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REVIEW

Peter D. Evans · Braudel Maqueira

Insect octopamine receptors: a new classification scheme based on studies of cloned *Drosophila* G-protein coupled receptors

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Summary Insect octopamine receptors are G-protein coupled receptors. They can be coupled to second messenger pathways to mediate either increases or decreases in intracellular cyclic AMP levels or the generation of intracellular calcium signals. Insect octopamine receptors were originally classified on the basis of second messenger changes induced in a variety of intact tissue preparations. Such a classification system is problematic if more than one receptor subtype is present in the same tissue preparation. Recent progress on the cloning and characterization in heterologous cell systems of octopamine receptors from *Drosophila* and other insects is reviewed. A new classification system for insect octopamine receptors into "α-adrenergic-like octopamine receptors (OctαRs)", "β-adrenergic-like octopamine receptors (OctβRs)" and "octopamine/tyramine (or tyraminergic) receptors" is proposed based on their similarities in structure and in signalling properties with vertebrate adrenergic receptors. In future studies on the molecular basis of octopamine signalling in individual tissues it will be essential to identify the relative expression levels of the different classes of octopamine receptor present. In addition, it will be essential to identify if co-expression of such receptors in the same cells results in the formation of oligomeric receptors with specific emergent pharmacological and signalling properties.

Keywords Insects · Octopamine receptors · Cloned *Drosophila* receptors · G-protein coupled receptors · cyclic AMP

P. D. Evans (✉) · B. Maqueira
The Inositide Laboratory, The Babraham Institute,
Cambridge, CB2 4AT, UK
E-mail: peter.evans@bbsrc.ac.uk
Tel.: +44-1223-496406
Fax: +44-1223-496509

Introduction

Octopamine is one of the major biogenic amines present in insects and it carries out many of the functional roles associated with both noradrenaline and adrenaline in vertebrates (Evans 1980; David and Coulon 1985; Roeder 1999, 2005; Roeder et al. 2003). Thus, octopamine can function as a circulatory hormone that is elevated under stressful conditions and can control lipid and carbohydrate metabolism. It can also function as an important neuromodulator being released from specific octopaminergic neurons at both peripheral neuromuscular junctions, and at central synapses in insects, controlling arousal levels and the function of central pattern generators, such as those involved in the generation of flight activity. Octopamine has also been shown to have important roles in memory and learning processes in both *Drosophila* and the honey bee, *Apis mellifera*. Octopamine also plays an important role in the control of egg laying in insects and acts as a transmitter initiating light production in firefly light organs.

The actions of octopamine are thought to be mainly mediated by interactions with G-protein coupled receptors which are coupled to either increases or decreases in intracellular levels of the second messenger cyclic AMP, or to the generation of intracellular calcium signals. Studies on insect octopamine receptors have been reviewed extensively (Evans 1993; Evans and Robb 1993; Roeder 1994, 2005; Roeder et al. 1995; Blenau and Baumann 2001; Vanden Broeck 2001).

The present review will outline the original classification scheme for insect octopamine receptors, which was based on pharmacological and signalling differences observed after octopamine application to whole tissue preparations. It will then review recent data on the cloning and heterologous expression of a range of *Drosophila* octopamine receptors. Finally, it will use the information from such studies on cloned receptors to propose a new classification scheme for insect octopamine receptors, which emphazises their structural and

signalling similarities to vertebrate adrenergic receptor subtypes.

Original octopamine receptor classification scheme based on whole tissue responses

An initial classification scheme for insect octopamine receptors was suggested by Evans (1981). This scheme was based on results obtained from a pharmacological characterization of the multiple octopamine receptors which were found to underlie the octopaminergic responses obtained from the extensor-tibiae muscle preparation of the hindleg of the locust. It was proposed to designate the octopamine receptors responsible for the slowing of the myogenic rhythm in this preparation the $OCTOPAMINE_1$ receptors. These receptors were subsequently shown to be likely to mediate their effects via a mechanism that elevated intracellular calcium levels (Evans 1984a). The receptors responsible for the octopaminergic modulation of slow motorneurone mediated neuromuscular transmission were designated the $OCTOPAMINE_2$ receptors. The latter class was subdivided into the $OCTOPAMINE_{2A}$ receptors on the presynaptic terminals of the slow motorneurone, mediating an increase in transmitter release, and the $OCTOPAMINE_{2B}$ receptors located postsynaptically on the muscle, mediating an increase in the relaxation rate of tension. Later studies showed that both the 2A and 2B receptor subtypes mediated their actions by increasing the levels of the second messenger cyclic AMP due to an activation of adenylyl cyclase activity (Evans 1984b, c, 1987).

The physiological distinction between the $OCTOPAMINE_1$ and $OCTOPAMINE_2$ receptor subtypes was supported by a clear pharmacological distinction when examined using a range of different agonists and antagonists. Thus, metoclopramide blocked $OCTOPAMINE_2$ but not $OCTOPAMINE_1$ receptors and the converse was true for yohimbine. In addition, clonidine was a much better agonist at $OCTOPAMINE_1$ receptors than naphazoline, and naphazoline was much better than tolazoline at $OCTOPAMINE_2$ receptors. However, the pharmacological differences between the 2A and 2B receptor subtypes were less dramatic. When this classification scheme was reviewed by Evans and Robb (1993), they suggested that the latter differences might be the result of tissue specific variants of a single subclass of receptor. This latter review also noted that in a much wider range of responses to octopamine, in a range of different insect tissues, that the basic distinction between the $OCTOPAMINE_1$ and the $OCTOPAMINE_2$ receptor subtypes working via different second messenger systems still seemed to be valid. However, although there were basic underlying similarities between the pharmacological profiles of $OCTOPAMINE_2$ receptors obtained in different insect tissues, there was likely to be an element of tissue specific variation for receptors of this class, which was larger for antagonists than for agonists. This review also concluded that an attempt to classify certain neuronal octopamine receptors in the central nervous systems of locusts and other insects, as a distinct central neuronal $OCTOPAMINE_3$ class of receptors (Roeder 1992), was not justified pharmacologically in view of their general similarity to other members of the $OCTOPAMINE_2$ subclass. In addition, different pharmacological profiles for this class were obtained by different groups of workers using different techniques on the same tissues. It was thus suggested that these receptors might be referred to as the $OCTOPAMINE_{2C}$subclass. The review of Evans and Robb (1993) concluded that due to the likely presence of multiple receptors for octopamine being present in complex tissues, together with other variable tissue specific factors, such as differential metabolism, that whole tissue pharmacological studies were not appropriate to reveal the true pharmacology of individual octopamine receptor subclasses. It further suggested that the resolution of the definitive classification of insect octopamine receptors must await the cloning of the genes encoding these receptors and a determination of their pharmacological characterization in appropriate heterologous expression systems.

Studies on cloned octopamine receptors

Octopamine/tyramine receptors or tyramine receptors?

The first potential insect octopamine receptor to be cloned from *Drosophila* (OAR_DROME, CG7485) (Arakawa et al. 1990; Saudou et al. 1990) showed a substantial pharmacological and structural homology with vertebrate α-adrenergic receptors. When expressed in Chinese hamster ovary (CHO) cells it could be activated to induce a reduction in cyclic AMP levels and the generation of an intracellular calcium signal (Robb et al. 1994). However, in both binding studies and in studies on the inhibition of adenylyl cyclase activity, tyramine, the metabolic precursor of octopamine, was much more potent than octopamine at stimulating the receptor. This lead to suggestions that this might actually be a specific tyraminergic receptor (Saudou et al. 1990; see also Nagaya et al. 2002; Roeder et al. 2003; Roeder 2004). However, a comparison of the relative abilities of octopamine and tyramine to activate different second messengers through this receptor demonstrated that the receptor exhibited "agonist-specific coupling" (Robb et al. 1994) or "agonist trafficking". This suggests that different agonists can induce different conformations of the receptor which can couple differentially to different second messenger systems (see Evans et al. 1995; Kenakin 1995). In this case tyramine preferentially coupled the receptor to the inhibition of adenylyl cyclase, whilst octopamine was more potent at coupling the receptor to the induction of a calcium signal. Thus, it would appear that this receptor might function as a dual octopamine/tyramine receptor depending upon the

neuromodulatory or neurotransmitter input that it receives in any particular location in the *Drosophila* nervous system where it has a widespread distribution (Hannan and Hall 1996). Interestingly, the *hono* mutation of this receptor causes defects in olfactory behaviour (Kutsukake et al. 2000).

Since the initial cloning of this *Drosophila* octopamine/tyramine or tyramine receptor numerous species homologues have been cloned from other insects and all show a preference for tyramine over octopamine in the inhibition of adenylyl cyclase activity (e.g. locust, Vanden Broeck et al. 1995; *Heliothis virescens*, von Nickisch-Rosenegk et al. 1996; *Bombyx mori*, von Nickisch-Rosenegk et al. 1996; *A. mellifera*, Blenau et al. 2000; Ohta et al. 2003; *Mamestra brassicae*, Grosmaitre and Jacquin-Joly 2001; *Papilio xuthus*, Ono and Yoshikawa 2004).

The suggestion that members of the above class of receptors may function as specific tyraminergic receptors is supported by evidence which suggests that tyramine and octopamine may have different physiological actions on some preparations (e.g. Saraswati et al. 2003; Cole et al. 2005) and by immunocytochemical evidence for tyramine containing neurons which apparently do not express octopamine (Monastirioti et al. 1996; Nagaya et al. 2002). In addition, recent evidence for the existence of specific tyraminergic neurones has also been presented in *Caenorhabditis elegans* (Alkema et al. 2005). Here the tyraminergic neurones play a specific role in the inhibition of egg laying, the modulation of reversal behaviour and the suppression of head oscillations in response to anterior touch. However, a definitive designation of the above insect receptors awaits the demonstration that they are selectively activated by tyramine in vivo in locations where tyramine is released selectively in preference to octopamine from identified neurones.

Mushroom body octopamine receptor, OAMB

The first insect octopamine receptor to be cloned from *Drosophila* that showed a preference for octopamine over tyramine, was the octopamine receptor from the mushroom bodies (OAMB or CG3856; Han et al. 1998). This receptor when activated was claimed to be coupled to an elevation of cyclic AMP levels when expressed in *Drosophila* S2 cells and to be coupled both to an elevation of cyclic AMP levels and to the generation of an intracellular calcium signal when expressed in HEK293 cells. However, the relationship and the relative time courses of the generation of the latter calcium signal, and the stimulation of adenylyl cyclase activity, were not investigated. However, homologs of OAMB have now been cloned from a number of other insects (Blenau and Baumann 2001) and when the homologous receptor (Pa oa$_1$) from the cockroach, *Periplaneta americana*, was cloned and expressed in HEK293 cells, experiments with the intracellular calcium buffering agent, BAPTA, suggested that the two responses were generated independently (Bischof and Enan 2004). Since the insect mushroom bodies have been shown to be essential for various learning and memory tasks in insects (Davis 1996), and since octopamine has been shown to stimulate adenylyl cyclase activity in fly head homogenates (Dudai and Zvi 1984), it was suggested that OAMB might have an important function in synaptic modulation and underlie behavioural plasticity (Han et al. 1998; Lee et al. 2003). However, more recently, Balfanz et al. (2005) have suggested that the cyclic AMP increases mediated by OAMB are relatively small and that this receptor is more likely to mediate its physiological effects via increases in intracellular calcium. This suggests that OAMB might be better classified as an OCTOPAMINE$_1$ receptor mediating its effects via intracellular calcium than as an OCTOPAMINE$_2$ receptor acting via adenylyl cyclase activation. This raises the question of whether OAMB is actually the major receptor responsible for the generation of cyclic AMP responses in the *Drosophila* brain and whether it actually has a major role in the octopaminergic modulation of insect memory and learning (see below for other octopamine receptor candidates which may mediate these physiological effects).

OAMB was initially suggested to be specifically expressed in *Drosophila* brains, and in particular in mushroom bodies and ellipsoid bodies, with lower expression levels in other brain regions and with no significant expression in other tissues (Han et al. 1998). However, more sensitive expression methods have shown that it is also expressed in the thoracic and abdominal ganglia, mature eggs, oviducts and other regions of the reproductive system (Lee et al. 2003). The latter study also showed that OAMB was expressed as two different transcripts, OAMB-K3 and OAMB-AS, produced by alternative splicing of the last exon which leads to divergent sequences from the putative third intracellular loop. However, both isoforms appear to be normally present in all tissues where the gene is expressed. Lee et al. (2003) also described a set of *Drosophila* mutants containing various deletions in the *oamb* locus. These mutants showed normal courtship and copulation but were impaired in ovulation with many mature eggs being retained in their ovaries. They also suggested that OAMB was required in the body, but not in the brain, for normal female egg laying to occur. It would be of much interest to know if these *oamb* mutant strains demonstrate any defects in learning and memory tasks, since this might help to address the question of the role of OAMB in such processes.

Identification of a novel family of *Drosophila* β-adrenergic-like octopamine receptors

The lack of any obvious cloned *Drosophila* GPCRs that could be candidates for the adenylyl cyclase selectively coupled OCTOPAMINE$_2$ subclass of insect octopamine receptors lead us to examine the orphan *Drosophila* GPCRs that had been predicted on structural grounds

to possibly be aminergic receptors (Brody and Cravchik 2000). We were attracted to four sequences (CG6919, CG6989, CG7078 and CG18314) which showed a homology to vertebrate β-adrenergic receptor sequences (Maqueira et al. 2004, 2005; Srivastava et al. 2004, 2005) when compared against other cloned GPCRs in the Swissprot database using PSI-BLAST and against a set of profile Hidden Markov Models using HMMER (Eddy 1998). Vertebrate β-adrenergic receptors mediate many, but not all, of their actions through a specific coupling to adenylyl cyclase (Pierce et al. 2002). In addition, the two cloned octopamine receptors from *Aplysia*, which also share a structural homology to vertebrate β-adrenergic receptors, also couple exclusively to adenylyl cyclase and do not generate calcium signals (Chang et al. 2000). We have thus cloned and expressed full length GPCRs corresponding all four of the above putative *Drosophila* orphan receptors and begun a characterization of their properties to identify their cognate ligands (Maqueira et al. 2004, 2005; Srivastava et al. 2004, 2005).

CG18314 encodes a GPCR we have named the *Drosophila melanogaster* Dopamine/Ecdysteroid receptor (DmDopEcR) (Srivastava et al. 2004, 2005). This receptor can be activated by both the catecholamine, dopamine, and by the insect ecdysteroids, ecdysone and 20-hydroxyecdysone. The receptor exhibits "agonist-specific coupling" (agonist trafficking) (Evans et al. 1995; Kennakin 1995) whereby different agonists couple the receptor differentially to different second messenger pathways by presumably inducing different conformations of the receptor. Thus, in expression systems dopamine couples the receptor to an elevation of intracellular cyclic AMP levels and to the activation of the phosphoinositide 3-kinase pathway. Conversely, ecdysone and 20-hydroxyecdysone show a higher affinity for the receptor than dopamine in binding studies and can inhibit the effects of dopamine, as well as coupling the receptor to a rapid activation of the mitogen-activated protein kinase pathway. In functional terms DmDopEcR is likely to act as a cell-surface GPCR that may be responsible for some of the rapid non-genomic actions of ecdysteroids (Tomaschko 1999; Thummel and Chory 2002), during both embryonic and larval development, and in signalling in the mature adult *Drosophila* nervous system. We have identified likely species homologues of DmDopEcR from *Drosophila pseudoobscura* (EAL30129; 97% identical and 98% similarity), *Anopheles gambiae* (XM_315694; 75% identity and 88% similarity) and *A. mellifera* (XM_396491; 71% identity and 83% similarity), as well as from *C. elegans* (NP_510580; 34% identity and 57% similarity) and *C. briggsae* (CAE63350) (Evans and Srivastava, unpublished data).

CG6919, CG6989 and CG7078 turn out to encode a closely related family of novel β-adrenergic-like insect octopamine receptors (Maqueira et al. 2004, 2005) located close together on the right arm of *Drosophila* chromosome 3 (The Flybase Consortium 2003; http://flybase.bio.indiana.edu/). The receptors are preferen-

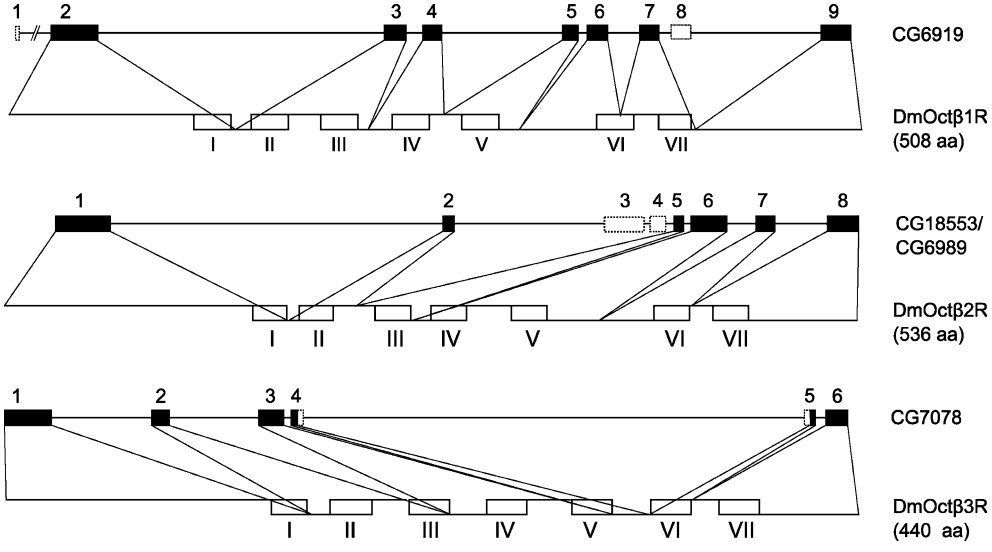

Fig. 1 The exon structure of the CG6919, CG6989 and CG7078 clones. The sequences of three independent clones for each gene were assembled using the Staden package and the exon structure diagrams created with PERL scripts based on the analysis generated with CHEXONS software. For each gene the top line represents the exon structure and the bottom line represents the predicted topologies of the receptors where the transmembrane regions are indicated as I, II, III, IV, V, VI and VII. CG6919 is composed of seven exons and encodes a full seven TM receptor. The alternatively transcribed clone CG6919-B (not shown) encodes an extra exon (*white box*) between the initially predicted exons 7 and 8 (which now becomes exon 9) and has a much reduced C-terminal. The original prediction for CG6989 only encoded four TMs and a full seven TM receptor can be found by extending the annotation into the adjacent annotation CG18553. The full length clone CG6898 is composed of six exons, but does not include exons 3 and 4 which are only found in alternatively spliced shorter forms of the receptor. The original annotation for CG7078 appears to be partially correct and the full length clone CG7078 is composed of six exons. The alternatively transcribed clones CG7078-B, -C and –D (not shown) each encode products with only five TMs. (Modified from Maqueria et al. 2005)

tially expressed in *Drosophila* heads suggesting a putative role in the modulation of neuronal activity. The full length 7TM receptor for CG6919 (CG6919A) is encoded by seven exons (Fig. 1) and corresponds to the original Flybase annotation (The Flybase Consortium, 2003; http://flybase.bio.indiana.edu/). We also identified an alternatively spliced variant of the receptor (CG6919B) with an extra exon which results in a C-terminally truncated version of the receptor. The original Flybase annotation for CG6989 only encoded a putative protein with five transmembrane (TM) regions. We found that the two N-terminal TM regions of the full length 7TM sequence for CG6989 were encoded in the adjacent annotation CG18553. The original annotation of CG7078 was recently split into three separate annotations (CG31348, CG31351 and CG31350), none of which encoded a full 7TM receptor. However, we found that the original annotation was partially correct and that we could identify a full length receptor (CG7078-A) encoded by six exons. We also identified a number of shorter alternative transcripts for this gene (CG7078B-D) which encode putative proteins with only five TMs. The functional roles of such short transcripts are not known at the present time, but similar transcripts from other receptors (e.g. the vasopressin V_2receptor and the chemokine receptor, CXCR3) have been suggested to both be capable of carrying out some signalling activities and of controlling the cell surface expression of the full length receptors (Ehlert et al. 2004; Sarmiento et al. 2004).

We stably expressed all three receptors in Chinese hamster ovary cells and have characterized their pharmacology and second messenger coupling abilities (Maqueira et al. 2004, 2005). All three receptors are preferentially activated by octopamine, rather than tyramine, to produce significant increases in intracellular cyclic AMP levels (EC_{50}s: CG6919, octopamine 5.56×10^{-9} M and tyramine 2.44×10^{-7} M; CG6989, octopamine 1.53×10^{-8} M and tyramine 2.02×10^{-7} M; CG7078, octopamine 1.40×10^{-8} M and tyramine 3.78×10^{-7} M). In addition, none of the receptors demonstrated any changes in intracellular calcium levels when exposed to octopamine at concentrations up to 1 μM. Similar results were also obtained by Balfanz et al. (2005) for only CG6919 in a preliminary study on this receptor. Further, studies will be required to determine if the receptors couple to additional second messenger pathways.

In pharmacological terms, each of the receptors showed a distinct pattern of responses to a range of agonists and antagonists. Thus, for the agonists tested the rank order of potency was: for CG6919, Naphazoline > Octopamine > Clonidine > Tolazoline; for CG6989, Octopamine > Naphazoline > Clonidine > Tolazoline; and for CG7078, Naphazoline > Octopamine > Tolazoline > Clonidine. The rank order of potency for the antagonists was: for CG6919, Mianserin >> Cyproheptadine > Phentolamine = Promethazine > Propranolol; for CG6989, Mianserin > Phentolamine; and for CG7078, Mianserin > Cyproheptadine > Phentolamine = Promethazine > Metoclopramide > Chlorpromazine. All three receptors are also capable of generating significant cyclic AMP increases in response to the catecholamines, adrenaline and noradrenaline, which might provide a signalling mechanism for the small amounts of noradrenaline reported to be present in the nervous systems of some insect species (Evans 1980). However, the classical β-adrenergic receptor agents, such as the agonist, isoproterenol, and the antagonist, propranolol, only had very weak effects on the responses of all three receptors. Another unusual property of all three newly cloned receptors was that they were all activated differentially by phentolamine, a traditional α-adrenergic antagonist. In addition, phentolamine reduced the octopamine stimulated increase in cyclic AMP levels generated by all three receptors. Although the new group of β-adrenergic-like octopamine receptors shows structural similarities to vertebrate β-adrenergic receptors, they can clearly be distinguished on pharmacological grounds which would make them important novel target sites for insect control.

The selective and substantive activation of the adenylyl cyclase pathway makes it likely that the novel β-adrenergic-like octopamine receptors, CG6919, CG6989 and CG7078, are the receptors that underlie the so called $OCTOPAMINE_2$—type cyclic AMP mediated effects, including those of the presumed "neuronal" $OCTOPAMINE_{2C}$ subclass (also referred to as the $OCTOPAMINE_3$ subclass, Roeder 1992; Roeder and Nathanson 1993) previously described in intact insect tissues (Evans 1981, 1993; Evans and Robb 1993). At present the relative contribution of the *Drosophila* mushroom body octopamine receptor, OAMB (Han et al. 1998) to the octopamine mediated increases in neuronal cyclic AMP levels is not clear, but would seem likely to be very low. Thus, the suggestions that insects express only one classical octopamine receptor, although expressed in different splice variants, and that most actions of octopamine are mediated through one neuronal type of receptor in the nervous system that has identical pharmacological features in different preparations (Roeder at al. 2003) are unlikely to be true. The fact that the pharmacological responses of the individual receptors of the new class of *Drosophila* β-adrenergic-like octopamine receptors do not correspond exactly with any of the published pharmacology of octopamine responses from the various studies on intact tissues (Evans and Robb 1993), suggests that the responses obtained in intact tissues will depend on the relative expression levels of these novel receptors and OAMB in individual tissues.

New classification scheme for insect octopamine receptors

The studies on the individual cloned *Drosophila* octopamine receptors reviewed earlier, suggest that it might now be appropriate to revise the initial classification

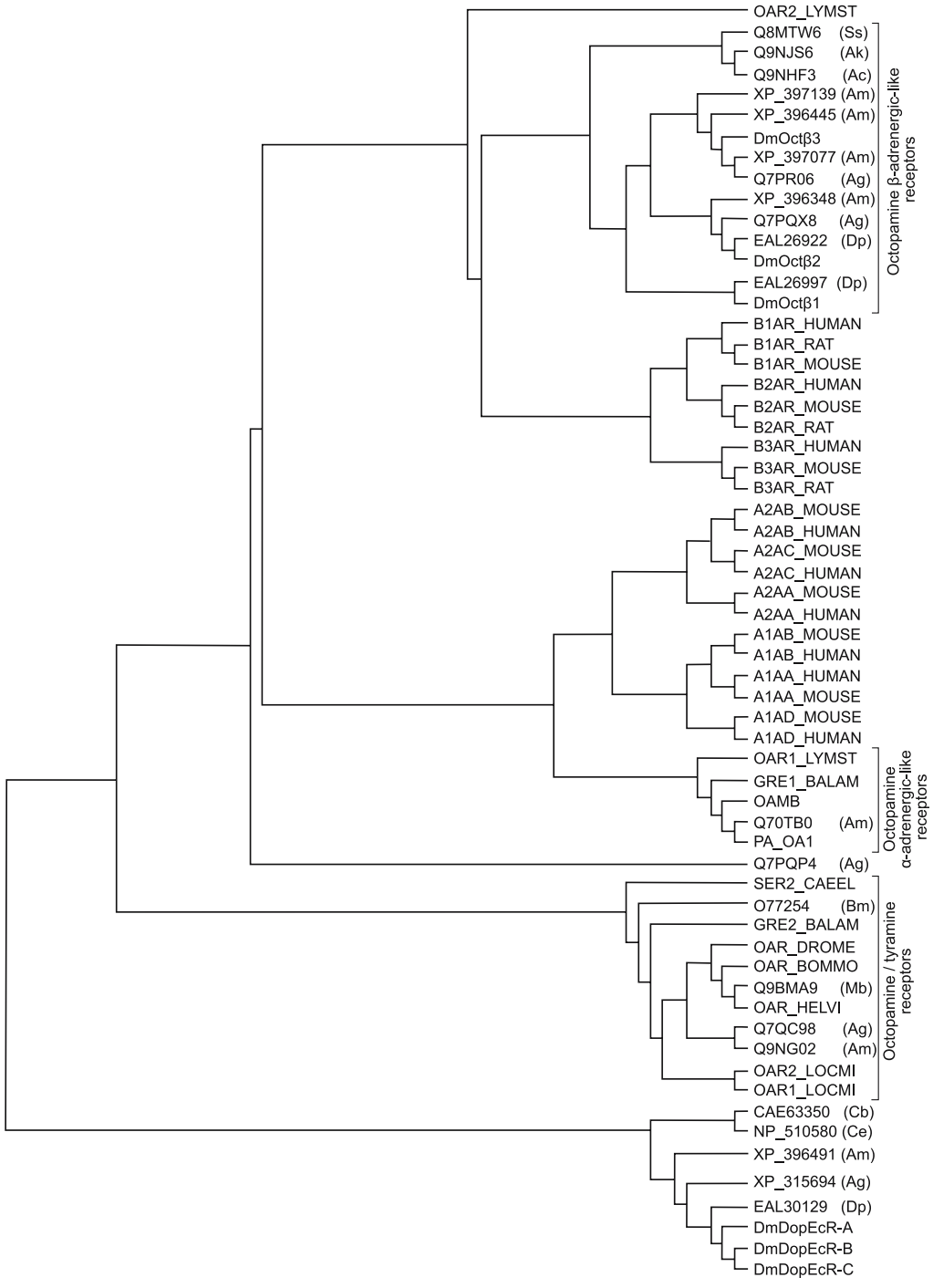

Fig. 2 Phylogenetic tree comparison of representative members of the different adrenoceptor families, the *β*-adrenergic-like and the α-adrenergic-like octopamine receptors and the octopamine/tyramine families together with several putative predicted insect biogenic amine receptor sequences. The CG6919 (DmOctβ_1), CG6989 (DmOctβ_2) and CG7078 (DmOctβ_3) sequences cluster with the *β*-adrenergic receptors and with a number of orphan receptors from other insects and the octopamine receptors from *Aplysia*. The sequences were aligned with ClustalW and the phylogenetic tree calculated using the Phylip package (bootstrap = 1,000, Fitch-Margoliash method). *Dm Drosophila melanogaster*; *Dp Drosophila pseudoobscura*; *Ag Anopheles gambiae*; *Ap Apis mellifera*; *Pa Periplaneta americana*; *Mb Mamestra brassicae*; *Bm Boophilus microplus*; *Ss Spisula solidissima*; *Ac Aplysia californica*; *Ak Aplysia kurodai*; *Bm Bombyx mori*; *Ce Caenorhabditis elegans*; *Cb Caenorhabditis briggsae* (modified from Maqueira et al. 2005)

scheme for insect octopamine receptors. In view of the structural and signalling similarities between CG6919, CG6989, CG7078 and *β*-adrenergic receptors, we have proposed that this new class of receptors should be known as the insect *β*-adrenergic-like octopamine receptors (Oct*β*Rs). We have proposed the following names for the *D. melanogaster* receptors: DmOct*β*1R (CG6919), DmOct*β*2R (CG6989) and DmOct*β*3R

(CG7078) (Maqueira et al. 2005). A comparison of the sequences of the DmOctβRs with those of other cloned G-protein coupled receptors, against both the Swissprot database and against a set of Hidden Markov Models using HMMER (Eddy 1998), shows that the novel receptors have the highest homologies to vertebrate β-adrenergic receptors (Fig. 2). It is clear from the phylogenetic tree that these receptors form three related subgroups with a range of predicted aminergic GPCRs from *D. pseudoobscura* (EAL26922, EAL26997), *A. mellifera* (XP__396348, XP_396445, XP_397139 and XP_397077) and *A. gambiae* (XP_312026, XP_312025), which probably represent related species homologs, and that all three groups are closely related to the two octopamine receptors from *Aplysia* (Q9NJS6 and Q9NHF3) which only couple to cyclic AMP increases (Chang et al. 2000). It can be seen that all these receptors are closely related structurally to vertebrate β-adrenergic receptors, which also correlates with their ability to selectively activate adenylyl cyclase activity and their responsiveness to adrenaline and noradrenaline. It will be interesting to see if the homologous receptors from the other insect species when expressed and characterized, also show the same pharmacological differences as CG6919, CG6989 and CG7078. This might suggest whether this novel group of insect octopamine receptors can be further subdivided into pharmacological subclasses in parallel with the subdivisions of the vertebrate β- and α-adrenergic classes into the $\beta_{1,2,3}$, $\alpha_{1A,1B,1C,1D}$ and $\alpha_{2A,2B,2C}$ adrenergic receptor subclasses.

It is clear from the phylogenetic tree that the representatives of the second main group of insect octopamine receptors that have been cloned, such as the *Drosophila* mushroom body receptor (OAMB), show a greater structural similarity to vertebrate α-adrenergic receptors, which is also supported by their pharmacology (Han et al. 1998), and are likely to mediate many of their effects via increases in intracellular calcium levels. We propose that this second group of insect octopamine receptors should be known as the insect α-adrenergic-like octopamine receptors (OctαRs). In addition, the so called octopamine/tyramine group of receptors (see above), including the *Drosophila* octopamine/tyramine receptor (OAR Drome) (Arakawa et al. 1990; Robb et al. 1994) would form a third class of receptors, which also show a greater structural similarity to vertebrate α-adrenergic receptors. This classification is again supported by their pharmacology. These ideas are further supported by the observation that the novel cloned DmOctβ receptors have a relatively short third intracellular loop, as do vertebrate β-adrenergic receptors, whilst the insect α-adrenergic-like octopamine receptors and the octopamine/tyramine receptors, have much longer third intracellular loops, as do vertebrate α-adrenergic receptors. Further, as discussed above, it has been suggested that, the octopamine/tyramine group of receptors may well actually represent a distinct class of tyraminergic receptors since they are preferentially activated by tyramine compared to octopamine in most of the cases that have been examined to date (see Nagaya et al. 2002; Roeder et al. 2003).

The other β-adrenergic-like *Drosophila* receptor (DmDopEcR – spliced variants A, B and C) which is activated by both dopamine and ecdysteroids forms a specific subgroup along with predicted species homologs from *D. pseudoobscura* (EAL30129), *A. mellifera* (XP__396491) and *A. gambiae* (XP_315694), together with predicted homologs from *C. elegans* (NP_510580) and *C. briggsae* (CAE63350).

It is interesting to note that the many aspects of the original classification of insect octopamine receptors (Evans 1981), such as the distinction between the subgroups that mediate their actions via the specific activation of adenylyl cyclase versus those that signal predominantly via increases in intracellular calcium, still appear to be valid. However, the advances made by a comparison of the structural sequences of cloned insect octopamine receptors and their individual signalling properties in heterologous expression systems, suggests that much care is needed in the interpretation of the molecular basis of signalling in tissues where multiple octopamine receptor subtypes may be expressed. In future studies on the molecular basis of octopamine signalling in individual tissues it will be essential to identify the relative expression levels of the different classes of octopamine receptor present. In addition, it will be essential to identify if co-expression of such receptors results in the formation of oligomeric receptors with specific emergent pharmacological and signalling properties.

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