Pharmacology of the octopamine receptor from locust central nervous tissue (OAR₃)

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1 The present study characterized highly effective agonists from different classes of compounds for the neuronal octopamine receptor (OAR₃) of the migratory locust (*Locusta migratoria* L.). Biogenic amines and phenyliminoimidazolidines (PIIs) were employed for the study of structure-activity relationships.

2 The highest affinity PIIs were predominantly those with substitutions at the positions 2 and 4 of the phenolic ring (e.g. NC 7, $K_i = 0.3$ nM, NC 8, $K_i = 0.81$ nM). Substitutions at these positions always had positive effects on the affinity of the respective agonists.

3 Substitutions at the positions 3, 5 and 6, however, always had negative effects on the affinity. At the position one of the phenolic ring, heterocyclic substituents are preferred.

4 Some PIIs had a more than 30 times higher affinity for OARs than for α -adrenoceptors which are the vertebrate homologues of the insect octopamine receptors.

5 The only non-PII with subnanomolar affinity was the aminooxazoline derivative AC 6 ($K_i = 0.92$ nM).

6 A variety of substances with known insecticidal activity such as chlordimeform, demethylchlordimeform, amitraz or AC 6 had high affinity for the locust neuronal octopamine receptor.

Keywords: Insect; nervous system; *Locusta*; phenyliminoimidazolidines; receptor; octopamine

Introduction

Since its discovery in the salivary glands of *Octopus* (Ers-pamer & Boretti, 1951), the biogenic monoamine octopamine (*p*-hydroxyethanolamine) has been well established as a neurotransmitter, neurohormone, and neuromodulator in various invertebrate species (Axelrod & Saavedra, 1977; Orchard, 1982; Evans, 1985; Saavedra, 1989). Especially in insects, the modulatory role of octopamine in different peripheral organs has been studied in great detail (Evans, 1985) and also in the central nervous system some physiological effects of octopamine have been described (Nathanson & Greengard, 1973; Orchard, 1982). It is essential for the coordination of locomotor behaviour, such as flying and running (Sombati & Hoyle, 1984) and even complex functions of the nervous system, such as learning and memory (Bicker & Menzel, 1989; Dudai *et al.*, 1987) are under the control of this neuroactive substance.

Receptors for octopamine were studied in neuronal (Nathanson & Greengard, 1973; Roeder & Gewecke, 1990) as well as in non-neuronal tissues (Evans, 1981; Nathanson, 1985a,b). In the latter, three different receptor classes (OAR₁, OAR_{2A} and OAR_{2B}) could be distinguished (Evans, 1981). Most octopamine receptors of non-neuronal tissues studied so far, have pharmacological features comparable to the class 2A receptor (Evans, 1985). In the nervous tissue a peculiar receptor class is present (Roeder, 1992). Its pharmacology is (in contrast to octopamine receptors from non-neuronal tissues such as the locust ext.-tibiae-muscle or the firefly lantern), less well studied (Nathanson, 1985a,b). In non-neuronal tissues, compounds such as the formamidines, the phenyliminoimidazolidines (Nathanson, 1985a,b; Evans, 1987) or the aminooxazolines (Jennings *et al.*, 1988) have high affinity properties.

Locust neuronal octopamine receptors have been studied using different methods. In addition to the study of octopamine-sensitive adenylate cyclases, radioligand-receptor assays with either [³H]-octopamine (Roeder & Gewecke,

1990) or the PII-derivative, [³H]-NC-5Z (Nathanson & Kaugars, 1989; Roeder & Nathanson, 1993) were successfully used. The identity of these binding sites with octopamine receptors located on isolated neuronal somata was recently confirmed by electrophysiological methods (Kaufmann & Benson, 1991).

As octopamine receptors are the only non-peptide receptors whose occurrence is restricted to invertebrates, they represent ideal targets for specific insecticides. Therefore, the detailed pharmacological characterization of octopamine receptors, especially of those from neuronal tissues, could result in the development of new leads for more specific insecticides.

Methods

Migratory locusts (*Locusta migratoria* L.) of either sex were reared in a light/dark cycle of 12h/12h (35°C) under crowded conditions. They were fed with a diet of fresh wheat and bran. Two to 3 weeks after imaginal moult, nervous tissue (supraoesophageal-, suboesophageal-, and thoracic ganglia) was dissected out and immediately stored on ice. The nervous tissue was homogenized in ice cold incubation buffer (HEPES/NaOH 10 mM, 5 mM MgSO₄, 100 μ M PMSF, pH 7.4) in a glass/teflon homogenizer followed by a glass/glass homogenizer (10 strokes each). This homogenate was centrifuged (20,000 g, 30 min), the supernatant discarded, and the pellet resuspended in the original volume. This procedure was repeated twice to obtain washed pellets that were stored frozen until use (–70°C).

The incubation was performed in a total volume of 100 μ l with 0.3–0.5 mg ml^{–1} protein, and 10 nM [³H]-octopamine at room temperature. The incubation proceeded for approximately 1 h and was stopped by filtration through glass fibre filters (Whatmann GF/C) preincubated in 0.3% polyethyleneimine. After addition of the sample, the filters were washed with ice cold buffer. All pharmacological experiments were performed in the presence of indicated

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concentrations of tested substances. Each substance was tested in 6–7 different concentrations at least 4–6 times in triplicate. Standard errors in the pharmacological studies were usually below 10% of the respective values. In order to increase clarity, error bars have been omitted. To determine non-specific binding, 10 μ M octopamine was added to the incubation medium. Radioactivity on the filters was quantified with a scintillation counter (app. 40% efficiency). K_i -values were determined using the LIGAND programme (Munson & Rodbard, 1980). A more detailed description of the procedures was given previously (Roeder & Gewecke, 1990).

Most of the PIIs were from Boehringer Ingelheim (Germany) or Shell Agriculture (Ingelheim), St 92 and NC 5Z were gifts of Dr J.A. Nathanson. Demethylchlordimeform was from CIBA-Geigy (Basel, Switzerland). AC 6 was a gift from Dr Jennings. Amitraz and BTS 23396 were from Cyanamid. Most of the other substances were obtained from SIGMA (Deisenhofen, Germany).

Results

Biogenic amines

Although the biogenic amines are substances with a structure related to that of octopamine, they vary substantially in their affinity for the octopamine receptor, and minor structural changes lead to different affinities. The natural occurring *p*-isomer of octopamine has an affinity that is approximately 500 times higher if compared with that of the *m*-isomer (Figure 1, Table 1). Comparison of the affinities of these two isomers with the non-hydroxylated analogue, phenylethanolamine, enabled quantification of the effects of hydroxylation at either position 4 or 3 of the phenolic ring. Whereas introduction of an hydroxyl-group at position 4 resulted in an approximately 15 fold increase in affinity, this substitution at position 3 led to an approximately 50 times decreased affinity (Figure 1). This negative effect of hydroxylation at position 3 of the phenolic ring is one of the major structural features of the octopamine receptor that demarcates it from catecholamine and especially from vertebrate adrenoceptors, to which octopamine receptors are presumably homologous.

Figure 1 Displacement of specific [3 H]-octopamine binding from locust (*Locusta migratoria* L.) nervous tissue membranes by two octopamine isomers (*meta*- (■) and *para*- (□) octopamine) and phenylethanolamine (●). Each point represents the mean of at least 3–4 separate experiments performed in triplicate. Standard errors are not indicated, but they represent usually less than 10% of the respective values.

The biogenic amine with highest affinity for the locust neuronal octopamine receptor is the N-methylated analogue of octopamine, synephrine ($K_i = 3.38$ nM). Its affinity is approximately twice as high as that of octopamine ($K_i = 7.9$ nM). This two fold increase in affinity caused by N-methylation was also observed for noradrenaline and its N-methylated analogue, adrenaline ($K_i = 416$ and 908 nM respectively; Table 1). Tyramine, the biological precursor of octopamine and itself a neurotransmitter candidate in the insect nervous system (Saudou *et al.*, 1990), has an affinity that is approximately 8 times lower than that of octopamine. The stereospecificity of the binding was shown by the two fold increase in affinity of the (–)-isomer of noradrenaline compared with the (±)-racemate. Ahlquist (1948) used in his pioneering work (which classified adrenoceptors into α - and β -receptors) the three biogenic amines adrenaline, noradrenaline and isoprenaline. Interestingly, the locust neuronal octopamine receptor has the same rank order of affinities for these agonists as the vertebrate α -adrenoceptors.

Phenyliminoimidazolidines

Currently, phenyliminoimidazolidines (PIIs) are the most interesting agonists for octopamine receptors. Among them are various compounds with subnanomolar affinities. To quantify the effects of a given substitution, the affinities of those PIIs were compared that are different at only one position of the phenolic ring. As a simple example, the positive effect of bromination at position 4 of the phenolic ring was demonstrated by comparing the affinities of the 4-bromo-substituted analogue NC 12 ($K_i = 14.9$ nM) with the nonsubstituted PII, NC 10 ($K_i = 23.4$ nM, Table 2). Sets of other PIIs could be used to quantify the effects of other substitutions. The chlorine substituted PII derivatives, clonidine (2, 6-dichlor), NC 11 (2,4,6-trichlor), NC 3 (2,4,5-trichlor) and NC 8 (2,4-dichlor) vary substantially in their affinities. The 2,4-chlor derivative (NC 8, $K_i = 0.812$ nM) has higher affinity than either the 2, 4, 5-trichlor (NC 3, $K_i = 2.27$ nM) or the 2, 4, 6-trichlor derivative (NC 11, $K_i = 18.7$ nM). The negative effect of chlorination at position 6 is even greater than the negative effect of this substitution at position 5 (Table 2). The positive effect of a chlorine-group at position 4 is shown by the higher affinity of the 2, 4, 6-trichlor derivative NC 13 compared with the 2, 6-dichlor PII (clonidine, $K_i = 47.4$ nM).

Regarding three different PIIs it was possible to evaluate the effect of methylation at different positions of the phenolic ring. Whereas the increase in affinity of the 2, 4, 6-trimethyl PII (NC 13, $K_i = 4.38$ nM) compared with the 2, 6-dimethyl derivative (NC 4, $K_i = 19.8$ nM) indicates the positive effect of *para*-methylation, methylation at position 6 results in a decreased affinity (the 2, 4-dimethyl PII, NC 9, $K_i = 1.02$ nM compared with NC 13, $K_i = 4.38$ nM; Figure 2). The positive

Table 1 Affinity of various biogenic amines for the locust neuronal octopamine receptor that share structural similarities with octopamine

Substance	K_i (nM)	$K_{i\text{oct}}/K_{ix}$
Synephrine	3.38 ± 0.64	2.33
<i>p</i> -Octopamine	7.9 ± 0.9	1
Tyramine	51.6 ± 17.5	0.15
Phenylethanolamine	115 ± 39.1	0.07
Adrenaline	416 ± 74.8	0.02
(–)-Noradrenaline	475 ± 42	0.016
(±)-Noradrenaline	908 ± 245	0.009
<i>m</i> -Octopamine	5050 ± 1860	0.002
Isoprenaline	18600 ± 4840	0.001

K_i -values $\pm$ s.d. are given. Each substance is tested in at least 5–7 different concentrations 3–4 times in triplicate. $K_{i\text{oct}}$ means K_i -value of octopamine, K_{ix} means K_i -value of the respective substance.

effects of methylation or chlorination at position 4 are obvious. If the two derivatives NC 7 (4-chlor, 2-methyl-P II, $K_i = 0.3$ nM) and NC 9 (2, 4-dimethyl derivative, $K_i = 1.02$) are compared a ranking of these two substitutions is possible. The chlorine substitution is approximately 3 times more efficient than the methyl-substitution at the *para* position. The opposite is true for position 2, where methylation is more effective than chloride substitution (NC 7 compared with NC 8, the 2, 4-dichlor derivative, $K_i = 0.81$ nM).

In the firefly lantern, 2, 6 di-alkyl substituted substances have especially high affinities (Nathanson, 1985b). For the locust neuronal octopamine receptor, the affinity rises about 20 times from the 2, 6-dimethyl P II, NC 4 ($K_i = 19.8$ nM) to the 2, 6-diethyl derivative, NC 5 ($K_i = 0.87$ nM). A further enlargement of the substituents at this position results in an approximately 150 fold decreased affinity as shown for the 2, 6-diisopropyl P II, NC 20 ($K_i = 132$ nM) compared with NC 5. The *para*-position of the phenolic ring appears to be the most interesting one. Even bulky substituents such as the azido-group or the amino-group are tolerated without loss of affinity (*p*-azido- and *p*-amino-clonidine, K_i values 44.5 and 58 nM respectively compared with clonidine $K_i = 47.4$ nM). All other substituents tested, although of different chemical structure (halogen substituents such as bromide and chloride

as well as alkyl substituents such as methyl and ethyl) have positive effects on the affinity (Table 2).

Insecticides

A variety of substances from different classes of compounds such as the formamidines or the aminooxazolines combine high affinity for the locust neuronal octopamine receptor with insecticidal activity. The two formamidine derivatives, chlordimeform and demethylchlordimeform, are especially potent insecticides (Table 3). Although chlordimeform has a lower affinity ($K_i = 137$ nM), it is thought to be metabolized in the insect to the more potent demethylchlordimeform ($K_i = 1.97$ nM). The substance with highest affinity in this group, and the only non-P II with a subnanomolar affinity, is the aminooxazoline derivative, AC-6 ($K_i = 0.95$ nM). Even substances such as BTS 23376 ($K_i = 8.9$ nM) and amitraz ($K_i = 21.6$ nM) which are known to have a certain insecticidal activity, have relatively high affinity for the locust neuronal octopamine receptor. It is suggested that at least some of these substances exert their insecticidal activity through interaction with the neuronal octopamine receptor.

If the affinities of these substances are compared with each other it is possible to quantify the effects of substitutions at position 1 of the phenolic ring. This is possible because all, except amitraz, have a 4-chlor, 2-methyl substitution at the phenolic ring (Figure 3). Substances with heterocyclic substituents such as the 2-aminooxazolines (AC-6) and phenyliminoimidazolidines (NC 7) have highest affinities (Table 3, Figure 3). Only slight structural changes such as the N-methylation of demethylchlordimeform result in a strongly decreased affinity. Interestingly, the introduction of the

Figure 2 Comparison of the relative potencies of different methylated phenyliminoimidazolidines (PIIs) in competing with [3 H]-octopamine for its specific binding site. Each point represents the mean of 3–4 experiments performed in triplicate: (■) 2, 4-dimethyl-P II; (□) 2, 4, 6-trimethyl-P II; (●) 2, 6-dimethyl-P II.

Table 3 Affinity of selected insecticides derived from different classes of compounds for the locust neuronal octopamine receptor

Substance	K_i (nM)	K_{loc}/K_{Ix}
<i>Formamidines</i>		
Demethylchlordimeform	1.97 ± 0.76	4.01
Chlordimeform	137 ± 70	0.06
<i>Aminooxazolines</i>		
AC-6 (4-chlor, 2-methyl-)	0.95 ± 0.24	8.3
<i>Miscellaneous</i>		
BTS 23376	8.9 ± 0.62	0.89
Amitraz	21.6 ± 5	0.37

K_i -values $\pm$ s.d. of substances from three different classes of compounds are given (see Table 1).

Table 2 Affinity of various phenyliminoimidazolidines for the locust neuronal octopamine receptor

Substance	Substitution	K_i (nM)	K_{loc}/K_{Ix}
NC 7	4-chlor, 2-methyl-	0.3 ± 0.04	26.4
St 92	2, 4, 6-triethyl	0.56 ± 0.14	14.2
NC 8	2, 4-dichlor	0.81 ± 0.18	9.73
NC 5	2, 6-diethyl	0.87 ± 0.32	9.04
NC 9	2, 4-dimethyl-	1.02 ± 0.42	7.75
NC-5Z	4-azido, 2, 6-dimethyl-	1.05 ± 0.47	7.52
NC 3	2, 4, 5-trichlor-	2.27 ± 0.89	3.48
NC 13	2, 4, 6-trimethyl-	4.38 ± 1.3	1.8
NC 12	4-bromo-	14.9 ± 3.3	0.53
NC 11	2, 4, 6-trichlor-	18.7 ± 3	0.42
NC 4	2, 6-dimethyl-	19.8 ± 6.5	0.4
NC 10	—	23.4 ± 4.7	0.34
<i>p</i> -Azido-clonidine	4-azido, 2, 6-dichlor-	44.5 ± 7.1	0.18
Clonidine	2, 6-dichlor-	47.4 ± 17.5	0.17
<i>p</i> -Aminoclonidine	4-amino, 2, 6-dichlor-	58 ± 16.2	0.14
NC 20	2, 6-diisopropyl-	132 ± 35.6	0.06

The K_i -values $\pm$ s.d. of the different substances are given (see Table 1).

they never reach the specificity of octopamine (Table 5). On the other hand, PIIIs such as the well known adrenoceptor ligands, clonidine and *p*-aminoclonidine, have a high degree of specificity for vertebrate adrenoceptors.

Discussion

Octopamine receptors are thought to represent ideal targets for highly specific insecticides because they are the only known non-peptide ligand receptors whose occurrence is restricted to invertebrates (Evans, 1985). Much effort has been made to characterize the pharmacology of octopamine receptors from non-neuronal tissues (Axelrod & Saavedra, 1977; Harmar & Horn, 1977; Evans, 1981; 1985; Nathanson, 1985a,b). Although, a class of highly specific antagonists was characterized for locust neuronal octopamine receptors (Roeder, 1990), these receptors have been less well studied. A variety of highly specific agonists for octopamine receptors such as the formamidines (Harmar & Horn, 1977; Nathanson & Hunnicutt, 1981), the phenyliminoimidazolidines (Nathanson, 1985a,b) or the 2-aminooxazolines (Jennings *et al.*, 1988) are available. In the current study the affinities of these compounds for a different type of octopamine receptor, the

neuronal class 3 receptor (Roeder, 1992) are evaluated, and structure-affinity evaluations have been made.

Biogenic amines

The most interesting pharmacological feature obtained from the study of biogenic amines is their rank order of affinities that makes a simple type of classification possible. With only three amines, octopamine, tyramine and noradrenaline it is possible to distinguish octopamine receptors from their closest relatives, the vertebrate α -adrenoceptors and the insect tyramine receptors (Saudou *et al.*, 1992; Hiripi *et al.*, 1994). Octopamine has the highest affinity, followed by tyramine and noradrenaline for the octopamine receptor, but octopamine and tyramine change places in their affinity for the insect tyramine receptor. Noradrenaline is the substance with the highest affinity for adrenoceptors. Interestingly, octopamine and tyramine are the substances with maximal specificity for insect neuronal octopamine receptors (Table 5). Octopamine receptors and tyramine receptors of insects are both thought to be homologues of vertebrate adrenoceptors receptors. While octopamine receptors appear to be homologous with vertebrate α -adrenoceptors, tyramine receptors are more closely related to vertebrate β -adrenoceptors. Clas-

Figure 5 Structure affinity relationship of agonists for the locust neuronal octopamine receptor. The substitutions of each position of the phenolic ring are listed, in the order of their efficacy, at the respective position. ++ indicates an increase in the affinity caused by the substitution of more than 10 times when compared with the nonsubstituted situation (positions 2–6). For position one, comparison is made with the affinity of the natural agonist octopamine; + indicates an increase in affinity between 2 and 10 times; o indicates no change in affinity (0.5–2 times the affinity); – indicates a decrease in affinity (0.5–0.1), and -- indicates a marked decrease in affinity (more than 10 times less affinity).

Table 5 Relative potencies of octopamine agonists in comparison with mammalian α -adrenoceptors

Substance	$K_1(\alpha_2)/K_1(\text{OA}_3 \text{ locust})$	$K_1(\alpha_{1A})/K_1(\text{OA}_3 \text{ locust})$
<i>Biogenic amines</i>		
Octopamine	197	1392
Tyramine	114	445
Phenylethanolamine	9.6	200
Adrenaline	0.014	1.42
Noradrenaline	0.036	2.1
Isoprenaline	0.3	—
<i>Phenyliminoimidazolidines</i>		
NC 5	33.5	—
NC 8	2.78	—
NC 9	5.5	—
NC 10	5.4	—
Clonidine	0.12	9.07
<i>p</i> -Aminoclonidine	0.015	—
<i>2-Benzylimidazolines</i>		
Tolazoline	9.7	113.5
Naphazoline	1.88	363

K_1 -values for α_2 -binding ($[^3\text{H}]$ -clonidine) were obtained from DeJong & Soudijn (1981), from U'Prichard *et al.* (1977), Jarrott *et al.* (1980) and from Rouot & Snyder (1979). For α_1 -binding ($[^3\text{H}]$ -WB-4101), the data were obtained from U'Prichard *et al.* (1977).

sical octopamine receptor agonists, such as octopamine itself, demethylchlordimeform, naphazoline and synephrine are relatively ineffective at the locust tyramine receptor (Hiripi *et al.*, 1994). To distinguish the octopamine and tyramine receptor in the locust nervous tissue, even better antagonists are available. Yohimbine, the antagonist with subnanomolar affinity for the insect tyramine receptor (Saudou *et al.*, 1992; Hiripi *et al.*, 1994) has very low affinity for the neuronal octopamine receptor of locusts ($K_1 = 82 \mu\text{M}$). On the other hand, high affinity antagonists of the locust neuronal octopamine receptor such as mianserin and phentolamine (Roeder & Gewecke, 1990) have only low affinity for the locust tyramine receptor (Hiripi *et al.*, 1994).

Phenyliminoimidazolidines

The other classes of compounds tested allowed more sophisticated pharmacological questions to be asked. The large number of high affinity phenyliminoimidazolidines used was necessary to obtain quantitative data about structure-activity relationships. PIIIs such as NC 5 have, as do octopamine and tyramine, high specificity for octopamine receptors if compared with vertebrate α -adrenoceptors (NC 5 has a more than 30 times higher affinity for neuronal octopamine receptors than for vertebrate α -adrenoceptors, Table 5). Substitutions at only two positions of the phenolic ring have a positive effect on the affinity. These are positions 2 and 4 of the phenolic ring (Figure 5). This is in contrast to the octopamine receptor of the firefly lantern, where PIIIs substituted at positions 2 and 6 are most effective (Nathanson, 1985a,b). The neuronal octopamine-sensitive adenylate cyclase of the tobacco hawkmoth *Manduca sexta* has a pharmacological profile that appears to be more similar to that of the locust nervous tissue (Hollingworth & Johnstone, 1983). In both preparations, substances that are substituted at positions 2 and 4 of the phenolic ring are most effective. In addition, the ability to stimulate the octopamine-sensitive tissue, adenylate cyclase is correlated with the insecticidal activity of these compounds (Hollingworth & Johnstone, 1983; Hollingworth *et al.*, 1984). Another class of compounds, the phenylaminomethylimidazolidines were found to have very high affinity for octopamine receptors from cockroaches, whereas their affinity for *Manduca* octopamine receptors is much lower (Orr *et al.*, 1991). Interestingly, the phenylaminomethylimidazolines have a different substitution preference at the phenolic ring. Substitutions at positions 2 and 3 seems to be optimal for this class of compounds (Hollingworth *et al.*, 1984; Orr *et al.*, 1991).

Insecticides and miscellaneous

It is likely that the insecticidal activity of substances such as the formamidines, amitraz, BTS 23376, or AC-6 result from interaction with octopamine receptors. The coincidence of insecticidal activity with high affinity for the octopamine receptor supports the assumption that the neuronal octopamine receptor is the target of these insecticides. Application at sublethal concentrations of the well-studied formamidines result in behavioural changes, such as hyperactivity, that could be attributed to the interaction with neuronal type octopamine receptors (Dadai *et al.*, 1987). Therefore, it seems likely that only the neuronal octopamine receptor is the target of octopamine insecticides, as these behavioural changes could not result from interaction with octopamine receptors from non-neuronal tissues.

As the most interesting structural feature of this study, various substituents at position 1 of the phenolic ring were tested. Heterocyclic substitutions such as the 2-iminoimidazolidines or the 2-benzylimidazolidines are the most effective. The spacing group between these two ringsystems is of great importance. Reduction of the spacing leads to a drastic decrease in affinity. The effect of further increasing the spacing by one group was not studied because the respective substances were not available. A further increase of the affinity should result because it has been shown for the cockroach nerve cord that a substance with this kind of structure also has very high affinity (XAMI; Orr *et al.*, 1991). Further studies are needed to clarify the pharmacological characteristics of octopamine receptors. It is inappropriate to combine pharmacological data from different octopamine receptor subtypes such as non-neuronal and neuronal octopamine receptors (Roeder, 1992; Nathanson, 1993) or from studies performed with octopamine receptor preparations from different insect species. Therefore, well-defined octopamine receptor preparations such as the locust neuronal octopamine receptor should be used to obtain quantitative pharmacological data of a variety of different classes of compounds. Studies like these might result in the development of substances with even higher affinity and specificity for octopamine receptors that could serve as new leads for better insecticides as well as better probes to study octopamine receptors in more detail.

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