamine receptors in cockroach nerve cord. Although mianserin also acts as a 5-HT and histamine antagonist in some preparations [13], the limited ability of these ligands to displace [^{3}H]mianserin-binding (Table 2) indicates poor binding to these receptors in the cockroach nerve cord.

[N-Methyl-^{3}H]mianserin exhibits time-dependent specific binding to nerve cord homogenates. The decrease in specific binding, following treatment of homogenates with trypsin, heat, and the two detergents, SDS and N-heptanesulfonic acid, indicates that the binding site is proteinaceous. Mercuric chloride also reduces binding significantly, suggesting that essential SH groups are associated with the binding site. The nonlinear Scatchard plot suggests that the mianserin binding sites are of two affinities, at the least. The Hill coefficient of less than 1.0 indicates that there is negative cooperativity between these sites or multiple receptor populations [24].

Incorporation of 10 μM Gpp(NH)p decreases the affinity of these binding sites. A similar trend has been reported by Hashemzadeh and Hollingworth [29] in studies of [^{3}H]octopamine-binding in the firefly light organ and in similar studies using fruitfly head homogenates [16]. One possible explanation may be the conversion of the binding sites from a high-affinity to a low-affinity state in vitro, as has been shown for several vertebrate hormone receptors that are coupled to adenylate cyclase [25].

The rank order of potency of various compounds in displacing [^{3}H]mianserin is similar to that reported for displacement of [^{3}H]octopamine-binding in firefly light organ [26]. These compounds also demonstrate an ability to interact with octopamine-sensitive adenylate cyclase in several insect tissues [12,27,28], including cockroach nerve cord [24]. However, some differences are apparent in the two systems; these differences may be due to the binding of mianserin to octopamine receptors that are not coupled or are negatively coupled to adenylate cyclase. Furthermore, the degree of cyclic AMP production does not necessarily reflect receptor affinity.

The ability of antagonists to inhibit octopamine-sensitive adenylate cyclase and to displace [^{3}H]mianserin-binding compares well between the two systems. The most potent inhibitor of the octopamine-sensitive adenylate cyclase is mianserin [24,27], the ligand used in the present binding study. Phentolamine, which has been used to define the nonspecific binding of

[^{3}H]octopamine to *Drosophila* head fractions [16], used here to define the nonspecific component of [^{3}H]mianserin-binding, is the most effective displacer of [^{3}H]mianserin-binding and is only slightly less effective than mianserin as an antagonist of the octopamine-sensitive adenylate cyclase. Cis-flupentixol and (+)-butaclamol, which are potent inhibitors of the dopamine-sensitive adenylate cyclase in *Locusta* salivary glands [29] and nerve cord [24], are poor displacers of [^{3}H]mianserin. Propanolol, a poor inhibitor of octopamine-mediated events [10,24] is also of limited effectiveness in the present study. Hence these results are consistent with the hypothesis that, in cockroach nerve cord homogenates, [^{3}H]mianserin labels sites with a pharmacological profile similar to that described for octopamine receptors coupled to adenylate cyclase.

LITERATURE CITED

1. Evans PD: Properties of modulatory octopamine receptors in the locust. In: Neuropharmacology of Insects. Ciba Foundation Symposium, Pitman, London, pp 48–69 (1982).
2. Orchard I: Octopamine in insects: Neurotransmitter, neurohormone, and neuromodulator. Can J Zool *60*, 659 (1982).
3. Nathanson JA, Hunnicut EJ: Neural control of light emission in *Photuris* larvae: Identification of octopamine-sensitive adenylate cyclase. J Exp Zool *208*, 255 (1979).
4. Orchard I, Loughton BG, Webb RA: Octopamine and short-term hyperlipaemia in the locust. Gen Comp Endocrinol *45*, 175 (1981).
5. Downer RGH: Induction of hypertrehalosemia by excitation in *Periplaneta americana*. J Insect Physiol *25*, 59 (1979).
6. Robertson HA, Steele JE: Octopamine in insect central nervous system: Distribution, biosynthesis and possible physiological role. J Physiol *237*, 34P (1973).
7. Evans PD: Octopamine receptors in insects. In: Receptors for Neurotransmitters, Hormones and Pheromones in Insects. Sattele DB, et al., eds. Elsevier/North Holland Biomedical Press, Amsterdam, pp 245–258 (1980).
8. Evans PD: Multiple receptor types for octopamine in the locust. J Physiol [Lond] *318*, 99 (1981).
9. Orchard I, Lange AB: Pharmacological profile of octopamine receptors on the lateral oviducts of the locust, *Locusta migratoria*. J Insect Physiol *32*, 741 (1986).
10. Bodnaryk RP: Biogenic amine-sensitive adenylate cyclases in insects. Insect Biochem *12*, 1 (1982).
11. Gole JWD, Orr GL, Downer RGH: Interaction of formamidines with octopamine-sensitive adenylate cyclase receptor in the nerve cord of *Periplaneta americana* L. Life Sci *32*, 2939 (1983).
12. Orr GL, Gole JWD, Downer RGH: Characterisation of an octopamine-sensitive adenylate cyclase in haemocyte membrane fragments of the American cockroach, *Periplaneta americana*. Insect Biochem *15*, 695 (1985).
13. Goodman LS, Gilman A: The Pharmacological Basis of Therapeutics. 7th Edition. Goodman Gilman A, Goodman LS, Rall TW, Murad F, eds. MacMillan Publishing Co., New York, pp 633–635 (1985).
14. Downer RGH, Gole JWD, Orr GL: Interaction of formamidines with octopamine-, dopamine- and 5-hydroxytryptamine-sensitive adenylate cyclase in the nerve cord of *Periplaneta americana*. Pestic Sci *16*, 472 (1985).
15. Dudai Y, Zvi S: High-affinity [^{3}H]octopamine-binding sites in *Drosophila melanogaster*: Interaction with ligands and relationship to octopamine receptors. Comp Biochem Physiol *77C*, 145 (1984).
16. Hiripi L: Biochemical charcterization of the octopaminergic and serotonergic system in the CNS of *Locusta migratoria*. In: Neuropharmacology and Pesticide Action (Neurotox 85). Proceedings of a Society of Chemical Industry Symposium, University of Bath, England, pp 81–82.

17. Hashemzadeh H, Hollingworth RM: Binding of octopamine to membranes from the housefly head. Soc Neurosci Abstr *12*, 244 (1986).
18. Matthews JR, Downer RGH: Hyperglycaemia induced by anaesthesia in the American cockroach, *Periplaneta americana*. Can J Zool *51*, 395 (1973).
19. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem *72*, 248 (1976).
20. Downer RGH: Trehalose production in isolated fat body of the American cockroach, *Periplaneta americana*. Comp Biochem Physiol *62C*, 31 (1979).
21. Chen YC, Prusoff WH: Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol *22*, 3099 (1973).
22. Nathanson JA: Phenyliminoimidazolidines: Characterization of a class of potent agonists of octopamine-sensitive adenylate cyclase and their use in understanding the pharmacology of octopamine receptors. Mol Pharmacol *28*, 254 (1984).
23. Lafon-Cazal M, Bockaert J: Pharmacological characterization of octopamine-sensitive adenylate cyclase in the flight muscle of *Locusta migratoria* L. Eur J Pharmacol *119*, 53 (1985).
24. Weiland GA, Molinoff PB: Quantitative analysis of drug-receptor interactions: 1. Determination of kinetic and equilibrium properties. Life Sci *29*, 313 (1981).
25. Spiegel AM: Signal transduction by guanine nucleotide binding proteins. Mol Cell Endocrinol *49*, 1 (1987).
26. Hashemzadeh H, Hollingworth RM, Volivia A: Receptors for ^{3}H-octopamine in the adult firefly light organ. Life Sci *37*, 433 (1985).
27. Downer RGH, Orr GL, Cole JWD, Orchard I: The role of octopamine and cyclic AMP in regulating hormone release from the corpus cardiacum of the American cockroach. J Insect Physiol *30*, 457 (1984).
28. Uzzan A, Dudai Y: Aminergic receptors in *Drosophila melanogaster*: Responsiveness of adenylate cyclase to putative neurotransmitters. J Neurochem *38*, 6 (1982).
29. Lafon-Cazal M, Bockaert J: Pharmacological characterization of dopamine-sensitive adenylate cyclase in the salivary gland of *Locusta migratoria* L. Insect Biochem *14*, 5 (1984).