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Isolation, Cloning, and Tissue Expression of a Putative Octopamine/Tyramine Receptor from Locust Visceral Muscle Tissues

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Octopamine has been shown to play major roles in invertebrate nervous systems as a neurotransmitter, neuromodulator, and neurohormone. Tyramine is the biochemical precursor of octopamine and its neuromodulatory role is now being investigated and clarified in invertebrates, particularly in insects. Both octopamine and tyramine mediate their actions via G protein-coupled receptors (GPCRs) and are believed to play important functions in the regulation of physiological processes in locust oviduct. Here we report the isolation, cloning, and tissue expression of a putative octopamine/tyramine receptor from the locust, *Locusta migratoria*. Degenerate oligonucleotides in PCR reactions were first used to obtain partial cDNA sequences and then these partial sequences were used in screens to obtain a full-length cDNA. The cloned cDNA is about 3.1 kb long and encodes a protein of 484 amino acid residues with typical characteristics of GPCRs including seven transmembrane domains and many signature residues. The amino acid sequence of the cloned cDNA displays sequence similarities with known GPCRs, particularly octopamine/tyramine receptors. Screening of the locust genomic DNA library resulted in isolation of a genomic DNA with the same size as the cDNA, indicating that the gene is intronless. RT-PCR and Northern blot analyses revealed the expression of the receptor mRNA in brain, ventral nerve cord, oviduct, and midgut tissues. Southern blot analyses using *Eco*RI and *Hind*III restriction endonucleases recognized at least two distinct gene bands. Arch. Insect Biochem. Physiol. 59:132–149, 2005.

KEYWORDS: *Locusta migratoria*; amine; G protein-coupled receptors; oviduct; midgut

INTRODUCTION

Receptor characterization and transduction mechanism studies will prove vital in gaining insight into the complexity of physiological processes. Most receptors mediate their intracellular actions in a pathway that involves activation of one or more guanine nucleotide-binding regulatory proteins or G proteins, the largest group of proteins in animals. There are predicted to be approximately 700, 1,100, 160, and 276 G protein-coupled receptors (GPCRs) in the *Homo sapiens, Caenorhabditis elegans, Drosophila melanogaster*, and *Anopheles gambiae* genomes, respectively (Hill et al., 2002; Gaillard et al., 2004). There is, however, no common consensus on the relationship among GPCRs. These receptors may be derived from very divergent ancestors or, alternatively, they may have independently undergone functional convergence in order to maintain interaction with G proteins (Kolakowski, 1994). However, GPCRs share considerable structural similarity, which suggests a common mechanism of action. Cloning and sequence analyses have indicated that transmembrane domains (TMs) in GPCRs, including biogenic amine receptors, are much conserved whereas their extracellu-

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lar and intracellular loops show diversity in sequences (Wess, 1998).

Biogenic amines, as a group of neuroactive chemicals that mediate their actions via activation of GPCRs, play functional roles as neurotransmitters, neuromodulators, and neurohormones by regulating or modulating many signaling pathways in the central as well as peripheral nervous system. These include the integration of sensory information, control of muscular and glandular activities, and complex processes such as learning, memory, and behavior (Blenau and Baumann, 2001). Octopamine and tyramine are two biogenic amines present in insect nervous and peripheral tissues (Roeder, 1994). Octopamine is known as the fight or flight hormone in insects (Roeder, 1999). In addition, octopamine is involved in the modulation of many other physiological and behavioural processes such as proboscis extension, sting response, and juvenile hormone release from the corpora allata (Roeder, 1999; Roeder et al., 2003). It is also involved in olfactory learning since the discrimination of nest mates from unrelated bees has been attributed to octopamine (Grohmann et al., 2003).

In the locust, *Locusta migratoria*, octopamine and tyramine inhibit spontaneous and induced-contractions and relax the basal tonus of the oviduct muscle tissue (Lange and Tsang, 1993; Lange and Nykamp, 1996; Donini and Lange, 2004). Studies on the relaxation of the locust oviduct by octopamine indicate that this biogenic amine acts as a neurotransmitter and binds to octopamine-2 receptors leading to an increase in cyclic AMP (Lange and Nykamp, 1996). Dorsal unpaired median (DUMOV1 and DUMOV2) neurons located in the posterior region of the seventh abdominal ganglion in the locust, *L. migratoria*, have been shown to contain axons projecting to the oviducts (Lange and Orchard, 1984). The presence of octopamine in cell bodies of DUMOV, in the oviducal nerve, and in the innervated oviduct muscle has been documented (Orchard and Lange, 1985).

The biological precursor of octopamine is tyramine, and, although its biosynthesis, release, uptake, and distribution within the locust central nervous system have been reported, its physiological role is not well understood (Downer et al., 1993; Roeder et al., 2003). Tyramine has been recently shown to have multiple and complex functions in the locust oviduct; at low doses, tyramine is capable of attenuating the forskolin-induced increase in cyclic AMP levels in a dose-dependent manner, suggesting the presence of specific tyramine receptor(s) in this tissue (Donini and Lange, 2004). Nerve processes containing tyramine-like immunoreactive materials are present on the locust oviduct. These processes also originate from DUM neurons in the posterior region of the seventh abdominal ganglion (Donini and Lange, 2004). Recent physiological studies suggest a neurotransmitter/neuromodulator role for tyramine in the locust oviduct with potential binding to two putative tyramine receptors (Donini and Lange, 2004), and with molecular cloning of specific tyramine receptors, including the one involved in cocaine sensitization in *D. melanogaster* (McClung and Hirsh, 1999), and the idea that tyramine might act as an independent neuroactive substance is being reconsidered (Roeder, 1999).

cDNA clones encoding octopamine receptors (Arakawa et al., 1990; von Nickisch-Rosenegk et al., 1996; Gerhardt et al., 1997a,b; Han et al., 1998; Baxter and Barker, 1999; Chang et al., 2000; Grosmaitre et al., 2001; Grohmann et al., 2003) and tyramine receptors (Saudou et al., 1990; Vanden Broeck et al., 1995; Blenau et al., 2000; Ohta et al., 2003) have been isolated from a variety of invertebrate species. Pharmacological studies have been performed to examine the characteristics of the octopamine and tyramine receptors in insects. The preferential binding of yohimbine to the tyramine receptor has been a distinguishing factor of the tyramine receptor from other biogenic amine receptors (Hiripi et al., 1994). Pharmacological studies indicate the presence of both octopamine and tyramine receptors in locust brain with tyramine-binding sites showing higher affinity to yohimbine (Hiripi et al., 1994). Cloned tyramine receptors in functional expression studies negatively couple to adenylate cyclase and reduce forskolin-induced cyclic AMP increase in most cases (Saudou et al., 1990; Vanden Broeck et al., 1995; Blenau et al.,

2000; Poels et al., 2001; Rex and Komuniecki, 2002; Ohta et al., 2003). In contrast, activation of octopamine receptors, in physiological preparations and in cell lines, has been shown to lead to increases in cyclic AMP and/or [Ca^{2+}] (Roeder, 1999; Blenau and Baumann, 2003). Tyramine receptors in *D. melanogaster* and *L. migratoria* expressed in various cell lines also have been shown to increase intracellular calcium levels (Robb et al., 1994; Reale et al., 1997; Poels et al., 2001). Since cloned tyramine receptors activate second messenger pathways such as cyclic AMP via G_i-protein to reduce intracellular cyclic AMP levels, it is reasonable to speculate that tyramine, as a neuroactive amine, has a function that differs from that of octopamine (Ohta et al., 2003).

To understand the molecular mechanisms controlled by octopaminergic/tyraminergic systems, we describe molecular cloning and tissue distribution of a putative octopamine/tyramine receptor in locust muscle tissues. The cloned cDNA encodes for an open reading frame (ORF) of 484 amino acid residues and shares considerable sequence similarity with known octopamine/tyramine receptors.

MATERIALS AND METHODS

Animals

Experiments were conducted on mature adult female locusts, *L. migratoria*. The locusts were raised in a crowded laboratory colony at the University of Toronto at Mississauga, Canada. They were kept on a 12/12-h light/dark regime at 30°C, and fed fresh wheat seedlings supplemented with bran and carrots.

Isolation of the mRNAs From the Locust Midgut and Oviduct Tissues

QuickPrep mRNA Purification Kit (Pharmacia, Uppsala, Sweden) was used for mRNA isolation according to the manufacturer's recommendation. Locust tissues (oviducts and midgut) were dissected out in physiological locust saline (150 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 4 mM $NaHCO_3$, 5 mM HEPES (pH 7.2), 90 mM sucrose, and 5 mM trehalose) via a mid-ventral incision, through the abdomen and thorax, after removal of the head, legs, and wings. Following removal of the luminal contents of the midgut and the fat and trachea on oviduct and midgut, the tissues were frozen in liquid nitrogen and stored separately at –70°C. About 0.5 g (wet weight) of the dissected tissues was homogenized over liquid nitrogen in a pre-chilled manual homogenizer and subjected to the mRNA isolation procedure.

cDNA Synthesis

SuperScript™ Choice System Kit (Gibco BRL, Rockville, MD) was used for cDNA synthesis according to the manufacturer's recommendation. Following the synthesis of the second strand cDNA, the reaction mixture was purified by phenol/chloroform extraction, precipitated, and then reconstituted in an appropriate volume of diethylpyrocarbonate (DEPC)-treated water. The *Eco* RI (*Not* I) adapter addition procedure was carried out followed by column chromatography according to the manufacturer's recommendation. The cDNAs from the fractions that contained appropriate sizes and concentrations for λ vector ligation were pooled, precipitated, washed, and reconstituted in an appropriate volume of either DEPC-treated water or TEN buffer (40 mM Tris-HCl [pH 7.5], 1 mM EDTA [pH 8.0], and 150 mM NaCl). The synthesized cDNA was then used as a template to construct the locust midgut and oviduct cDNA libraries using the λ ZAP II system (Stratagene, La Jolla, CA).

Screening of the cDNA Libraries by PCR

Isolation and purification of the λ DNA was carried out using λ Quick Spin Kit (Bio 101, Vista, CA) according to the manufacturer's recommendation. The isolated λ DNA was used as DNA template in PCR reactions. Two degenerate PCR primers corresponding to DNA sequences within the conserved regions of TM III and TM VI of known GPCRs, 5′- CCG GAT CCG YSA TYR SSI TKG ACN GST A-3′, and 5′-ACG AAT TCG GSM ICC ARC AGA ISR YRA A-3′ were used to amplify

fragments of the template cDNA. After one denaturation step at 95°C for 5 min, 32 cycles of denaturation at 94°C for 40 s, annealing at 50°C for 2 min, extension at 72°C for 3 min, and the final cycle of extension at 72°C for 10 min were performed using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) at the ramp speed of 3–5°C/sec. Multiple PCR products were generated and those of expected sizes (400–1,000 bp) were recovered from low melting temperature agarose gels using Sephaglas™ Band Prep Kit (Pharmacia, Uppsala, Sweden). PCR products were cloned into the *Eco* RI sites of the pCR® 2.1 vector (Invitrogen, San Diego, CA), and plasmid DNAs were isolated from the overnight cell cultures and sequenced at the Core Molecular Biology and DNA Sequencing Facility (York University, Toronto, Canada) or at the DNA Sequencing Facility (The Centre for Applied Genomics, Hospital for Sick Children, Toronto, ON, Canada) using the sequencer Model 377, Version 3.3, SemiAdaptive Version 3.2, ABI Prism (Applied Biosystems).

Screening of the cDNA Libraries by the Conventional Method

Following the titer determination, screening of the library was carried out by using a probe based on a partial cDNA obtained from initial screening of the libraries. In order to prepare the probe, plasmid vector, pCR® 2.1 (TA, Invitrogen) containing insert was digested with *Eco* RI restriction endonuclease followed by gel purification of the insert. The gel purified insert was subjected to random prime labeling procedure (Sambrook et al., 1989) using Klenow enzyme, hexanucleotide mix (Roche, Laval, QC, Canada) and [α-^{32}P] dCTP (activity at 3,000 ci/mmol (10 mci/ml) Amersham Pharmacia, Uppsala, Sweden). Membranes were incubated in pre-hybridization solution [6× SSC (saline sodium citrate; 20× SSC is 3 M NaCl and 0.3 M sodium citrate, pH 7.4), 5× Denhardt's medium, 0.5% SDS, 10 µg/ml salmon sperm DNA] at 65°C for 1–2 h. This was followed by hybridization at 65°C for 18 h in a solution that contained 6× SSC, 0.5% SDS, and the denatured ^{32}P labeled probe. Following the hybridization, membranes were washed 2–3 times in 0.1× SSC, 0.1% SDS at 65°C. Exposures were made at –80°C for 48 h using X-OMAT AR films (Kodak, Rochester, NY). Positive clones were identified and subjected to two additional screens as above. To isolate the corresponding full-length cDNA clones, cDNA inserts from these clones were excised in vivo as phagemids (λ ZAP II system manual, Stratagene) and sequenced. DNA sequences were determined from both strands and sequence analysis was performed using the BLAST search at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov /BLAST).

Isolation and Cloning of the Genomic DNA

Genomic DNA was isolated from locust tissues using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's recommendation. A PCR-based approach was used to screen the genomic DNA. PCR primers were 5′-CTG CGC ACA TCG AGT TCT AAT ATC-3′ and 5′-CAG TCA GTC TCC CAA TCT GCC-3′ (located at nucleotide positions of 120–143 and 3,028–3,048, respectively) designed based on the full-length gcr3 cDNA sequence. After one denaturation step at 95°C for 10 min, 35 cycles of denaturation at 94°C for 10 s, annealing at 61°C for 1 min, and extension at 68°C for 3:30 min were performed. The final cycle was completed with 10 min of extension at 68°C. PCR amplified product was gel purified using QIAEX® II Gel Extraction Kit (QIAGEN, Mississauga, ON, Canada) and subcloned to the pCR® 2.1 cloning vector (Invitrogen). Plasmid DNA minipreparation was performed on the overnight culture and sequenced.

Reverse Transcription (RT)-PCR

Locust tissues were dissected in physiological locust saline and frozen in liquid nitrogen. Total RNA was isolated from frozen tissues using guanidinium isothiocyanate-acid phenol, TRIZOL® Reagent (GIBCO BRL, Rockville, MD) according to the manufacturer's recommendation. Isolated RNA was digested with RNase-free DNase I (Sigma-

Aldrich, Mississauga, ON, Canada) and quantified using spectrophotometer analysis and gel electrophoresis. RT-PCR was performed using the One Step RT-PCR kit (QIAGEN, Mississauga, ON, Canada) in 50 µl reaction mixture containing 1.5 µg total RNA, 400 µM of each dNTP, 0.6 µM of each primer, 1× RT-PCR buffer, and 2 µl of enzyme mix. RT-PCR primers were 5′-CTG CGC ACA TCG AGT TCT AAT ATC-3′ and 5′-GCA CGT CAG CCA CAT CTT G-3′ (located at nucleotide positions of 120–143 and 1,103–1,121, respectively) designed on the full-length gcr3 (Tyr-Loc2) cDNA. Primers for positive control were 5′-CTA GTG GAA AGG CAG CCA AG-3′ and 5′-GTG TCA GGA TGG ACC TGC TT-3′ designed on the histone H2B gene. The absence of DNA in the RNA samples was verified by running PCR directly without reverse transcription. Thermocycling reaction included reverse transcription at 50°C for 30 min, PCR reaction activation at 95°C for 15 min, denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1:30 min. The final cycle was completed with 10 min of extension at 72°C. PCR reactions of 24, 27, 30 cycles were performed in order to assure measurement in the exponential phase of the reaction. PCR amplified products were subjected to electrophoresis on a 1.3% agarose gel and visualized by staining with ethidium bromide. The specificity of the amplified fragments was verified by sequence analysis. RT-PCR experiments were repeated 4–5 times.

Northern Blot Analysis

Total RNA was isolated from the locust tissues as above. Aliquots (20 µg) of RNA were size-fractionated on a 1% agarose gel containing formaldehyde and transferred overnight in 20× SSC to a positively charged nylon membrane (Roche Molecular Biochemicals, Laval, QC, Canada) by capillary blotting and then immobilized by baking in a vacuum oven at 80°C for 2 h. Probe was a fragment amplified from the full-length gcr3 (Tyr-Loc2) cDNA by PCR using primers, 5′-CCT GGA AGT TGA TGA AGT GGT AGA C-3′ and 5′-GCA CGT CAT CGT GCG ACA CAC-3′ (located at nucleotide positions of 425–449 and 633–653, respectively) as forward and reverse primers. The probe was labeled with [α-^{32}P] dCTP (Amersham Pharmacia Biotech., Montreal, QC, Canada) by random priming. Blots were prehybridized at 42°C in hybridization solution (DIG-Easy Hyb buffer, Roche, Laval, QC, Canada) for 60 min, followed by hybridization using 10^7 cpm/mL^{-1} of heat denatured ^{32}P-labeled probes at 42°C for 18 h. Blots were then rinsed twice in 2× SSC/0.1% SDS at room temperature for 15 min, and once in 1× SSC/0.1% SDS at 50°C for 30 min, respectively. Exposures were made at –80°C for 48 h using X-OMAT AR films (Kodak, Rochester, NY). To strip off the probe, blots were treated in 0.1% SDS at 80°C for 30 min and reprobed with a histone H2B cDNA probe to monitor the integrity and quantity of RNA.

Southern Blot Analysis

Genomic DNA was isolated from adult female locusts as above. Genomic DNA (30 µg) was digested with *Eco* RI or *Hind* III endonucleases, transferred onto a positively charged nylon membrane (Roche, Laval, QC, Canada) and fixated by UV crosslinking using the Hyperlinker HL 2000 (UVP Laboratory Products, Upland, CA) at autocrosslink setting of 120,000 µJ for about 30 s. A set of primer pair, 5′-GAA GTG CTT GTT GAC GTC ATA AGG-3′ and 5′-CAG TCA GTC TCC CAA TCT GCC-3′ designed on the full-length gcr3 (Tyr-Loc2) cDNA was used to amplify a region of the clone in order to use as a probe. The resulting fragment was gel purified and 25 ng of the purified DNA was random-primed using the Rediprime II DNA Labeling System (Amersham Biosciences, Baie d'Urfé, QC, Canada) in the presence of [α-^{32}P] dCTP. Hybridization was carried out using the radio-labeled probe at 68°C for ~16 h using DIG-Easy Hyb buffer. The blot was then washed under stringency conditions with two washes of 2× SSC, 0.1% SDS at room temperature for 10 min, one wash of 1× SSC, 0.1% SDS at room temperature for 10 min, followed by a final brief wash in 0.2× SSC, 0.1% SDS at 50°C (Sambrook et al., 1989). Exposures

were made at –80°C for 72 h using X-OMAT AR films (Kodak).

RESULTS

Molecular and Structural Properties of the Cloned Octopamine/Tyramine Receptor cDNA and Genomic DNA

A PCR-based cloning strategy was implemented for screening of the locust oviduct and midgut cDNA libraries by taking advantage of the highly conserved amino acid sequences located in the transmembrane regions (between TM III and TM VI) of GPCRs, specifically biogenic amine receptors, using degenerate oligonucleotide primers. Several partial cDNA sequences with sequence similarities to known GPCRs, particularly to biogenic amine receptors including octopamine/tyramine receptors, were obtained. Further screening of the libraries using one of the partial cDNA sequences as a probe resulted in cloning of a putative octopamine/tyramine receptor subtype that we have named gcr3 (Tyr-Loc2). The nucleotide and predicted amino acid sequence of the cloned receptor cDNA are shown in Figure 1. Sequence analysis revealed a 1,452-bp open reading frame (ORF) encoding 484 amino acid residues. The predicted major ORF contains a translation initiation (ATG, methionine) codon and is terminated by a translation termination (stop) codon (TGA) at nucleotide positions 1,453–1,455. The 5′ and 3′ nontranslated regions of the cloned cDNA consist of 731 and 867 nucleotides, respectively (Fig. 1). The molecular mass of the cloned receptor protein is predicted to be 53.6 kDa, with a calculated PI of 9.41. A hydropathy plot of the deduced amino acid sequence of the clone revealed the presence of seven transmembrane domains connected by extracellular and intracellular loops in accordance with all GPCRs. The deduced amino acid sequence of the cloned receptor shows other characteristic features common to biogenic amine receptors. There is a highly conserved DRY domain immediately downstream of TM III that is thought to be important for binding to and activation of G proteins. Several serine and threonine residues in the carboxy-terminal loop are targets for phosphorylation by cyclic AMP-dependent Protein Kinase A (PKA) and Protein Kinase C (PKC). In addition, conserved cysteine (C) residues ($C_{124,\ 203}$) predicted to form a disulfide bond are also found in this receptor (Fig. 1).

Screening of a locust genomic library by PCR using primers designed at the beginning of the 5′ and the end of 3′ of the gcr3 cDNA sequence as forward and reverse primers, respectively, resulted in the isolation of a genomic clone. Sequence analysis of the genomic DNA revealed that there is no intron at least in the region amplified by the designed primers (results not shown). This is in accordance with the findings of studies indicating that some GPCRs are either intronless or contain small introns (Bryson-Richardson et al., 2004).

Characterization of the Cloned Octopamine/Tyramine Receptor

A BLAST search of GenBank with the deduced amino acid sequence revealed sequence similarities with several known vertebrate and invertebrate GPCRs including the biogenic amine receptors. The most similar insect and other invertebrate receptors were aligned by CLUSTAL W (Fig. 2). The highest sequence similarity was between the cloned receptor and a previously cloned tyramine receptor (gcr1, Tyr-Loc1, Vanden Broeck et al., 1995) from *L. migratoria*. The cloned receptor in the present study (gcr3, Tyr-Loc2) showed differences in at least 15 amino acid residues (97% sequence similarity) with gcr1 (Tyr-Loc1). In the open reading frame, 10 amino acid replacements are nonconservative substitutions. Sequence similarities among the cloned receptor and invertebrate biogenic amine receptors ranged from 30% for the serotonin receptor from *Lymnaea stagnalis* (Sugamori et al., 1993) to 59% for the tyramine receptor from *Apis mellifera* (Blenau et al., 2000) (Table 1). The BLAST search of the GenBank also revealed high sequence similarity (97.3% at the amino acid level) to gcr2 (GenBank accession no. X69521). No functional analysis has been reported for gcr2.

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CCAGGGAAAACCAAGCTTATTGCCCCAATGATTTTACCGCCCAAGGCCTGGGTTCCCCGA
GGCTTCGGAATCCCCACTTAAGTAACCGGCCCGCCCAAGTGTGCTGGAATTCGGGTTTTC
TGCGCACATCGAGTTCTAATATCTCTGGCCGGGCCCGACCCCTGTTGTCGCCGCTGATAA
CGTCGAGACTCCGGTGGCTGGGACTGCCTCTGAGGGGGGACTACTGCGCGATTGCTGCCT
CCGGTTACCTAGAGAAGTGGGACGTAAATATTGGGTCTTCCTTGTAAACCAGATGTAACC
GTAGGTGATGGTATTAAAGAGGACAATTTACTCGCAGCACCATACCTGTTTTGTCTCTCT
TTTGTAGGTCTCAAATTTCCAGTGAAACTAAATTTTCATATGGGTGTCATTGACGTCGCA
CTTGCCTGGAAGTTGATGAAGTGGTAGACATCTTGCGATAAATTTCTGAGGGTGTGGTGG
TATTTGTAGGTAATTTTGTGTGAGAGGACTCCTACAATATCAGGCACTAGGGTTGGCAAG
TGTTTCCATACGGGGGTGCACCATGTGGCTGGTGTTAGCGACACTGAAGTAAGGATCTGC
AGGTCGGTGGCGAGTGGTAAGGAGGGATAGGCGTGTGTCGCACGATGACGTGCTCCAGAG
ACGTAGTTCCGACCGGCTAGTGGGGTGGGCGTGTGCGGTTCCCGGGTCAGCGAGCGGCGG
CGGGCGTAGGC

  1  ATGGTGCGCGTCGAGCTGCAGGCGGTGTCGCTGATGAACGGGTCTTCGGCTGCGGAGGAG
  1   M  V  R  V  E  L  Q  A  V  S  L  M  N  G  S  S  A  A  E  E

 61  CCGCAGGACGCGCTGGTGGGCGGCGACGCGTACGGTGGCCGGCGGCCCCCCAGCGTGCTG
 21   P  Q  D  A  L  V  G  G  D  A  Y  G  G  R  R  P  P  S  V  L

121  GGCGTGCGCCTCGCCGTGCCCGAGTGGGAGGTGGCCGTCACCGCCGTCACGCTCTCGCTC
 41   G  V  R  L  A  V  P  E  W  E  V  A  V  T  A  V  T  L  S  L
                                                  I
181  ATCACCCTCATCACCATCGTCGGCAACGTGCTCGTCGTCCTCAGCGTCTTCACCTACAAG
 61   I  T  L  I  T  I  V  G  N  V  L  V  V  L  S  V  F  T  Y  K

241  CCGCTGCGCATCGTCCAGAACTCCTTCATCGTCTCCCTCGCCGTCGCCGATCTGACGGTG
 81   P  L  R  I  V  Q  N  S  F  I  V  S  L  A  V  A  D  L  T  V
                                          II
301  GCGGTGCTGGTGATGCCGTTCAACGTGGCGTACTCGCTGATCCAGCGCTGGGTGTTCGGC
101   A  V  L  V  M  P  F  N  V  A  Y  S  L  I  Q  R  W  V  F  G

361  ATCGTGGTGTGCAAGATGTGGCTGACGTGCGACGTGCTGTGCTGCACGGCGTCCATCCTG
121   I  V  V  C  K  M  W  L  T  C  D  V  L  C  C  T  A  S  I  L
                                          III
421  AACCTGTGCGCCATCGCGCTCGACCGCTACTGGGCCATCACCGACCCCATCAACTACGCG
141   N  L  C  A  I  A  L  D  R  Y  W  A  I  T  D  P  I  N  Y  A

481  CAGAAGCGCACGCTGCGCCGCGTGCTCGCCATGATCGCCGGCGTCTGGCTGCTCTCCGGC
161   Q  K  R  T  L  R  R  V  L  A  M  I  A  G  V  W  L  L  S  G
                                          IV
541  GCCATCAGCTCGCCGCCGCTCATCGGCTGGAACGACTGGCCAATGGAGTTCAACGACACG
181   A  I  S  S  P  P  L  I  G  W  N  D  W  P  M  E  F  N  D  T

601  ACGCCCTGCCAGCTGACTGAGGAGCAGGGCTACGTCATCTACTCGTCGCTGGTCTCCTTC
201   T  P  C  Q  L  T  E  E  Q  G  Y  V  I  Y  S  S  L  V  S  F
                                          V
661  TTCATCCCGCTGTTCATCATGACCATCGTCTACGTCGAAATATTCATCGCGACGAAGCGG
221   F  I  P  L  F  I  M  T  I  V  Y  V  E  I  F  I  A  T  K  R

721  CGCCTGCGCGAGCGCGCGAAGGCATCCAAGCTGAACTCCGCGATGAAGCAGCAGATGGCC
241   R  L  R  E  R  A  K  A  S  K  L  N  S  A  M  K  Q  Q  M  A

781  GTTCAAGCAGTGCCGTCGTCCGTGCCGTCGCACGACCAGGAGTCGGTGAGCAGCGAGACC
261   V  Q  A  V  P  S  S  V  P  S  H  D  Q  E  S  V  S  S  E  T
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Figure 1.

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841   AACCACAACGAGCTGCCGCCGCCGCTTGCGCCGCCGTCGAAGGAGAAGAGGCGCAAGACG
281    N  H  N  E  L  P  P  P  L  A  P  P  S  K  E  K  R  R  K  T

901   AAGAAGAAGTCCAAGAAGAAGGACCAGACAGCCGAGGAGGGCCGCTTCCTGGCGCCCGCC
301    K  K  K  S  K  K  K  D  Q  T  A  E  E  G  R  F  L  A  P  A

961   ATGGTGGCCGAGGACTCCGTCACCGACAACAGCGTCAGCGTGGGGCCCGTCGCCAAGAAC
321    M  V  A  E  D  S  V  T  D  N  S  V  S  V  G  P  V  A  K  N

1021  CACCTGGCCGAGGATGGCTACACATGTACGACGACCACGACGACGACGACGACGACGACT
341    H  L  A  E  D  G  Y  T  C  T  T  T  T  T  T  T  T  T  T  T

1081  GCGGTGACGGACTCCCCGCGGTCGCGCACCGCCAGCCAGAAGGGCAGCACGGCGCCGCCC
361    A  V  T  D  S  P  R  S  R  T  A  S  Q  K  G  S  T  A  P  P

1141  ACGCCGGTGCAGCCCAAGTCGATCCCGGTGTACCAGTTCATCGAGGAGAGGCGGCGCATC
381    T  P  V  Q  P  K  S  I  P  V  Y  Q  F  I  E  E  R  R  R  I

1201  TCGCTGTCCAAGGAGCGGCGCGCGGCGCGCACGCTCGGCATCATCATGGGCGTCTTCGTC
401    S  L  S  K  E  R  R  A  A  R  T  L  G  I  I  M  G  V  F  V
                                        VI
1261  GTCTGCTGGCTGCCCTTCTTCCTCATGTACGTCATCGTGCCGTTCTGCAACCCCAGCTGC
421    V  C  W  L  P  F  F  L  M  Y  V  I  V  P  F  C  N  P  S  C

1321  AAGCCGTCGCCCAAGCTCGTCAACTTCATCACCTGGCTCGGCTACATCAACTCCGCGCTC
441    K  P  S  P  K  L  V  N  F  I  T  W  L  G  Y  I  N  S  A  L
                                           VII
1381  AACCCCATCATCTACACCATCTTCAACCTCGACTTCCGGCGCGCTTTCAAAAAGCTGCTC
461    N  P  I  I  Y  T  I  F  N  L  D  F  R  R  A  F  K  K  L  L

1441  CACTTCAAGACATGA
481    H  F  K  T

      AGTGTTTGTGCAGGTCGAAAGGCTCTTCTTTGACTGTGATACTCAGTGTTAATAGAAGTG
      CTTGTTGACGTCATAAGGGACTGTGTTTCGAGTTGCAAGTGCTTTCGAAAGAACACCGTG
      ATGCTTTTCGAAAACCTGCGAAACTTTATCAGGAAATACTGAAACGTGTTATACATTTTG
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      TGTACATTAACATTTAACTTTAACAATTAAGAGATAACAAAGGTTAGCGTCCTAACAGAA
      AGGAGAACATGTAATGTCAATGGCTTTTTATATCTTTGGTCAGCAGATCAGCGGGTATGG
      GTTACCTATGAATTTAAGAACTTACGTATAAGTCCACTGTTCACATGTGGCTTGGAGTCC
      GAAGACGTGTGTGATAGCTGACGAGAAAAATCATTTAGTTCAGGACAGTCTGAGAAGCTA
      AAGGAGTACGACAGGCGTTTCTTATTTGGTACAAACTCTTTATTTCAGAGTGCCGCTGTT
      TCCACATTGTCACATGCGAGTTTATATAGACGTATTCATGAAACCTGCGTAATTTACGTT
      CAAATAATATGTAGATTTTATTTCAATAACTATTTTAATAATGCGTATTAGAAGCGTAAT
      GTCTATGTACACATAGGAATTAATAGGAACTTATTATGTCACTGAACGTTGTAATTTTTT
      CTACTGTACAGAGACTAATATTTCATTACAAATATCTGCATGTTTCATACTGTAGATATG
      AGGCAGATTGGGAGACTGACTGAAAAA
```

Fig. 1. Nucleotide sequence of the locust, *L. migratoria*, octopamine/tyramine receptor cDNA, and deduced amino acid sequence of the coding region. Putative transmembrane domains are underlined and numbered from I to VII. Potential sites for N-linked glycosylation are shown in open boxes. Consensus sites for phosphorylation by Protein Kinase C and Protein Kinase A are indicated by shaded boxes.

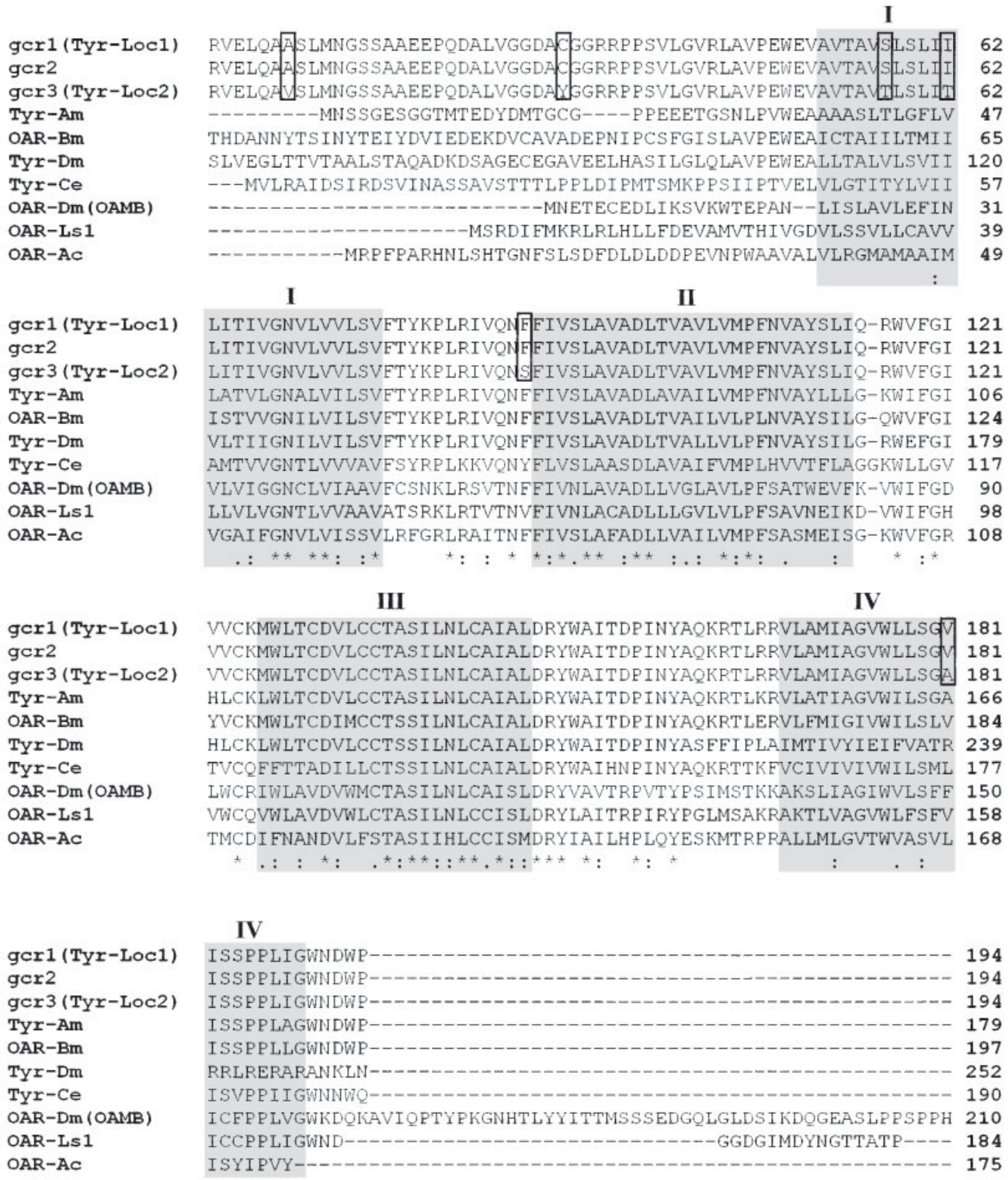
I
gcr1(Tyr-Loc1) RVELQAASLMNGSSAAEEPQDALVGGDACGGRRPPSVLGVRLAVPEWEVAVTAVSLSLII 62
gcr2 RVELQAASLMNGSSAAEEPQDALVGGDACGGRRPPSVLGVRLAVPEWEVAVTAVSLSLII 62
gcr3(Tyr-Loc2) RVELQAVSLMNGSSAAEEPQDALVGGDAYGGRRPPSVLGVRLAVPEWEVAVTAVTLSLIT 62
Tyr-Am ---------MNSSGESGGTMTEDYDMTGCG----PPEEETGSNLPVWEAAAASLTLGFLV 47
OAR-Bm THDANNYTSINYTEIYDVIEDEKDVCAVADEPNIPCSFGISLAVPEWEAICTAIILTMII 65
Tyr-Dm SLVEGLTTVTAALSTAQADKDSAGECEGAVEELHASILGLQLAVPEWEALLTALVLSVII 120
Tyr-Ce ---MVLRAIDSIRDSVINASSAVSTTTLPPLDIPMTSMKPPSIIPTVELVLGTITYLVII 57
OAR-Dm(OAMB) ----------MNETECEDLIKSVKWTEPAN--LISLAVLEFIN 31
OAR-Ls1 ----------MSRDIFMKRLRLHLLFDEVAMVTHIVGDVLSSVLLCAVV 39
OAR-Ac ----------MRPFPARHNLSHTGNFSLSDFDLDLDDPEVNPWAAVALVLRGMAMAAIM 49
I II
gcr1(Tyr-Loc1) LITIVGNVLVVLSVFTYKPLRIVQNFFIVSLAVADLTVAVLVMPFNVAYSLIQ-RWVFGI 121
gcr2 LITIVGNVLVVLSVFTYKPLRIVQNFFIVSLAVADLTVAVLVMPFNVAYSLIQ-RWVFGI 121
gcr3(Tyr-Loc2) LITIVGNVLVVLSVFTYKPLRIVQNSFIVSLAVADLTVAVLVMPFNVAYSLIQ-RWVFGI 121
Tyr-Am LATVLGNALVILSVFTYRPLRIVQNFFIVSLAVADLAVAILVMPFNVAYLLLG-KWIFGI 106
OAR-Bm ISTVVGNILVILSVFTYKPLRIVQNFFIVSLAVADLTVAILVLPLNVAYSILG-QWVFGI 124
Tyr-Dm VLTIIGNILVILSVFTYKPLRIVQNFFIVSLAVADLTVALLVLPFNVAYSILG-RWEFGI 179
Tyr-Ce AMTVVGNTLVVVAVFSYRPLKKVQNYFLVSLAASDLAVAIFVMPLHVVTFLAGGKWLLGV 117
OAR-Dm(OAMB) VLVIGGNCLVIAAVFCSNKLRSVTNFFIVNLAVADLLVGLAVLPFSATWEVFK-VWIFGD 90
OAR-Ls1 LLVLVGNTLVVAAVATSRKLRTVTNVFIVNLACADLLLGVLVLPFSAVNEIKD-VWIFGH 98
OAR-Ac VGAIFGNVLVISSVLRFGRLRAITNFFIVSLAFADLLVAILVMPFSASMEISG-KWVFGR 108
III IV
gcr1(Tyr-Loc1) VVCKMWLTCDVLCCTASILNLCAIALDRYWAITDPINYAQKRTLRRVLAMIAGVWLLSGV 181
gcr2 VVCKMWLTCDVLCCTASILNLCAIALDRYWAITDPINYAQKRTLRRVLAMIAGVWLLSGV 181
gcr3(Tyr-Loc2) VVCKMWLTCDVLCCTASILNLCAIALDRYWAITDPINYAQKRTLRRVLAMIAGVWLLSGA 181
Tyr-Am HLCKLWLTCDVLCCTASILNLCAIALDRYWAITDPINYAQKRTLKRVLATIAGVWILSGA 166
OAR-Bm YVCKMWLTCDIMCCTSSILNLCAIALDRYWAITDPINYAQKRTLERVLFMIGIVWILSLV 184
Tyr-Dm HLCKLWLTCDVLCCTSSILNLCAIALDRYWAITDPINYASFFIPLAIMTIVYIEIFVATR 239
Tyr-Ce TVCQFFTTADILLCTSSILNLCAIALDRYWAIHNPINYAQKRTTKFVCIVIVIVWILSML 177
OAR-Dm(OAMB) LWCRIWLAVDVWMCTASILNLCAISLDRYVAVTRPVTYPSIMSTKKAKSLIAGIWVLSFF 150
OAR-Ls1 VWCQVWLAVDVWLCTASILNLCCISLDRYLAITRPIRYPGLMSAKRAKTLVAGVWLFSFV 158
OAR-Ac TMCDIFNANDVLFSTASIIHLCCISMDRYIAILHPLQYESKMTRPRALLMLGVTWVASVL 168
IV
gcr1(Tyr-Loc1) ISSPPLIGWNDWP---------- 194
gcr2 ISSPPLIGWNDWP---------- 194
gcr3(Tyr-Loc2) ISSPPLIGWNDWP---------- 194
Tyr-Am ISSPPLAGWNDWP---------- 179
OAR-Bm ISSPPLLGWNDWP---------- 197
Tyr-Dm RRLRERARANKLN---------- 252
Tyr-Ce ISVPPIIGWNNWQ---------- 190
OAR-Dm(OAMB) ICFPPLVGWKDQKAVIQPTYPKGNHTLYYITTMSSSEDGQLGLDSIKDQGEASLPPSPPH 210
OAR-Ls1 ICCPPLIGWND----------GGDGIMDYNGTTATP---- 184
OAR-Ac ISYIPVY---------- 175

Figure 2.

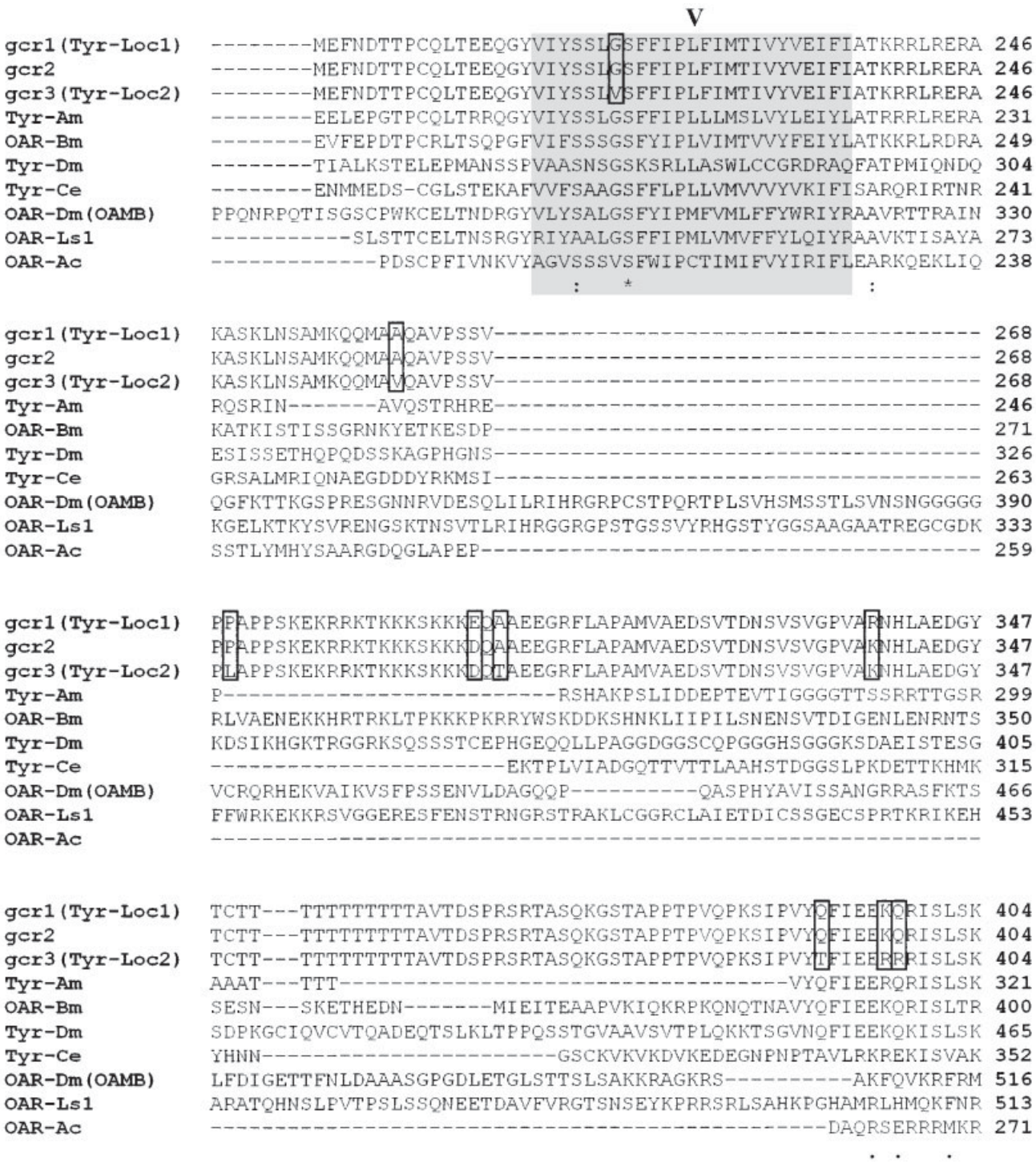

Figure 2. *(continued)*

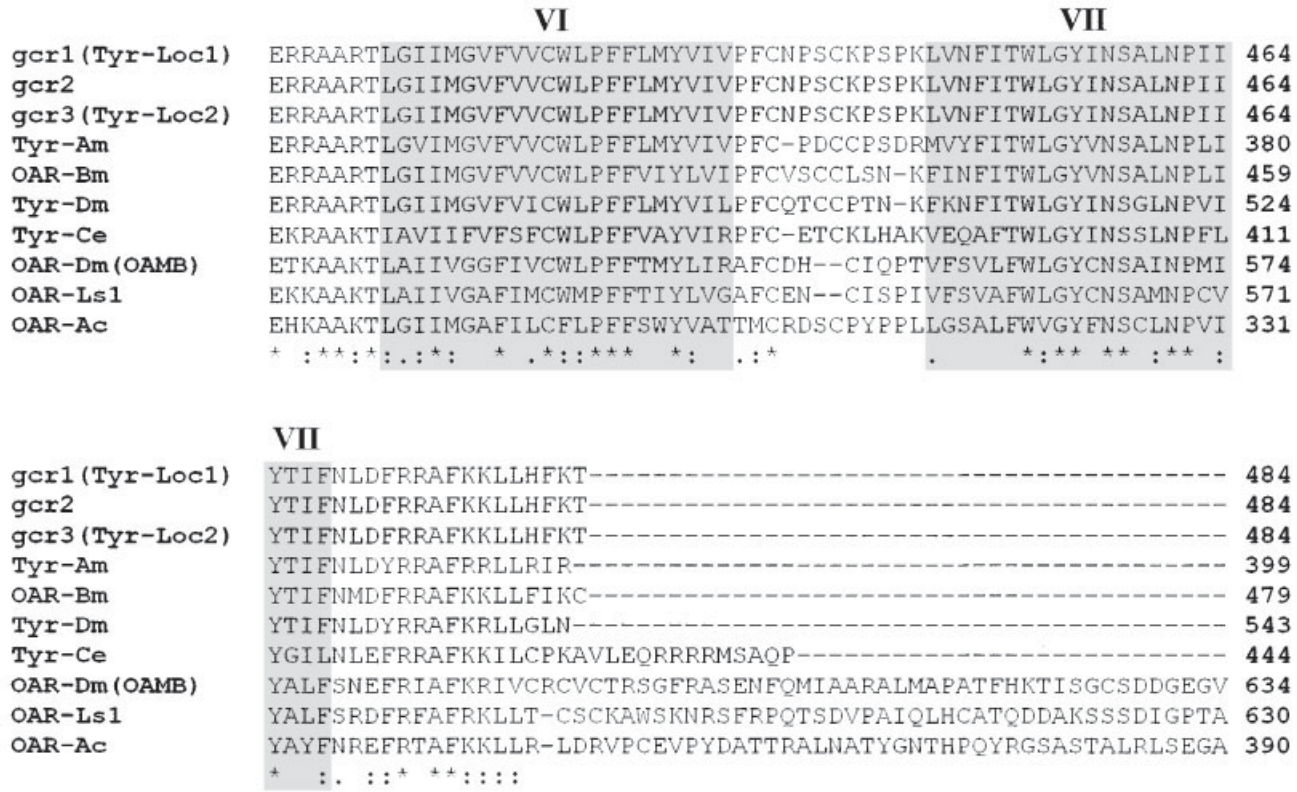

Fig. 2. Amino acid sequence alignment of the locust octopamine/tyramine receptor with other biogenic amine receptors. *L. migratoria* tyramine receptor (gcr1 or Tyr-Loc1 and gcr2; Vanden Broeck et al., 1995), *L. migratoria* octopamine/tyramine receptor (gcr3, Tyr-Loc2), *A. mellifera* tyramine receptor (Tyr-Am; Blenau et al., 2000), *B. mori* octopamine receptor (OAR-Bm; von Nickisch-Rosenegk et al., 1996), *D. melanogaster* tyramine receptor (Tyr-Dm; Saudou et al., 1990), *C. elegans* tyramine receptor (Tyr-Ce; Rex and Komuniecki, 2002), *D. melanogaster* octopamine receptor (OAR-Dm or OAMB; Han et al., 1998), *L. stagnalis* octopamine receptor (OAR-Ls1; Gerhardt et al., 1997a), and *A. californica* octopamine receptor (OAR-Ac; Chang et al., 2000). Stars (*) indicate conservation of identical residues in all 10 sequences and the colons (:) and periods (.) indicate the residues are "strongly" or "weakly" conserved, respectively. Those residues that differ in gcr1, gcr2, and gcr3 are shown in boxes. Seven transmembrane domain sequences are shaded in grey and numbered in Roman numerals. The non-conserved residues are not shown. Multiple sequence alignment was performed using the European Molecular Biology Laboratories-European Bioinformatics Institute (EMBL-EBI) CLUSTAL W alignment program at http://www.ebi.ac.uk/clustalw/index.html.

Tissue Expression of the Receptor Gene Determined by Reverse Transcriptase (RT)-PCR and Northern Hybridization

The expression pattern of gcr3 in locust tissues was analyzed by RT-PCR. The receptor transcript was detected in all tissues examined including brain, ventral nerve cord (VNC), oviduct, and midgut. It appeared that the expression of the receptor transcript was the highest in brain tissue followed by VNC. The expression levels of receptor mRNA in oviduct and midgut tissues were relatively similar as examined under a semi-quantitative RT-PCR condition (Fig. 3).

Northern blot analysis using total RNA isolated from locust tissues including brain, VNC, oviduct, and midgut was performed to determine the presence of mRNA encoding the receptor. The gcr3 transcript estimated to be 4.3 kb long, is longer than the cDNA sequence that has been determined, indicating that the transcript may have an extensive 5′ non-coding region. The mRNA was detected

TABLE 1. Percentage Amino Acid Sequence Similarity of the Locust Octopamine/Tyramine Receptor With Other Biogenic Amine Receptors as Determined by Multiple Sequence Alignment Using the CLUSTAL W Program (Higgins and Sharp, 1988)

Receptor	Species	% similarity	Reference
Tyramine	*L. migratoria*	97	Vanden Broeck et al. (1995)
Tyramine	*A. mellifera*	59	Blenau et al. (2000)
Tyramine	*D. melanogaster*	52	Saudou et al. (1990)
Tyramine	*C. elegans*	35	Rex and Komuniecki (2002)
Octopamine	*D. melanogaster*	52	Arakawa et al. (1990)
Octopamine	*M. brassicae*	52	Grosmaitre et al. (2001)
Octopamine	*B. microplus*	51	Baxter and Barker (1999)
Octopamine	*H. virescens*	51	von Nickisch-Rosenegk et al. (1996)
Octopamine	*B. mori*	50	von Nickisch-Rosenegk et al. (1996)
Serotonin	*L. stagnalis*	30	Sugamori et al. (1993)
Serotonin	*A. californica*	30	Barbas et al. (2002)

in all tissues examined indicating a broad range of distribution for the receptor in various locust tissues including both nervous and visceral muscle tissues (Fig. 4).

Southern Blot Analysis of the Receptor Gene

Southern blot analysis was carried out using genomic DNA isolated from individual locusts. For this purpose, locust genomic DNA was digested with *Eco* RI and *Hind* III restriction endonucleases and hybridized to a gcr3 probe (Fig. 5). Digests with either *Eco* RI or *Hind* III restriction endonucleases resulted in two bands. Since there is no recognition site for these enzymes within the full-length gcr3 receptor sequence and the gene is intronless, the results indicate that the probe utilized in these studies identifies two DNA fragments that share sequence similarity to the receptor probe.

Phylogenetic Relationship of the Cloned Receptor With Other Octopamine/Tyramine Receptors

Phylogenetic analysis of the cloned receptor and octopamine/tyramine receptors from other invertebrates using the complete amino acid sequences suggest that relatedness at the primary sequence level among octopamine/tyramine receptors can be used to group them into three major groups (Fig. 6). Octopamine receptors from *Aplysia californica* (OAR-Ac) and *A. kurodai* (OAR-Ak) form the first

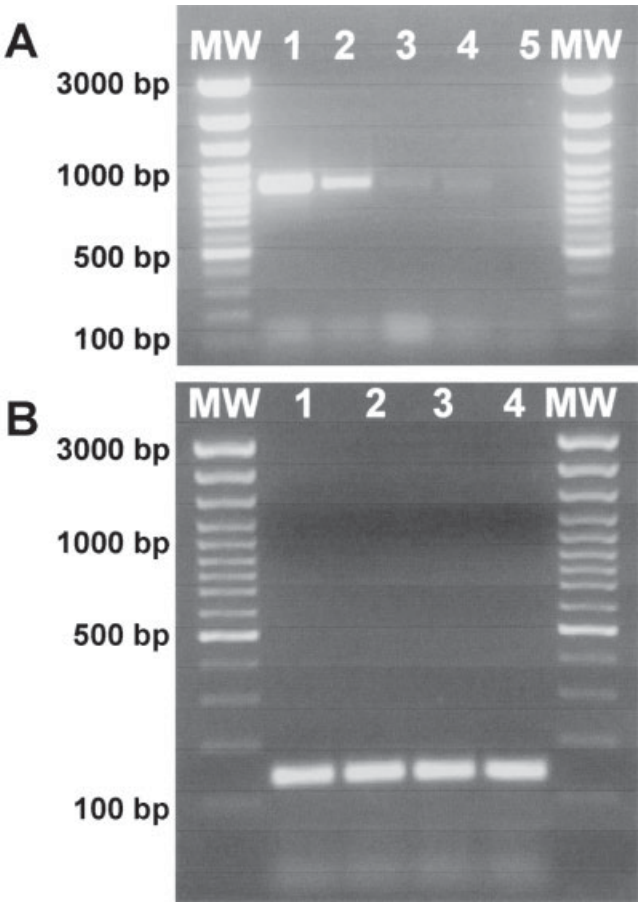

Fig. 3. Tissue-specific expression of the locust octopamine/tyramine receptor examined by RT-PCR. Locust tissues including (**lane 1**) brain, (**lane 2**) VNC, (**lane 3**) oviduct, and (**lane 4**) midgut were used to generate RNA for RT-PCR as described in Materials and Methods. A no-template negative control (**lane 5**) ensuring the specificity of the amplicons did not generate any products (**A**). PCR reactions without reverse transcription step were also performed. PCR primers designed on the gcr3 cDNA sequence were used in RT-PCR reaction. Positive control products were amplified using primer pairs based on the histone H2B gene (180 bp) and numbers indicate the source of RNAs from locust tissues as above (**B**). Amplified products were visualized under UV illumination after staining with ethidium bromide. The position of the molecular weight markers (MW, GeneRuler 100 bp DNA Ladder Plus, MBI, Fermentas, Hanover, MD) are indicated on both sides.

group in this analysis. Octopamine receptors from *Bombyx mori* (OAR-Bm), *Heliothis virescens* (OAR-Hv), *L. stagnalis* (OAR-Ls2) and tyramine receptors from *L. migratoria* (Tyr-Loc1 (gcr1) and Tyr-Loc2

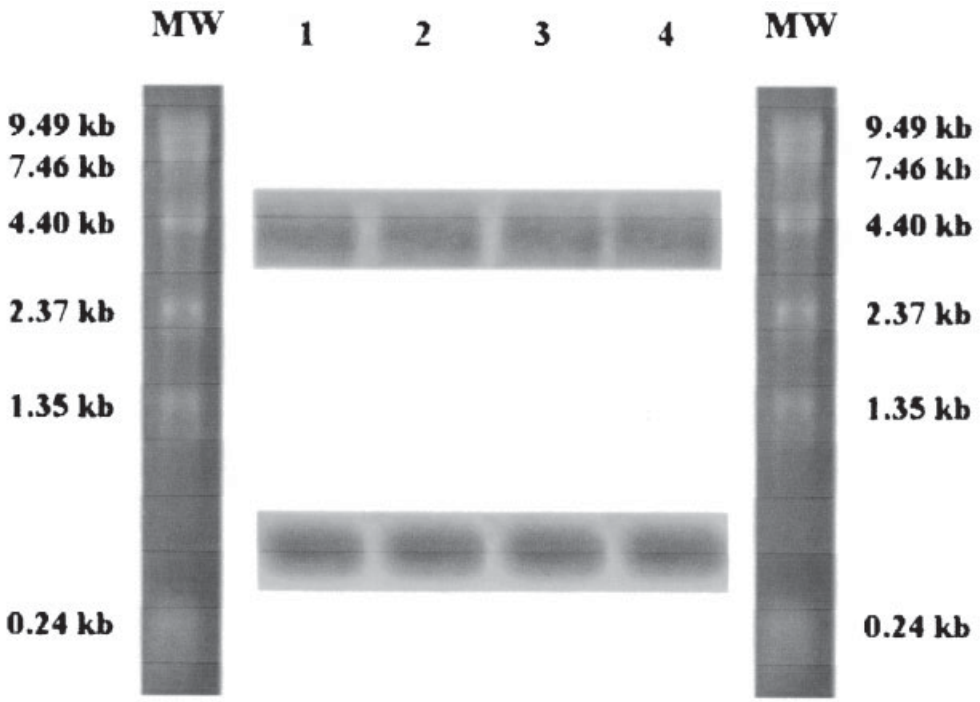

Fig. 4. Tissue distribution of locust octopamine/tyramine receptor determined by Northern blot analysis. A Northern blot of 20 μg of total RNA from locust tissues including (**lane 1**) brain, (**lane 2**) ventral nerve cord (VNC), (**lane 3**) oviduct, and (**lane 4**) midgut probed with a probe based on the sequence of the cloned receptor cDNA (gcr3) as described in Materials and Methods. This blot was exposed to a Kodak (Rochester, NY) BioMax film for 2 days at -80°C. After stripping, each blot was hybridized with a histone H2B cDNA probe for normalization, shown below the corresponding blot. The position of the RNA molecular weight markers (MW, Invitrogen, San Diego, CA) are indicated on both sides.

(gcr3), *A. mellifera* (Tyr-Am), *C. elegans* (Tyr-Ce), and *D. melanogaster* (Tyr-Dm) form the second major group. The cloned tyramine receptor (gcr3, Tyr-Loc2) clusters with the first tyramine receptor from *L. migratoria* (gcr1 or Tyr-Loc1, Vanden Broeck et al., 1995) exclusively within this group. Finally, octopamine receptors from *D. melanogaster* (OAR-Dm1 or OAMB and OAR-Dm2) and *L. stagnalis* (OAR-Ls1) form the third distinct group. Interestingly, two of the major groups are comprised entirely of octopamine receptors. This is in contrast to the second major group, which is comprised of both receptor types. However, it is worth noting that the octopamine receptors from *B. mori* and *H. virescens* (von Nickisch-Rosenegk et al., 1996) that cluster within the mostly tyramine receptors' group could now be considered as tyramine receptors based on the new evidence (Ohta et al., 2003). A serotonin receptor from *D. melanogaster* (5-HT2A-Dm) has been used as an out-group in this analysis.

DISCUSSION

The present study identifies a GPCR that is expressed in the *L. migratoria* nervous system and in the visceral muscle tissues, oviduct, and midgut. The presence of certain amino acid residues in the receptor sequence is important in transducing the extracellular response to effector enzymes and interaction of the receptor with G proteins. In addition to seven transmembrane domains common to all GPCRs, this receptor displays a series of conserved signature residues specific to octopamine/tyramine receptors in insects. There are a number of cysteine residues in the cloned receptor (C_{124},

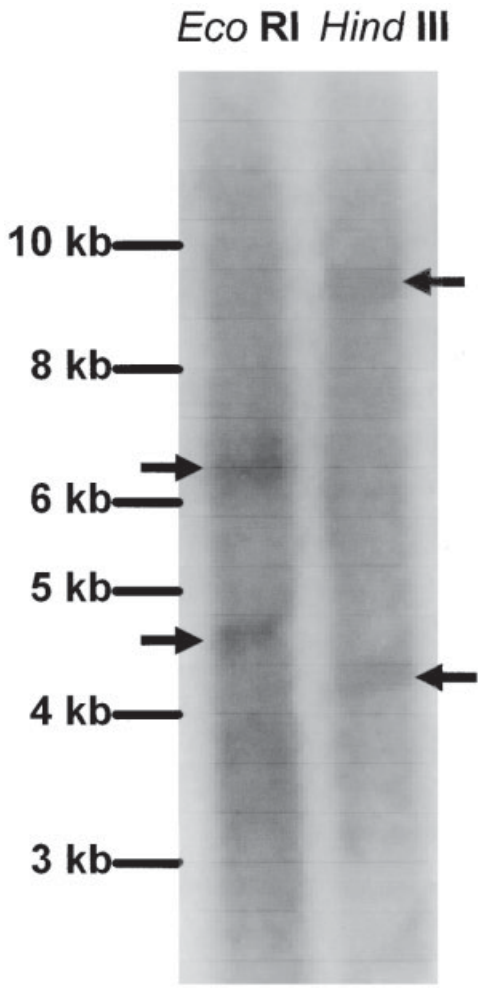

Fig. 5. Southern blot hybridization of a probe based on the putative octopamine/tyramine receptor (gcr3) to *Eco* RI and *Hind* III digested locust genomic DNAs. A Southern blot of 30 μg locust genomic DNAs digested with *Eco* RI and *Hind* III restriction endonucleases was probed with a probe amplified from the full-length receptor cDNA (gcr3) as described in Materials and Methods section. This blot was exposed to a Kodak (Rochester, NY) BioMax film for 72 h at –80°C. Arrows indicate two distinct bands in each gel lane. The position of a molecular weight marker is indicated on the side.

$_{203}$) believed to be important in maintaining disulfide bridges, which are essential for the stability of the receptor protein (Dixon et al., 1987). A sequence motif of L_{93}-X-X-X-D found in the TM II of the cloned receptor is also a conserved motif common to GPCRs (Spence et al., 1998). The aspartate residue in this motif is believed to play a role in sodium sensitivity (Schetz and Sibley, 1999). Furthermore, in the cloned receptor, two conserved motifs of D_{148}RY at the carboxy-terminal of TM III, and N_{461}-X-X-X-Y at the TM VII are among highly conserved amino acid residues in β-adrenergic receptors (Fraser et al., 1988; Oliveira et al., 1999). The cloned receptor also contains serine residues ($S_{215, 216, 219}$) in TM5 with known spacing patterns in a signature sequence motif of S_{215}SLVS that might be involved in maintaining a hydrogen bond between the serine residue and hydroxyl group of the benzoyl ring of tyramine (Blenau et al., 2000). In addition, a number of threonine (T_{228}) and serine ($S_{215, 216, 219}$) residues at TM V of this receptor are among the conserved residues in amine receptors (Bouchard et al., 2003). A conserved motif of F_{419}-X-X-X-W-L-P followed by two phenylalanine residues ($F_{426, 427}$) at TM VI of the cloned receptor is also commonly found in biogenic amine receptors (Bouchard et al., 2003).

Genbank records show the existence of two tyramine receptor genes from *L. migratoria* (gcr1 and gcr2, accession nos. X69520 and X69521, respectively). These two sequences exhibit sequence identities of 98 and 99.59% at the nucleotide and amino acid levels, respectively. Only the gcr1 sequence has been used in functional expression experiments (Vanden Broeck et al., 1995). The cloned putative octopamine/tyramine receptor (gcr3) described in this report shows differences from gcr1 in at least 15 amino acid residues, and these differences are due to non-conservative substitutions in 10 cases. Physiological data on the action of tyramine suggests that there might be two tyramine receptor types in the locust oviduct (Donini and Lange, 2004). The first type is sensitive to low doses of tyramine, leading to the inhibition of adenylate cyclase activity and modulation of the excitatory junction potentials (EJPs). The second type is sensitive to high doses of tyramine, causing the inhibition of oviduct contraction with unknown signaling properties (Donini and Lange, 2004).

High-stringency Southern blot analyses on the locust genomic DNA using restriction endonucleases *Eco* RI and *Hind* III recognize two distinct gene band sequences in each case. Given that the cloned receptor transcript does not contain recog-

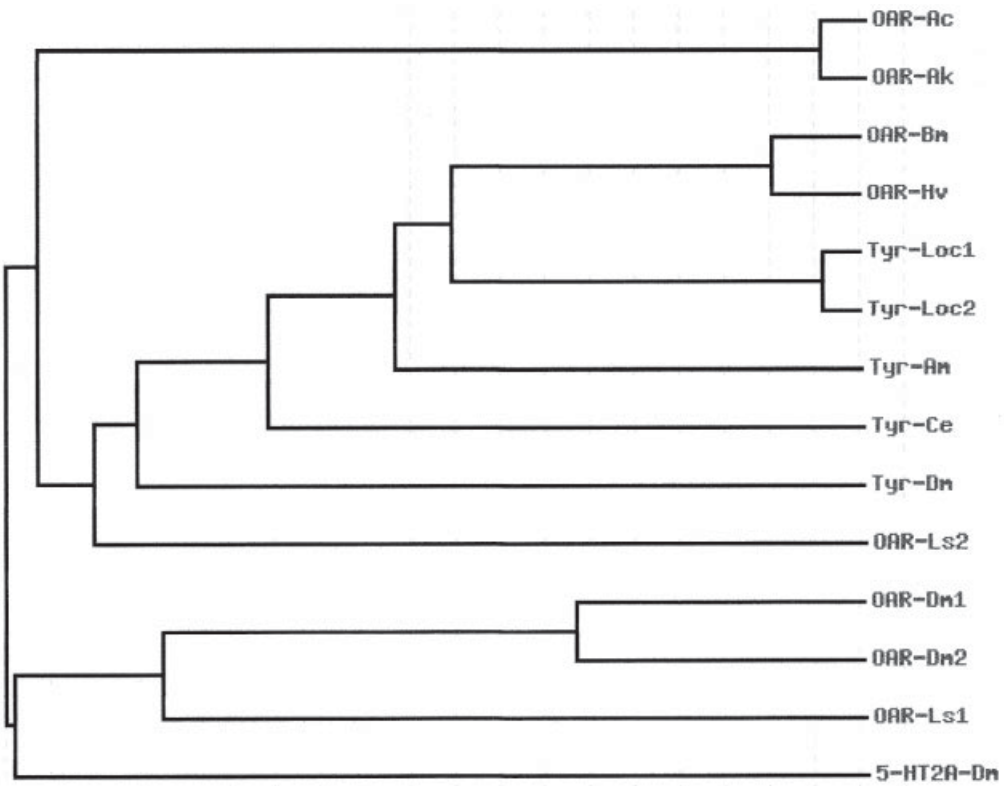

Fig. 6. Dendrogram shows the phylogenetic relationship of the cloned GPCR with other octopamine/tyramine receptors from invertebrates. Amino acid sequence alignment was carried out according to the CLUSTAL W algorithm and the dendrogram was constructed by Neighbour-Joining using Phylip program at http://www.genebee.msu.su/services/ phtree_reduced.html. The lengths of horizontal lines are inversely proportional to the sequence homology between two sequences or between groups of sequences. Abbreviations and GenBank accession numbers (#) for the receptor sequences are: OAR-Ac, octopamine receptor from the sea slug, *A. californica* (# AAF37686); OAR-Ak, octopamine receptor from the sea slug, *A. kurodai* (# AAF28802); OAR-Bm octopamine receptor from the silkworm, *B. mori* (# Q17232); OAR-Hv, octopamine receptor from the tobacco budworm, *H. virescens* (# Q25188); Tyr-Loc1 (gcr1), tyramine receptor from the locust, *L. migratoria* (# Q25321); Tyr-Loc2 (gcr3), putative octopamine/tyramine receptor cloned from the locust, *L. migratoria* in our studies; Tyr-Am, tyramine receptor from the honeybee *A. mellifera* (# AJ245824); Tyr-Ce, tyramine receptor from the nematode, *C. elegans* (# NM171978); Tyr-Dm, tyramine receptor from *D. melanogaster* (# S12004); OAR-Ls2, octopamine receptor from *L. stagnalis* (# O01670); OAR-Dm1, octopamine receptor from *D. melanogaster* (# AF065443); OAR-Dm2, octopamine receptor from *D. melanogaster* (# AJ007617); and OAR-Ls1 octopamine receptor from *L. stagnalis* (# O77408). 5-HT2A-Dm, serotonin receptor from *D. melanogaster* (# S19155) has been used as an out-group.

nition sites for either of these endonucleases, and the fact that this gene is intronless, the probe utilized in this analyses is identifying two distinct DNA fragments that share sequence similarity to the cloned receptor probe. It is also known that several genes are duplicated within the *L. migratoria* genome. Therefore, it is conceivable to expect several fragments hybridize to the probe. Furthermore, sequence alignment of the residues flanking TM5 and TM6 located in the intracellular loop 3 of the cloned receptor and tyramine receptors revealed a high degree of sequence conservation among these receptors. Amino acid residues flanking TM5 and TM6 are important for functional coupling of the receptors (Blenau and Baumann, 2003). Based upon this evidence, we suggest that there might

be at least two, if not more receptor subtypes and that the cloned receptor in the present study and the previously cloned tyramine receptor (Tyr-Loc; Vanden Broeck et al., 1995) may represent two tyramine receptor isoforms or subtypes. The presence of different receptor subtypes for biogenic amines has been indicated in insects and these receptor subtypes may bring about a variety of effects and activate different groups of second messenger systems (Roeder, 2002).

High-sequence similarity between the cloned receptor in our studies and gcr1 (Vanden Broeck et al., 1995) is not surprising. For instance, the amino acid sequences of two octopamine/tyramine receptors isolated from *B. mori* and *H. virescens* showed 96.3% homology to each other (von Nickisch-Rosenegk et al., 1996). More recently, the occurrence of an octopamine receptor (AmOA1) from the brain of the honeybee, *A. mellifera*, was examined by Western blot analysis using an antiserum raised against a peptide selected from the AmOA1 sequence (Farooqui et al., 2004). In addition to an expected band of 78 kDa, 5 additional bands were identified. There was no significant sequence identity between the peptide sequence used to generate the antiserum and other biogenic amine receptor sequences from honeybee or other insects. Furthermore, the expression of these proteins was reduced by AmOA1 dsRNA treatment. Based on the above evidence, it was concluded that these proteins may be different subtypes, different splice variants, and/or post-translational covalent modifications of the AmOA1 receptor (Farooqui et al., 2004).

Little expression data are available on gcr1 (Tyr-Loc1) to serve as a basis for comparison. The mRNA expression of the Tyr-Loc1 receptor has been detected in the locust nervous system with widespread distribution in the brain and the ventral nerve cord (Vanden Broeck et al., 1995). The tissue distribution of our cloned receptor mRNA indicates that the expression of the receptor transcript was the highest in brain tissue followed by VNC. The expression levels of receptor mRNA in oviduct and midgut tissues was relatively similar as examined under a semi-quantitative RT-PCR and Northern blot analysis. Furthermore, the expression of the receptor mRNA was detected by in situ hybridization in a small number of cells likely being endocrine-like cells in the locust midgut and in a small group of spindle-shape cells in the common oviduct (preliminary observations). Further work will be aimed at assigning a functional role for the receptor and its respective ligand in regulating/modulating physiological processes such as the peristaltic movements and the passage of food through the midgut lumen, digestive breakdown and uptake of dietary nutrients in midgut, and modulating physiological processes such as contraction and facilitating the process of egg deposition in the oviduct. It is also worth noting that the probe(s) utilized in expression experiments probably can not discriminate between gcr1, 2, or 3 in the above studies.

In conclusion, a novel GPCR from the locust visceral muscle has been cloned and shown to be expressed in the central nervous system and visceral muscle tissues. Sequence analysis strongly suggests that the receptor belongs to the biogenic amine receptor family and specifically to the octopamine/tyramine receptor group. Phylogenetic analysis supports this receptor as being a tyramine receptor. Our data suggest that this receptor is different from the previously identified locust tyramine receptor and may represent a sub-type of this family. The distribution of the receptor mRNA in locust tissues, particularly oviduct, verifies the physiological responsiveness of these tissues to octopamine and tyramine (Lange and Nykamp, 1996; Donini and Lange 2004). Together these studies suggest that the cloned receptor and its possible ligand, tyramine, may assume important functions in mediating physiological processes in locust visceral muscles, oviduct, and midgut.

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