Molecular Cloning and Pharmacological Characterization of a Molluscan Octopamine Receptor

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SUMMARY

We describe the cloning and functional expression of a cDNA encoding a novel G protein-coupled receptor, which was isolated from the central nervous system of the pond snail Lymnaea stagnalis. The amino acid sequence predicted by this cDNA shows highest similarity with the sequence of the Drosophila tyramine receptor, the Drosophila tyramine/octopamine receptor, and the mammalian a-adrenergic receptors. On expression in mammalian cells, [3H]rauwolscine, an a2-adrenergic receptor antagonist, binds with high affinity (KD = 2.9 × 10−9 M) to the receptor. Of several tested neurotransmitters, octopamine (which is considered to be the invertebrate counterpart of norepinephrine) showed the highest affinity (1.9 × 10−8 M) for the receptor. Therefore, we consider this receptor to be the first true octopamine receptor to be cloned. The ligand binding properties of the novel receptor, designated Lym oa1, seem to be distinct from any of the binding profiles described for octopamine receptors in tissue preparations. Although the pharmacological profile of Lym oa1 shows some similarity with that of Tyr/Oct-Dro and Tyr-Loc, there are also clear differences. In particular, phenolamine, chlorpromazine, and mianserin display markedly higher affinities for Lym oa1 than for the insect receptors. As far as the vertebrate adrenergic receptors are concerned, the ligand binding properties of Lym oa1 resemble a2-adrenergic receptors more than they do a1- or b-adrenergic receptors. Octopaminergic stimulation of Lym oa1 induces an increase in both inositol phosphates and cAMP (EC50 = 9.1 × 10−7 M and 5.1 × 10−6 M, respectively). This is in contrast to the signal transduction pathways described for the related tyramine- and a2-adrenergic receptors, which couple in an inhibitory way to adenylyl cyclase.

Octopamine, the monohydroxylated analogue of norepinephrine, is a well-established neurotransmitter, neuropeptide, and neuromodulator in many invertebrate species (1). In particular, various insect preparations have been used to study the role of octopamine. Because many of the octopamine-mediated responses are connected to adaptation to stressful circumstances, the octopaminergic system has been considered to be the invertebrate equivalent of the vertebrate sympathetic nervous system. Although some early reports describe effects of octopamine in vertebrates (1), it is present in only trace amounts and probably plays no significant role in neurotransmission. This raises the possibility of specifically interfering with octopamine neurotransmission in invertebrates, an issue of considerable interest for the pesticide industry.

Octopamine specifically interacts with octopamine receptors, which belong to the superfamily of G protein-coupled receptors. The pharmacology of octopamine receptors is well documented in several insect species, especially in locusts. Four octopamine receptor subtypes (oct-1, oct-2A, oct-2B, oct-3) have been characterized, each with pharmacological properties (for a review, see Ref. 2). Stimulation of oct-1 receptors induces an increase in intracellular calcium concentration, whereas oct-2A, oct-2B, and oct-3 receptors are positively coupled to AC.

Octopaminergic neurotransmission has been studied not only in insects; biochemical and immunocytochemical studies have demonstrated the presence of octopamine in the brain of several molluscan species (3–7). In addition, the physiology and pharmacology of octopamine receptors have been examined (3, 8–11).

Despite the large interest in the octopaminergic system, the molecular structure of octopamine receptors has not been elucidated. However, two closely related receptors that exhibit highest affinity for tyramine were cloned from Drosophila (12, 13) and Locusta (14). Tyramine, which is the precur-

ABBREVIATIONS: AC, adenylyl cyclase; bp, base-pairs; CNS, central nervous system; TM, transmembrane region; HEK, human embryonic kidney; PLC, phospholipase C; IP, inositol phosphate; PCR, polymerase chain reaction; DMEM, Dulbecco’s modified Eagle medium.

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sor of octopamine, was also suggested to play a role as a neurotransmitter in insects (15), but it is less extensively studied than octopamine. Although tyramine exhibits a higher affinity for the *Drosophila* receptor than does octopamine and tyramine is a more potent inhibitor of AC, both transmitters exhibit similar potencies for raising intracellular calcium levels in cells expressing this receptor (16). Therefore, the *Drosophila* tyramine receptor (13) has been suggested to function as a combined tyramine/octopamine receptor and will be referred to as Tyr/Oct-Dro.

We present the cloning and functional expression of a cDNA encoding a novel G protein-coupled bioamine receptor that is expressed in the CNS of the freshwater snail *Lymnaea stagnalis*. This receptor is designated Lym oa1 according to the most recent recommendations by the Committee for Receptor Nomenclature of the International Union of Pharmacology (17). The predicted amino acid sequence of Lym oa1 exhibits highest sequence similarity with the sequence of Tyr/Oct-Dro (12, 13), Tyr-Loc (14), and mammalian α-adrenergic receptors. However, Lym oa1 clearly differs from these receptors in its high affinity for octopamine and its unique signaling properties: octopamine stimulates both PLC and AC. On the basis of these data, we concluded that we had cloned an octopamine receptor.2

**Materials and Methods**

**Animals.** Adult *Lymnaea stagnalis* (shell height, 28–34 mm) were bred in the laboratory under standard conditions (18).

**Isolation and sequence analysis of the cDNA encoding Lym oa1.** Total RNA (5 μg) was isolated from *L. stagnalis* CNS (19) and reverse-transcribed into cDNA using oligo(dT) primers and SuperScript reverse transcriptase (GIBCO BRL, Baltimore, MD). This cDNA was used as a template in a PCR with degenerate oligonucleotides that recognize conserved amino acids in TMs 6 and 7 of G protein-coupled bioamine receptors. The primers and PCR conditions were described previously (20). Sequence analysis (Sequenase, GIBCO BRL) of the PCR products revealed that one of the products showed significant similarity to the mammalian adrenergic receptors. Based on the sequence of this fragment, a specific antisense primer (5′-CAGAAGGATCCGACCTGGTAAATGGTG-3′, based on bp 2563–1584; Fig. 1) was used to isolate the corresponding full-length cDNA clone. A PCR-based library screening was performed on a cDNA library of *L. stagnalis* CNS, cloned in λ-ZAP according to a method described by Gibbons et al. (21). The cDNA insert was excised *in vivo* as a pBluescript SK+ phagemid (designated pBS-Lym oa1) and sequenced on both strands (Sequenase) using a walking primer strategy.

**Construction of Lym oa1 expression vector and expression of Lym oa1 in mammalian cells.** The 5′ part of the open reading frame of Lym oa1 was amplified in a PCR using pBS-Lym oa1 as a template and the following two primers: (i) a sense primer (5′-GCA GGATCCACCAGACGTATTTCTGCTGCAAGAATGGAT TGTCAGCTGACATCTCTGATA-3′), which starts with a BamHI restriction site (bold letters), followed by a region encoding an eight-amino acid FLAG peptide (underlined letters), and a region based on bp 956–977 (see Fig. 1); and (ii) an antisense primer (5′-CAGAAGGATCCGGACTGCACCCACGACCAGAACCATGACTGA ACTGACATGCAAGATATGCAGACGAGAATGATGCAGT GTCAGCTGACATCTCTGATA-3′), which starts with a BamHI restriction site (bold letters), followed by a region encoding an eight-amino acid FLAG peptide (underlined letters), and a region based on bp 596–717 (see Fig. 1). The resulting PCR product was cloned as a BamHI/KpnI fragment into pBluescript SK+, and the resulting subclone (pBS-5′-Lym oa1) was sequenced on both strands. The 3′ part of the open reading frame of Lym ao1 was

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2 The sequence of Lym ao1 has been deposited in the GenBank under accession no. U62771.

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**Fig. 1.** Nucleotide sequence and predicted amino acid sequence of Lym ao1. Boxes, putative TM regions labeled I–VII; white letters, serine or threonine residues represent consensus sites for protein kinase C-mediated phosphorylation; gray circles, residues putatively involved in ligand binding.

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amplified similar to the manner described for the 5′ part, using the following primers: (i) a sense primer (5′-TACGCTCACTGGTGTAAGG-3′), based on bp 2715–2732 (see Fig. 1); and (ii) an antisense primer (5′-GCTCTAGACCTGTTTAGATCCG-3′), starting with an XbaI site (bold letters) and followed by a sequence comprising the predicted stop codon (italic letters), based on bp 2860–2879 (see Fig. 1). A 113-bp AAtII/XbaI fragment of the resulting PCR product was ligated to a KpnI/AatII fragment of the original cDNA clone, and the resulting subclone (pBS-3′Lym ao1) was sequenced. The final expression construct (pcDNA3-Lym ao1) was made by ligation of a BamHII/KpnI fragment of pBS-5′Lym ao1 and a KpnI/XbaI fragment of pBS-3′Lym ao1 into the BamHII/XbaI sites of pcDNA3 (Invitrogen, San Diego, CA). pcDNA3-Lym ao1 was introduced into HEK 293 cells by calcium phosphate-mediated transfection. Transfected cells were grown in the presence of 0.8 mg/ml G-418 (G-418, Gibco, Grand Island, NY), and membranes of clonal cell lines were selected for binding to [3H]rauwolscine (5 nM).

Radioligand binding studies. Stably transfected HEK 293 cells were harvested in 50 mM ice-cold Tris-HCl, pH 7.4, and centrifuged for 20 min at 26000 × g at 4°. Cells were lysed by sonication in 5 mM Tris-HCl, pH 7.4, and recentrifuged (20 min), and the membrane pellet was resuspended in 50 mM Tris-HCl, pH 7.4. To determine the dissociation constant (Kd) of [3H]rauwolscine, 20 μg of protein was incubated with increasing concentrations of [3H]rauwolscine (0.1–45 nM) in the presence of 50 mM Tris-HCl, pH 7.4, in a total volume of 250 μl for 30 min at 25°. Yohimbine (5 μM) was added to determine the nonspecific binding. To determine the affinities of several compounds for Lym ao1, increasing concentrations of inhibitor (10−10 to 10−4 M) were used to inhibit the binding of 4 nM [3H]rauwolscine. Receptor binding reactions were terminated by filtration over Whatman (Fairfield, NJ) GF/B filters, which were then washed three times with 50 mM ice-cold Tris-HCl, pH 7.4. The radioactivity retained on the filters was measured by liquid scintillation counting.

Kaleidagraph 3.0 (Abelbeck/Synergy, Reading, PA) was used to fit the obtained data. Saturation curves were fitted according to the equation: SB = Bmax × [L]/([L] + Kd), in which SB is specific binding (total binding minus nonspecific binding), Bmax is the total number of binding sites, and [L] is the concentration of [3H]rauwolscine. The affinity constant (Kd) was determined from Scatchard analysis plotting specific binding versus specific binding/[L]. Displacement curves were fitted according to the equation: B = TB − SB × ([L]/([L] + IC50)α′), in which B is bound radioactivity, TB is total binding, [L] is the concentration of the inhibitor, and α is the Hill coefficient (Kd = IC50/(1 + ([L]/IC50))).

Chinese hamster ovary cells stably expressing the human α2A-, α2C-, or α2δ-adrenergic receptor were used to study the pharmacological profile of the human α-adrenergic receptors. The receptor expression levels of these cell lines, derived from [3H]rauwolscine concentration binding isotherms, were as follows: α2A-adrenergic receptor, Bmax = 2.8 pmol/mg of protein (Kd = 0.8 nM); α2δ-adrenergic receptor, Bmax = 8.6 pmol/mg of protein (Kd = 1.6 nM); and α2δ-adrenergic receptor, Bmax = 9.1 pmol/mg of protein (Kd = 0.3 nM). For radioligand binding assays, 6–12 μg of membrane protein suspended in 25 mM glycglycine/NaOH buffer, pH 7.6, was incubated with [3H]rauwolscine (final concentration, 1 nM). Nonspecific binding was determined with oxymetazoline (α2δ-adrenergic receptor, 1 μM final concentration) or spiroxatrine (α2δ- and α2C-adrenergic receptors, 1 μM final concentration). Assay mixtures were incubated for 30 min at 25° and terminated by rapid filtration over GF/B filters. Filters were washed, and the radioactivity collected on the filters was counted in a liquid scintillation spectrometer. Sigmoidal inhibition curves were calculated by nonlinear regression analysis according to algorithms described by Oestreicher and Pinto (22).

Measurements of IP formation. HEK 293 cells stably expressing Lym ao1 were grown onto 24-well plates and incubated with 1 μCi of [3H]inositol (Amersham, Arlington Heights, IL)/ml of isosmotically free DMEM (GIBCO) supplemented with 10% dialyzed fetal calf serum for 24 hr. Cells were washed once with DMEM and incubated with 10 mM LiCl for 10 min at 37°. Agonists (10−3 M to 10−9 M) were added and incubated for 60 min at 37°. The medium was removed, and the cells were lysed by sonication in ice-cold chloroform/methanol (1:2). After chloroform extraction, the aqueous phase was incubated with Dowex AG 1X8 anion exchange resin. Resin was washed thoroughly with H2O, and IPs were eluted with 1 ml of 0.1 M formic acid/1 M ammonium formate. Radiolabeled IPs were measured by liquid scintillation counting.

Measurements of cAMP formation. The cellular concentration of cAMP in HEK 293 cells stably expressing Lym ao1 was determined as described by Leurs et al. (23). Briefly, cells were grown onto 24-well plates and incubated for 20 min at 37° with 300 μM 3-isobutyryl-1-methylyanthidine and agonists (10−3 M to 10−9 M) in DMEM. After stimulation of the cells, the medium was aspirated, and the cells were lysed by sonication in ice-cold 0.1 N HCl. After neutralization, the extract was incubated with protein kinase A and 30,000 dpm of [2,8-3H]cAMP for 2.5 hr on ice. Reactions were terminated by filtration over Whatman GF/B filter, and filters were washed three times with 50 mM ice-cold Tris-HCl, pH 7.4. The bound radioactivity was compared with a calibration curve in which 0.25–32 pmol of cAMP was incubated with protein kinase A and [2,8-3H]cAMP.

Results

Cloning of Lym ao1 cDNA. PCR with degenerated oligonucleotides recognizing conserved regions in TM5 and 7 of bioamine receptors resulted in the isolation of several partial receptor cDNA fragments that are expressed in the CNS of the pond snail L. stagnalis. One of these fragments showed considerable similarity to the vertebrate α-adrenergic receptors. A full-length cDNA clone (3416 bp) corresponding to this fragment was isolated from a L. stagnalis CNS cDNA library (Fig. 1). It consists of a leader sequence of 922 bp, an open reading frame of 1914 bp, and a trailer sequence of 580 bp. The predicted protein (638 amino acids) shows the typical seven hydrophobic regions putatively forming the seven TM domains characteristic for G protein-coupled receptors. An eighth hydrophobic region at the amino-terminal part of the protein, as is present in many invertebrate G protein-coupled neurotransmitter receptors, is not present in Lym ao1.

A comparison of the predicted amino acid sequence of Lym ao1 with the Swiss Prot data base revealed highest sequence identity (30–40%) with the Drosophila tyramine/octopamine receptor (Tyr/Oct-Dro) (12, 13), the Locusta tyramine receptor (Tyr-Loc) (14), and the vertebrate α-adrenergic receptors. When only the TM regions are considered, overall identities are 50% (Tyr/Oct-Dro), 53% (Tyr-Loc), and ~40% (human α2C-adrenergic receptors). A comparison of these amino acid sequences is given in Fig. 2. The second extracellular loop of Lym ao1 is larger than is usually observed in G protein-coupled neurotransmitter receptors. The amino-terminal region of Lym ao1 is relatively short and does not contain any consensus sites for N-linked glycosylation. These glycosylation sites are a common feature of G protein-coupled receptors and have been associated with membrane trafficking of the receptor. In the intracellular domains of Lym ao1, 11 consensus sites for phosphorylation by protein kinase C are present that may play a role in receptor desensitization.

Stable expression of Lym ao1 in HEK 293 cells; pharmacological profile. HEK 293 cells were transfected with pcDNA 3-Lym ao1 and selected for growth on medium containing 0.8 mg/ml G-418. Several resistant colonies were isolated, and membrane preparations of these clonal cell lines were tested for their ability to bind [3H]rauwolscine.
Onestablecellline (\(B_{\text{max}} = 3.6 \pm 0.2\) pmol/mg of protein; five experiments) was selected for further analysis of the pharmacological and signaling properties of Lymoa 1. Fig. 3 shows the saturation binding curve and Scatchard plot for \([3H]\)rauwolscine (\(K_D = 2.85\) nM; five experiments). HEK293cells not expressing Lymoa 1 did not show any binding to \([3H]\)rauwolscine (data not shown). To test which naturally occurring agonist could be the ligand for Lymoa 1, several bioamine neurotransmitters were tested for their ability to displace \([3H]\)rauwolscine binding (Table 1). Hill coefficients for all agonist inhibition curves were not significantly different than 1. The rank order of potencies of the tested transmitters was \((6)\) - p-octopamine > serotonin > p-tyramine > (2) - epinephrine > (2) - norepinephrine > dopamine > histamine. Because of the structural relationship between Lymoa 1 and the adrenergic receptors, we tested several other agonists and antagonists that are known to interact with adrenergic receptors (Table 1). The rank order of potency of those agonists was \((6)\) - p-synephrine $>$ clonidine $>$ xylometazoline $>$ phenylephrine $>$ oxymetazoline $>$ B-HT920 $>$ methoxamine, and the rank order of the antagonists was yohimbine $>$ chlorpromazine $>$ spiperone $>$ phentolamine $>$ mianserin $>$ rauwolscine $>$ prazosin $>$ propanolol $>$ alprenolol $>$ pindolol. This profile suggests that Lymoa 1 is more closely related to the \(\alpha_2\)- than to the \(\alpha_1\)- and \(\beta\)-adrenergic receptors. To determine whether Lymoa 1 exhibits differential homology to one of the \(\alpha_2\)-adrenergic receptor subtypes, we performed a similar pharmacological characterization for the human \(\alpha_2A\), \(\alpha_2B\), and \(\alpha_2C\)-adrenergic receptors (Table 1). No significant correlation could be found between the agonist binding properties of Lymoa 1 and the \(\alpha_2\)-adrenergic receptors. For the antagonists, however, Lymoa 1 was more closely correlated to the \(\alpha_2H\)-adrenergic (\(r_S = 0.950, p < 0.001\)) than
TABLE 1  
\( pK_i \) values of various compounds for Lym oa1 and human \( \alpha_{2A} \), \( \alpha_{2B} \), and \( \alpha_{2C} \)-adrenergic receptors as determined by displacement of [\( ^3H \)]rauwolscine binding  

\( K_i \) values are mean \( \pm \) standard deviation, as deduced from at least three independent experiments, each of which was performed in triplicate. In the case of Lym oa1, the concentration of [\( ^3H \)]rauwolscine was 4 nM. In the case of the human \( \alpha_{2A} \)-adrenergic receptors, the concentration was 1 nM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lym oa1</th>
<th>Human ( \alpha_{2A} )-adrenergic receptor</th>
<th>Human ( \alpha_{2B} )-adrenergic receptor</th>
<th>Human ( \alpha_{2C} )-adrenergic receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pindolol</td>
<td>4.56 ( \pm ) 0.26</td>
<td>7.78 ( \pm ) 0.12</td>
<td>7.00 ( \pm ) 0.12</td>
<td>(&lt;5)</td>
</tr>
<tr>
<td>Alprenolol</td>
<td>5.01 ( \pm ) 0.15</td>
<td>7.14 ( \pm ) 0.36</td>
<td>7.14 ( \pm ) 0.36</td>
<td>(&lt;5)</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>7.46 ( \pm ) 0.26</td>
<td>7.44 ( \pm ) 0.36</td>
<td>7.44 ( \pm ) 0.36</td>
<td>(&lt;5)</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>7.67 ( \pm ) 0.56</td>
<td>7.37 ( \pm ) 0.29</td>
<td>7.37 ( \pm ) 0.29</td>
<td>(&lt;5)</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>8.89 ( \pm ) 0.28</td>
<td>8.71 ( \pm ) 0.10</td>
<td>9.14 ( \pm ) 0.10</td>
<td>(&lt;5)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>8.53 ( \pm ) 0.26</td>
<td>7.98 ( \pm ) 0.49</td>
<td>7.14 ( \pm ) 0.36</td>
<td>(&lt;5)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>4.43 ( \pm ) 0.13</td>
<td>5.17 ( \pm ) 0.08</td>
<td>4.79 ( \pm ) 0.26</td>
<td>4.94 ( \pm ) 0.08</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>8.07 ( \pm ) 0.14</td>
<td>8.12 ( \pm ) 0.35</td>
<td>5.11 ( \pm ) 0.00</td>
<td>5.19 ( \pm ) 0.05</td>
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<tr>
<td>Mianserin</td>
<td>7.67 ( \pm ) 0.34</td>
<td>8.15 ( \pm ) 0.07</td>
<td>6.11 ( \pm ) 0.26</td>
<td>7.26 ( \pm ) 0.13</td>
</tr>
<tr>
<td>(–)-Epinephrine</td>
<td>4.44 ( \pm ) 0.17</td>
<td>5.62 ( \pm ) 0.06</td>
<td>4.94 ( \pm ) 0.08</td>
<td>4.94 ( \pm ) 0.08</td>
</tr>
<tr>
<td>(–)-Norepinephrine</td>
<td>4.09 ( \pm ) 0.39</td>
<td>6.67 ( \pm ) 0.26</td>
<td>6.60 ( \pm ) 0.36</td>
<td>6.60 ( \pm ) 0.36</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>(&lt;4)</td>
<td>(&lt;4)</td>
<td>(&lt;4)</td>
<td>(&lt;4)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>(&lt;4)</td>
<td>5.77 ( \pm ) 0.36</td>
<td>5.33 ( \pm ) 0.33</td>
<td>5.76 ( \pm ) 0.33</td>
</tr>
<tr>
<td>Histamine</td>
<td>(&lt;4)</td>
<td>(&lt;4)</td>
<td>(&lt;4)</td>
<td>(&lt;4)</td>
</tr>
<tr>
<td>Antagonist</td>
<td></td>
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</table>

Stable expression of Lym oa1 in HEK 293 cells: signal transduction. Activation of octopamine receptors classically results in elevation of the concentration of cAMP in the cell (1). Therefore, we tested the ability of octopamine to stimulate AC in HEK 293 cells stably expressing Lym oa1. Fig. 4 shows the dose-dependent increase in intracellular cAMP levels induced by octopamine and tyramine. Octopamine increases the intracellular cAMP concentration maximally to 9-fold over basal levels, with an EC_{50} value of 5.1 \( \pm \) 0.8 \( \times \) 10^{-6} M. Tyramine could induce only a slight increase in intracellular cAMP levels (2-fold over basal levels) at rather high concentrations (\( \geq 10^{-5} \) M). Stimulation of untransfected HEK 293 cells with octopamine or tyramine did not show any elevation in cAMP concentration (data not shown). Related neurotransmitters (epinephrine, norepinephrine, dopamine, serotonin) did not stimulate cAMP formation to a significantly higher degree in HEK 293 cells expressing Lym oa1 than in untransfected cells (data not shown).

Stimulation of the insect oct-1 receptor induces an increase in intracellular calcium levels (24). In addition, Baines and Downer (25) have shown that the effect of octopamine on phagocytosis in cockroach hemocytes is mediated by inositol trisphosphate. Therefore, we also tested whether octopamine could induce a change in the intracellular concentration of IPs. Indeed, octopamine was able to efficiently increase the amount of IPs to a maximal level of 45-fold over basal (Fig. 5). Also, tyramine, epinephrine, and norepinephrine stimulated IP production, albeit with a lower E_{max} value (\( \geq 30 \)-fold over basal). EC_{50} values were 9.1 \( \pm \) 0.10 M for octopamine, 3.0 \( \pm \) 1.1 \( \times \) 10^{-5} M for epinephrine, 5.4 \( \pm \) 1.7 \( \times \) 10^{-5} M for tyramine, and 9.9 \( \pm \) 4.0 \( \times \) 10^{-5} M for norepinephrine. In nontransfected HEK 293 cells, none of the agonists mentioned above was able to induce an increase in IP formation (data not shown).

Discussion

We described the cloning and functional expression of a cDNA that encodes a novel type of G protein-coupled receptor isolated from the CNS of L. stagnalis. The predicted protein sequence exhibits highest identity to the tyramine/octopamine receptor from Drosophila, the tyramine receptor from...
norepinephrine. Because octopamine is a mono-,
para-hydroxylated catecholamine, the conservation of Ser246 can be
considered as indicative for a similar binding of octopamine
and norepinephrine to their receptors.

Octopamine is a known neurotransmitter in \emph{L. stagnalis}.
Several identified neurons in the CNS have been shown to
contain octopamine \citep{7}, and recently, \[^{3}\text{H}]\text{octopamine binding}
 sites in the CNS were pharmacologically characterized. \cite{3} This
pharmacological profile, however, differs strongly from the
profile of Lyn \emph{oa}_1. In particular, the affinities of phentol-
amine and yohimbine are much higher for Lyn \emph{oa}_1 than for
the \[^{3}\text{H}]\text{octopamine binding sites in total } \emph{L. stagnalis} CNS.
This suggests that in addition to Lyn \emph{oa}_1, other octopamine
receptor or receptors will be present in \emph{L. stagnalis}.

The pharmacological properties of the oct-1, -2A/-2B, and
-3 receptor subtypes from insect tissues have been studied
extensively (for a review, see Ref. 2). Interestingly, the ligand
binding characteristics of Lyn \emph{oa}_1 show no homology with
the binding profiles of any of the insect octopamine receptors,
suggesting that Lyn \emph{oa}_1 is not a species variant of the pre-
viously characterized insect octopamine receptor subtypes.
Therefore, we suggest that Lyn \emph{oa}_1 is the first member of a
novel subclass of octopamine receptors.

Because of the structural similarity among octopamine,
tyramine, and (nor)epinephrine, we compared the ligand
binding properties of Lyn \emph{oa}_1 with those of the cloned
tyramine and mammalian adrenergic receptors. When the
pharmacological profile of Lyn \emph{oa}_1 is compared with the profiles
of Tyr/Oct-Dro and Tyr-Loc \citep{18–20}, there is a better corre-
lation between Lyn \emph{oa}_1 and Tyr/Oct-Dro \((r_S = 0.9, p < 0.001)
(12, 13) than between Lyn \emph{oa}_1 and Tyr-Loc \((r_S = 0.8, p =\)
0.07) (14). However, phentolamine, chlorpromazine, and
mianserin in particular have a markedly higher affinity for
Lyn \emph{oa}_1 than for the insect receptors. When the pharma-
cological profile of Lyn \emph{oa}_1 is compared with that of the human
adrenergic receptors, the affinities of both agonists and an-
tagons for Lyn \emph{oa}_1 indicate that Lyn \emph{oa}_1 is more closely
related to the \(\alpha\)-adrenergic receptors than to the \(\beta\)-adrenergic
receptors (illustrated by the high affinity of clonidine and the
low affinities of alpenolol, propanolol, and pindolol for Lyn
\emph{oa}_1). Furthermore, the high affinities of rauwolscine and
yohimbine and the moderate affinity of prazosin indicate a
closer relationship between Lyn \emph{oa}_1 and the \(\alpha_2\)-adrenergic
receptors than between Lyn \emph{oa}_1 and the \(\alpha_1\)-adrenergic
receptors. Therefore, we wanted to know whether Lyn \emph{oa}_1 was
most closely related to one of the three \(\alpha\)-adrenergic receptor
subtypes. Such information could be useful in unraveling
the structure-function relationships of \(\alpha\)-adrenergic receptors.
Concerning the antagonist binding affinities, a better corre-
lation can be found between Lyn \emph{oa}_1 and the \(\alpha_{2B}\)-adrenergic
receptor subtype \((r_S = 0.950, p < 0.001)\) than between Lyn
\emph{oa}_1 and the \(\alpha_{2A}\)-adrenergic receptor subtype \((r_S = 0.820, p =\)
0.007) and the \(\alpha_{2C}\)-adrenergic receptor subtype \((r_S = 0.683,\)
\(p < 0.042)\). However, there is no significant correlation be-
tween the agonist binding affinities of Lyn \emph{oa}_1 and the
\(\alpha_2\)-adrenergic receptors. The pharmacological and structural
relationships between Lyn \emph{oa}_1 and the \(\alpha_2\)-adrenergic recep-
tors suggest that the corresponding genes have evolved from
a common ancestor. The conservation of the functional prop-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.png}
\caption{Concentration-effect relationship of agonist-induced increase
in intracellular concentration of IPs in HEK 293 cells stably transfect-
ed with Lyn \emph{oa}_1. Error bars, standard deviation of the mean value obtained
from three experiments, each performed in triplicate. Stimulation was
performed with octopamine (\textbullet{}), epinephrine (\textcircled{a}),
tyramine (\textcircled{A}), and norepinephrine (\textcircled{o}). Maximal stimulation
was 46-, 33-, 35-, and 31-fold over basal, respectively. Maximal stimulation
is plotted as 100% for easier comparison of the concentration dependencies of the different
agonist-induced effects on intracellular IP concentration. Basal IP lev-
els were 2.3%, 3.3%, 3.3%, and 3.2%, respectively, and correspond to
292 ± 95 dpm of \[^{3}\text{H}]\text{IPs/well.}

Locusta, and the mammalian \(\alpha\)-adrenergic receptors. The
extent of this identity (40–50% in TM regions), however,
indicates that the \emph{L. stagnalis} receptor is not a species vari-
ant of these receptors. Indeed, on permanent expression of
the receptor in HEK 293 cells, it shows higher affinity for
octopamine than for tyramine or (nor)epinephrine. Fur-
more, octopamine is the most potent agonist in stimulation of
IP and cAMP formation. These results strongly suggest that
we identified an octopamine receptor, and we propose to call
it Lyn \emph{oa}_1.

The residues that are involved in the interaction between
catecholamines and their receptors have been studied exten-
sively. An important source of binding energy between the
ligand and the receptor is provided by the ionic interaction
with the amine of the ligand and an aspartic acid residue in
TM 3. However, both agonists and antagonists interact with
this aspartic acid residue. The actual receptor activation is
thought to involve an interaction between the catechol moi-
ey of the agonist and residues in TMs 6 and 7. More specif-
ically, the aromatic ring interacts with a phenylalanine resi-
due in TM 6, and the catechol hydroxyl groups form
hydrogen bonds with side chains of two serine residues in TM
5. In the \(\beta_2\)-adrenergic receptor, these two serine residues
(Ser204 and Ser207) were shown to interact with the \textit{meta-
and para}-hydroxyl group of the catechol moiety of norepi-
nephrine, respectively \citep{26}. In the \(\alpha_{2A}\)-adrenergic receptor,
Ser204 was shown to be involved in hydrogen bonding with
the \textit{para}-hydroxyl group (like its cognate residue in the
\(\beta_2\)-adrenergic receptor; \citep{27}). In contrast, Ser200 did not seem to
participate in receptor agonist interaction. In Lyn \emph{oa}_1, the
presence of Asp108 in TM 3 and Phe535 in TM 6 is conserved.
Interestingly, only one of the two conserved serine residues in
TM 5 is present \citep{Ser246}. This serine residue corresponds
to the serine residues in the \(\alpha\) - and \(\beta\)-adrenergic receptors
(Ser207 and Ser204, respectively) that are thought to inter-
act with the \textit{para}-hydroxyl group of the catechol moiety of

\footnotesize{\textsuperscript{3} Juhos, S., Z. Hiripi, M. Eckert, J. Rapus, and R. Elekes, personal commu-
nication.}
erties of octopamine receptors and norepinephrine receptors (e.g., their key role in stress adaptation) is in agreement with such an evolutionary connection.

Most octopamine receptors (i.e., the oct-2 and oct-3 receptor subtypes) have been shown to be positively coupled to AC (1, 2). Activation of the less well-characterized oct-1 receptor subtype has been described to result in an elevation of the intracellular concentrations of both calcium and IP(_3_)(24, 25). We have shown that octopamine induces an increase in both IP(_3_)s and cAMP in cells stably expressing Lym α_A. We did observe, however, that the coupling of Lym α_A to AC seemed to be less efficient than the coupling to PLC. This can be concluded from the fact that the maximal production of cAMP (~20 pmol/well, ~9-fold over basal) is markedly lower than that obtained by stimulation of endogenous (G_m coupled) β-adrenergic receptors in the same HEK 293 cell-line (~48 pmol/well, ~20-fold over basal; data not shown), although the latter receptors are expressed at much higher densities. In contrast, the octopamine-induced stimulation of IP formation is extremely efficient (45-fold over basal). Furthermore, octopamine is the only agonist that can induce a significant increase in cAMP levels in cells expressing Lym α_B compared with wild-type HEK 293 cells. Although the stimulation of AC by the activation of endogenous β-adrenergic receptors might mask the effect of (nor)epinephrine on tyramine. On the other hand, all tested agonists elicit a strong IP response to octopamine. Similar situations, in which receptor activation leads to a strong stimulation of phosphatidyl inositol biphosphate hydrolysis and a weak increase in cAMP levels, have been described for the α_2-adrenergic receptors (28–30), for the m1 and m3 muscarinic acetylcholine receptors (30–36), and for the 5-hydroxytryptamine_{2A} serotonin receptor (37). Such dual signaling can be explained by (i) coupling of the receptor to two different G proteins; (ii) coupling of the receptor to a single G protein that activates PLC, followed by cross-activation of AC by activated protein kinase C or receptor to a single G protein that activates PLC, followed by receptor to two different G proteins; (ii) coupling of the receptor to multiple second messenger systems.

References


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