
Octopamine Receptors in the Head of the Larva of the Common Cutworm, Spodoptera litura Fabricius (Lepidoptera: Noctuidae): Effects of Agonists and Antagonists

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Octopamine elevated cAMP level in head membranes of common cutworm (Spodoptera litura) larvae in a concentration-dependent manner, indicating the activation of receptor-linked adenylate cyclase. Several octopamine analogues, in which the hydroxyl groups of octopamine are replaced by other groups such as a chlorine atom, also showed agonist activities comparable to that of octopamine. However, no activation was seen with norepinephrine and dopamine. The rank order of potency of known agonists was: naphazoline > octopamine > 2-(2,6-diethylanilino)-2-imidazoline > de-N-methylchloridineform > clonidine > tolazoline. Mianserin appeared to be the most potent antagonist, although clear differences in potency between tested antagonists were not observed. The head of larval S. litura appears to contain heterogeneous octopamine receptors, with properties somewhat different from those of other insects.

Key words: octopamine receptors, adenylate cyclase, biogenic amines, common cutworm, Spodoptera litura

INTRODUCTION

Octopamine, a biogenic phenylethanolamine, plays an important role as a neurotransmitter, neuromodulator and neurohormone in invertebrates (Evans, 1980). Octopamine is thought to mediate its effects through the interaction with two major classes of receptors (Evans, 1981). The binding of octopamine to the class 1 (octopamine1) receptors is suggested to lead to an increase in intracellular calcium levels, while its binding to the class 2 (octopamine2) receptors leads to the activation of receptor-linked adenylate cyclase, which consequently increases levels of intracellular cAMP as a second messenger (Evans, 1981).

Chlordimeform is known as an insecticidal chemical (Beeman and Matsumura, 1978), causing antifeeding and abnormal behaviors, particularly in Lepidoptera and Hemiptera (Hollingworth, 1976). Shimizu and Fukami (1984) have reported that this compound induces the continuous burst of mandibular movements (CBMM) in the larva of the cabbage armyworm, Mamestra brassicae, and have suggested that abnormal electrogensis in the sabescophageal ganglion (SG) causes CBMM. They have also speculated that the antifeeding activity of chlordimeform is related to CBMM. Meanwhile, Nathanson and

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Hunnicutt (1981) have shown that the desmethyl analogue (DCDM) of chlordimeform acts as a partial agonist of octopamine2 receptors. If CBMM is induced by the octopaminergic action of the metabolite DCDM, the heads, including the SG, of lepidopterous larvae may be a good source for the octopamine2 receptor-adenylate cyclase system. In addition, chlordimeform-sensitive insects might have octopamine2 receptors with properties different from those of insensitive insects such as the American cockroach, Periplaneta americana.

To test such possibilities, we have examined the effects of agonists (including octopamine and its analogues) and antagonists on adenylate cyclase from the head of the larva of the common cutworm, Spodoptera litura, and now report the results.

MATERIALS AND METHODS

Insects. Eggs of S. litura were obtained from cultures maintained at Takarazuka Research Center, Sumitomo Chemical Co. Ltd. Larvae of S. litura were reared on an artificial diet (Kojima and Nakayama, 1979) at 25°C in a dark room.

Membrane preparation. Twelve heads of final instar larvae of S. litura were homogenized in 5 ml of a 10-mM Tris-maleate buffer (pH 8.0) with a glass homogenizer. The homogenate was centrifuged at 500 × g for 10 min, and the pellet was again homogenized and centrifuged. The combined supernatant was centrifuged at 15,000 × g for 10 min. The pellet was superficially washed with the buffer and suspended in the buffer. The suspension was recentrifuged at 15,000 × g for 10 min. The pellet was washed superficially and suspended finally in 1 ml of the buffer.

Standard adenylate cyclase assay. Adenylate cyclase assay was done by a modification of the method of Nathanson and Hunnicutt (1981). The membrane suspension (100 μl) was added to 100 μl of a 190-mM Tris-maleate buffer (pH 8.0) containing 25 mM theophylline, 20 mM magnesium chloride, 0.25 mM GTP and 1.25 mM EGTA. After 25 μl of an aqueous solution containing an agonist was added, the mixture was preincubated at 30°C for 1 min. The reaction (at 30°C for 10 min) was started by the addition of 25 μl of an aqueous solution containing 20 mM ATP and stopped by heating the mixture at 100°C for 2 min. The mixture was centrifuged at 15,000 × g for 10 min, and the supernatant was used to determine cAMP level. The amount of produced cAMP was measured according to the protocol of cyclic [3H]AMP assay system (Amersham International plc). Antagonists were similarly tested by incubating with 10 μM octopamine.

Compounds. dL-Octopamine (1) and dL-norepinephrine (2) were purchased from Aldrich Chemical Co., Inc.; dopamine (3), clonidine and naphazoline from Wako Pure Chemical Industries, Ltd.; tolazoline from Sigma Chemical Co.; and chlorpromazine, cyproheptadine, metoclopramide and mianserin from Research Biochemicals Inc. Aminoacetophenones 14–18 were synthesized by the Friedel-Crafts reaction of mono-substituted benzenes with bromo- or chloroacetyl chloride, followed by amination with hexamethylenetetramine (Corrigan et al., 1945). Phenylethanolamines 4–10 were prepared by NaBH4 reduction (Goto, 1954) of the corresponding aminoacetophenones. Phenylethanolamine 11 was obtained by Fe/HCl reduction of 10. Phenylethylamines 12 and 13 were prepared by chlorination (Ward, 1943) of 6 and 7 with thionyl chloride, respectively. 2-(2,6-Diethylamino)-2-imidazoline (NC-5) was synthesized by the method described by Nathanson and Kaugars (1989). De-N-methylchlordimeform (DCDM) was synthesized by the method of Benezet and Knowles (1976). All these compounds were hydrochloride salts. The structures of the synthesized compounds were confirmed by chemical ionization.
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mass spectrometry and proton nuclear magnetic resonance spectroscopy. The structures of numbered compounds are shown in Table 1.

RESULTS AND DISCUSSION

*Effects of octopamine and related biogenic amines*

Experiments were first done to examine the effect of octopamine on the activity of adenylate cyclase by measuring cAMP. Under the incubation conditions used, octopamine (100 μM) elevated cAMP level to 235 ± 93 ng (mean ± SD; n = 2) of basal level. The degree of activation of adenylate cyclase is lower than those reported in cockroach (*P.*

Table 1. Activation of adenylate cyclase by octopamine analogues in head membranes of *S. litura* larvae

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>X</th>
<th>Kₐ (μM)</th>
<th>Vₘₐₓ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OH</td>
<td>H</td>
<td>CH(OH)</td>
<td>0.41 ± 0.10²ᵇ</td>
<td>102 ± 4¹ᵇ</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>OH</td>
<td>CH(OH)</td>
<td>—</td>
<td>—¹ᵃ</td>
</tr>
<tr>
<td>3</td>
<td>OH</td>
<td>OH</td>
<td>CH₃</td>
<td>—</td>
<td>—¹ᵃ</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>H</td>
<td>CH(OH)</td>
<td>0.052 ± 0.007³ⁿ</td>
<td>82 ± 3⁴ⁿ</td>
</tr>
<tr>
<td>5</td>
<td>Me</td>
<td>H</td>
<td>CH(OH)</td>
<td>0.29 ± 0.53³ᵃ</td>
<td>85 ± 17²ⁿ</td>
</tr>
<tr>
<td>6</td>
<td>Cl</td>
<td>H</td>
<td>CH(OH)</td>
<td>0.17 ± 0.02³ⁿ</td>
<td>128 ± 9⁴ⁿ</td>
</tr>
<tr>
<td>7</td>
<td>Br</td>
<td>H</td>
<td>CH(OH)</td>
<td>0.20 ± 0.02³ⁿ</td>
<td>74 ± 5⁴ⁿ</td>
</tr>
<tr>
<td>8</td>
<td>OMe</td>
<td>H</td>
<td>CH(OH)</td>
<td>0.86 ± 0.11²ᵃ</td>
<td>46 ± 1²ᵃ</td>
</tr>
<tr>
<td>9</td>
<td>Ph</td>
<td>H</td>
<td>CH(OH)</td>
<td>—</td>
<td>1 ± 5³ᵈ</td>
</tr>
<tr>
<td>10</td>
<td>NO₂</td>
<td>H</td>
<td>CH(OH)</td>
<td>—</td>
<td>—⁶ ± 8⁴ᵈ</td>
</tr>
<tr>
<td>11</td>
<td>NH₂</td>
<td>H</td>
<td>CH(OH)</td>
<td>—</td>
<td>4 ± 3³ᵈ</td>
</tr>
<tr>
<td>12</td>
<td>Cl</td>
<td>H</td>
<td>CHCl</td>
<td>0.84 ± 0.52²ᵃ</td>
<td>117 ± 14²ᵇ</td>
</tr>
<tr>
<td>13</td>
<td>Br</td>
<td>H</td>
<td>CHCl</td>
<td>2.2 ± 1.0²ᵃ</td>
<td>125 ± 12²ᵇ</td>
</tr>
<tr>
<td>14</td>
<td>H</td>
<td>H</td>
<td>C-O</td>
<td>—</td>
<td>62 ± 25³ᵈ</td>
</tr>
<tr>
<td>15</td>
<td>Me</td>
<td>H</td>
<td>C-O</td>
<td>—</td>
<td>50 ± 9³ᵃ</td>
</tr>
<tr>
<td>16</td>
<td>Cl</td>
<td>H</td>
<td>C-O</td>
<td>—</td>
<td>72 ± 1⁴ᵈ</td>
</tr>
<tr>
<td>17</td>
<td>Br</td>
<td>H</td>
<td>C-O</td>
<td>—</td>
<td>31 ± 1⁴ᵈ</td>
</tr>
<tr>
<td>18</td>
<td>OMe</td>
<td>H</td>
<td>C-O</td>
<td>—</td>
<td>27 ± 8³ᵈ</td>
</tr>
</tbody>
</table>

ᵃ Values (mean ± standard deviation) were obtained from dose-response curves consisting of 4 data points, each being the mean of 3 experiments. The activity was measured relative to that of octopamine (100 μM). Two methods (described below as b and c) appropriate for each set of data were chosen to estimate *Kₐ* and *Vₘₐₓ* values.

ᵇ Values were estimated by the method of least squares with the TAYLOR expansion (SARODA and HIROMI, 1976).

ᶜ Not determined.

d cAMP production (mean ± standard deviation) at 100 μM is expressed relative to cAMP produced on addition of octopamine.

e Values were estimated by the double-reciprocal plots.
americana) thoracic ganglia (NATHANSON and GREENGARD, 1973) and firefly (Photuris sp.) light organs (NATHANSON, 1979), but is comparable to those in the brains of P. americana (HARMAR and HORN, 1977) and the bertha armyworm (M. configurata) pharate adult (BODNARYK, 1979). Octopamine-activated cAMP production was low (7.7 ± 0.3 pmol/min/mg protein [mean ± SD; n = 2]) compared to those reported for brains and ganglia. This low specific activity might result from the crude nature (i.e., neural plus non-neural tissues) of the head preparation. The activation was concentration-dependent as shown by closed circles in Fig. 1. The concentration for half-maximal activation ($K_c$) of octopamine (1 in Table 1) was 4- to 7-fold lower than those reported for other insects (NATHANSON and GREENGARD, 1973; HARMAR and HORN, 1977; NATHANSON, 1979; BODNARYK, 1979); thus, octopamine shows a relatively high affinity for its receptor in the head of larval S. litura.

Structurally related catecholamines, norepinephrine (2) and dopamine (3), failed to increase cAMP (Table 1), indicating that the head preparation of larval S. litura contains an octopamine-sensitive adenylate cyclase but neither norepinephrine- nor dopamine-sensitive ones. This is in contrast to findings with P. americana tissues (NATHANSON and GREENGARD, 1973; HARMAR and HORN, 1977).

Effects of octopamine analogues

To examine structural requirements for octopamine analogues to interact with its receptor, we tested $p$-substituted phenylethanolamines, phenylethylamines and aminoacetophenones for their agonist activity on the octopamine-sensitive adenylate cyclase (Table 1). Twelve out of the 15 analogues (4-18) significantly increased cAMP production, when tested at 100 μM. Removal of the $p$-hydroxyl group of octopamine did not cause an increase (i.e., a reduction in potency) but rather a decrease (i.e., an increase in potency) in $K_c$ without a large decrease in $V_{max}$ (maximum efficacy). The high activity of the resulting $p$-unsubstituted analogue (4) is inconsistent with findings in the thoracic ganglia (NATHANSON and GREENGARD, 1973), brain (HARMAR and HORN, 1977) and ventral nerve cord (HIRASHIMA et al., 1992) of P. americana. Substitution of the $p$-hydroxyl group of octopamine by a methyl group did not cause a substantial change in activity, as shown by
5. Similar substitutions by a chlorine and bromine atom gave active compounds (6 and 7) with slightly larger and slightly smaller \( V_{\text{max}} \) than the \( V_{\text{max}} \) of octopamine, respectively. \( p \)-Methoxy substitution to produce 8 resulted in a marked decrease in \( V_{\text{max}} \). The \( p \)-phenyl (9), \( p \)-nitro (10) and \( p \)-amino (11) analogues were inactive. Replacement of the \( \beta \)-hydroxyl group of the \( p \)-chloro analogue (6) by a chlorine atom did not remarkably change the efficacy and potency of 6, which has a \( V_{\text{max}} \) slightly larger than that of octopamine. A similar \( \beta \)-chlorine replacement of the \( p \)-bromo analogue (7) caused increases in \( V_{\text{max}} \) and \( K_{v} \). However, it should be noted that the dose-response curves of the resulting \( p \),\( \beta \)-dichloro (12) and \( p \)-bromo-\( \beta \)-chloro (13) analogues appear to be biphasic unlike those of octopamine and other \( \beta \)-hydroxy analogues (4-8) (Fig. 1). Interactions with two or more cAMP-producing systems could be involved in the action of 12 and 13. \( p \)-Substituted aminoacetophenones (14-18) also had activities, although the efficacy was lower than that of the corresponding phenylethanolamines (4-8).

\( p \)-Fluorophenylethanolamine is the only octopamine analogue reported to date to have a full agonist activity comparable to that of octopamine (in the brain of \( P. \) americana) (HARMAR and HORN, 1977). The \( p \)-fluoro analogue and several \( p \)-substituted phenylethanolamines have recently been reported to have activities as a partial agonist in \( P. \) americana ventral nerve cord, with \( V_{\text{max}} \) which are smaller (expressed as % of octopamine \( V_{\text{max}} \)) than those obtained in the current study (HIRASHIMA et al., 1992). The data presented here demonstrate that the \( p \)-hydroxy group of octopamine is not essential in activating \( S. \) litura adenylate cyclase, and that not only the \( p \)-hydroxyl group but also the \( \beta \)-hydroxyl group can be replaced by other groups such as a chlorine atom without significant loss of activity. In contrast, the introduction of a \( m \)-hydroxyl group causes a loss of ability to activate \( S. \) litura adenylate cyclase, as shown by norepinephrine (2) and dopamine (3).

**Effects of known agonists and antagonists**

To characterize the octopamine receptor pharmacologically, we examined the effects of several known agonists and antagonists on it. Three \( \alpha \)-adrenergic imidazolines were found to act as a full or almost full agonist in \( S. \) litura larvae, although NC-5 was a partial agonist (Table 2). The rank order of potency was: naphazoline \( \geq \) NC-5 \( > \) clonidine \( > \) tolazoline. Despite the high sensitivity of the SG of larval \( M. \) brassicaceae to chlordimeform (SHIMIZU and FUKAMI, 1984), DCDM was low in both potency (\( K_{v} \)) and efficacy (\( V_{\text{max}} \)) in the \( S. \) litura head preparation. More experiments with isolated SG will be needed to confirm

Table 2. Activation of adenylate cyclase by known agonists in head membranes of \( S. \) litura larvae

<table>
<thead>
<tr>
<th>Agonist</th>
<th>( K_{v} ) (( \mu )M)</th>
<th>( V_{\text{max}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octopamine</td>
<td>0.41 \pm 0.10</td>
<td>102 \pm 4</td>
</tr>
<tr>
<td>Naphazoline</td>
<td>0.30 \pm 0.17</td>
<td>97 \pm 9</td>
</tr>
<tr>
<td>NC-5</td>
<td>0.62 \pm 0.53</td>
<td>53 \pm 8</td>
</tr>
<tr>
<td>DCDM</td>
<td>1.1  \pm 0.6</td>
<td>44 \pm 5</td>
</tr>
<tr>
<td>Clonidine</td>
<td>3.3  \pm 0.5</td>
<td>76 \pm 2</td>
</tr>
<tr>
<td>Tolazoline</td>
<td>12.3 \pm 0.6</td>
<td>92 \pm 1</td>
</tr>
</tbody>
</table>

Values (mean \( \pm \) standard deviation) were estimated by applying the method of least squares with the TAYLOR expansion (SAKODA and HIROMI, 1976) to data sets consisting of 3-5 data points, each being the mean of 3 experiments. The activity was measured relative to that of octopamine (100 \( \mu \)M).
if the SG contains DCDM-sensitive octopamine receptors, since DCDM-insensitive octopamine receptors that could exist in other parts of the head probably affect the results. Also, the possibility cannot be ruled out that CBMM is induced by an action of chlor-dimeform at a site other than octopamine receptors. On the other hand, psychotropic agents inhibited the octopamine activation of adenylate cyclase (Table 3). Mianserin appears to be most potent, although clear differences in potency between antagonists are not observed.

The high potency of the agonist naphazoline and the antagonist mianserin is consistent with the characteristics of octopamine2 receptors (Evans, 1981). However, the rank order of potency of agonists and antagonists does not entirely agree with those for any subclasses of octopamine receptors reported, while it resembles those for octopamine2a (Evans, 1981) and octopamine3 receptors (Roeder, 1992). This might be due to the heterogeneity of octopamine receptors present in the head of larval S. litura; that is, the head of larval S. litura might contain multiple receptor subclasses with pharmacological properties somewhat different from those of other insects. It is necessary to investigate the pharmacological properties of adenylate cyclase from the SG to make clear what subclass(es) of receptors the SG of larval S. litura contains.

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Octopamine Receptors in *S. littoralis*


