Archives of Insect Biochemistry and Physiology 48:13–38 (2001)

Molecular and Pharmacological Properties of Insect Biogenic Amine Receptors: Lessons From *Drosophila melanogaster* and *Apis mellifera*

Wolfgang Blenau[1]* and Arnd Baumann[2]

[1]*Department of Zoology, University of Otago, Dunedin, New Zealand*
[2]*Institut für Biologische Informationsverarbeitung, Forschungszentrum Jülich, Jülich, Germany*

In the central nervous system (CNS) of both vertebrates and invertebrates, biogenic amines are important neuroactive molecules. Physiologically, they can act as neurotransmitters, neuromodulators, or neurohormones. Biogenic amines control and regulate various vital functions including circadian rhythms, endocrine secretion, cardiovascular control, emotions, as well as learning and memory. In insects, amines like dopamine, tyramine, octopamine, serotonin, and histamine exert their effects by binding to specific membrane proteins that primarily belong to the superfamily of G protein–coupled receptors. Especially in *Drosophila melanogaster* and *Apis mellifera* considerable progress has been achieved during the last few years towards the understanding of the functional role of these receptors and their intracellular signaling systems. In this review, the present knowledge on the biochemical, molecular, and pharmacological properties of biogenic amine receptors from *Drosophila* and *Apis* will be summarized. Arch. Insect Biochem. Physiol. 48:13–38, 2001. **© 2001 Wiley-Liss, Inc.**

Key words: dopamine; G protein-coupled receptors; histamine; honeybee; octopamine; serotonin; tyramine

INTRODUCTION

Communication between individual neurons as well as between neurons and non-neuronal target cells is mediated by unique electro-chemical signaling pathways. Action potentials lead to the release of chemical "messengers" from specialized cell regions of the excited neuron. These messengers include small organic or peptidergic compounds, which either act as neurotransmitters, neuromodulators, or neurohormones. Some compounds may even have overlapping properties, acting as neurotransmitters in locally confined interneuronal signaling or as neurohormones when released into the blood or haemolymph and transported via the circulatory system to their target tissues in the body. The messengers are rec-

Contract grant sponsor: German Research Foundation; Contract grant numbers: Er 79/4-1, 4-2; Ba 1541/2-1, 2-2; Bl 469/1; Contract grant sponsor: University of Otago; Contract grant number: ORG MFZ B00; Contract grant sponsor: Alexander von Humboldt-Foundation.

Institution from which paper emanated: Department of Zoology, University of Otago, Dunedin, New Zealand

*Correspondence to and current address: Dr. Wolfgang Blenau, Institut für Biochemie und Biologie, Universität Potsdam, Postfach 601553, D-14415 Potsdam, Germany. E-mail: blenau@rz.uni-potsdam.de

Received 20 December 2000; Accepted 1 April 2001

© 2001 Wiley-Liss, Inc.

ognized by specific receptors on the surface membrane of the target cell. Interaction between the messenger and its receptor translates the chemical signal into a specific electrical or biochemical response of the target cell.

Although the structural features of neuroactive substances are quite diverse, the cellular responses evoked by these compounds are generally mediated by members of only two large gene families encoding different types of membrane receptors. Binding of the messengers to ionotropic receptors (ligand-gated ion channels) leads to the opening of the channel pore and causes either excitation or inhibition of the target cell. In contrast to ion channel activation, binding of messengers to metabotropic receptors (G protein-coupled receptors; GPCRs) leads to slower cellular responses. Activated GPCRs transmit the signal to intracellular trimeric GTP-binding (G) proteins (see Structural Properties of Biogenic Amine Receptors). Once activated, the G proteins either stimulate or inhibit specific target proteins. This causes changes in the concentration of intracellular "second messengers," e.g., cyclic nucleotides (cAMP, cGMP), inositol 1,4,5-trisphosphate (IP_3), and diacylglycerol (DAG) (see Signaling Pathways Activated by Biogenic Amine Receptors). Finally, second messenger-dependent enzymes are activated and transiently modify the functional properties of various cytosolic, membrane-bound, or nuclear proteins. G protein subunits may also regulate ion channel activity directly (Hille, 1994; Clapham and Neer, 1997; Schneider et al., 1997).

One important group of messenger substances that primarily bind to GPCRs are the biogenic amines (for a review see Vernier et al., 1995). These molecules are synthesized from amino acids in single to multistep reactions (see Biosynthesis of Biogenic Amines), and are found in both vertebrates and invertebrates (see Table 1). In the central nervous system (CNS), biogenic amines control and modulate various functions, including cardiovascular homeostasis, circadian rhythms, emotional states, endocrine secretion, sexual behavior, thermoregulation, as well as learning and memory. In humans, the etiology of several neural diseases has been linked to impaired biogenic amine signaling. Much effort has been given to evaluating the molecular and functional properties of biogenic amine receptors and their downstream reaction partners in order to identify potential targets for the pharmacological treatment of such diseases. In recent years, considerable progress has been made in unraveling the physiological role of biogenic amines and their receptors in invertebrates. The aim of this review is to summarize the molecular, pharmacological, and functional properties of insect biogenic amine receptors. Since our own work is focused on the molecular and pharmacological characterization of receptors from the fruitfly (*Drosophila melanogaster*) and the honeybee (*Apis mellifera*), we will concentrate primarily on these two species.

TABLE 1. List of the Major Biogenic Amines Identified in Vertebrates and Invertebrates

Vertebrates	Invertebrates
Dopamine	Dopamine
Norepinephrine	Tyramine
Epinephrine	Octopamine
Serotonin	Serotonin
Histamine	Histamine

BIOSYNTHESIS OF BIOGENIC AMINES

In both vertebrates and invertebrates, the group of biogenic amine messengers consists of five members (see Table 1). In addition to molecules shared by both phylogenetic groups (dopamine, serotonin, histamine), some biogenic amines seem to be synthesized preferentially in either vertebrates (norepinephrine, epinephrine) or invertebrates (tyramine, octopamine). Biogenic amines are synthesized from three different amino acids and here we will briefly summarize these biosynthetic pathways. Additional information can be found in comprehensive textbooks (Siegel et al., 1998; Hardman et al., 1996) and the citations in this section will consider only publications on *Drosophila*.

Biogenic Amines Derived From Tyrosine

In vertebrates, tyrosine gives rise to the catecholamines dopamine, norepinephrine, and epinephrine (see Fig. 1). Biosynthesis starts with hydroxylation in the *meta*-position of tyrosine and is catalyzed by tyrosine hydroxylase (TH). TH is the rate-limiting enzyme in catecholamine synthesis. In a second step, 3,4-dihydroxy-L-phenylalanine (L-DOPA) is decarboxylated to dopamine. The conversion is mediated by the enzyme DOPA decarboxylase (DDC). In certain vertebrate neurons, dopamine is an intermediate reaction prod-

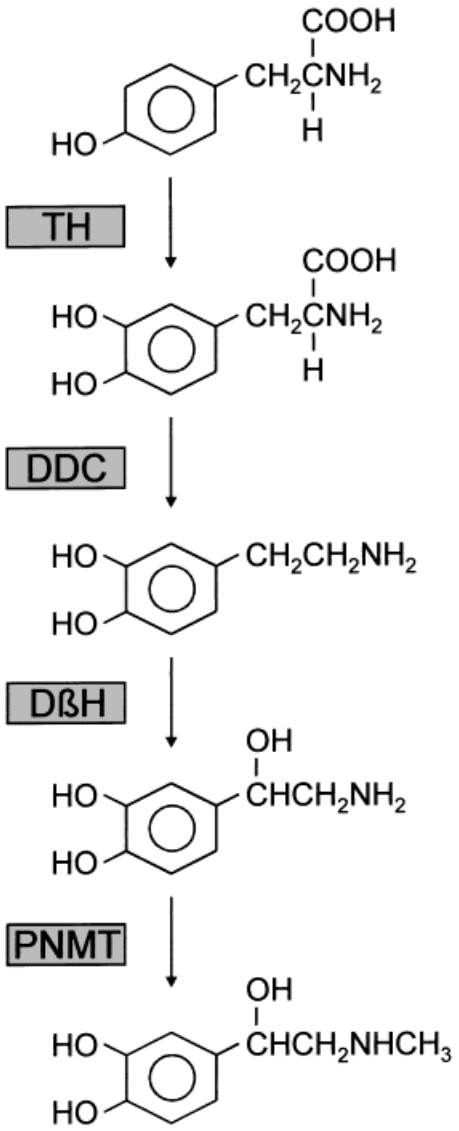

Fig. 1. Biosynthesis of catecholamines. Tyrosine is hydroxylated by tyrosine hydroxylase (TH) to L-DOPA. Decarboxylation of L-DOPA by DOPA decarboxylase (DDC) generates dopamine. Dopamine is hydroxylated by dopamine β-hydroxylase (DβH) to norepinephrine. Norepinephrine is methylated by phenylethanolamine N-methyltransferase (PNMT) to epinephrine.

uct and will be further metabolized. Dopamine β-hydroxylase (DβH) catalyzes the formation of norepinephrine and adds a hydroxyl group to the β-carbon on the side chain of dopamine. But even norepinephrine can be further modified. This is achieved by the enzyme phenylethanolamine N-methyltransferase (PNMT), which adds a methyl group to the nitrogen of norepinephrine, thereby forming the secondary amine epinephrine.

Whereas the identical pathway to synthesize dopamine also exists in invertebrates (Hirsh and Davidson, 1981; Livingstone and Tempel, 1983; Neckameyer and Quinn, 1989; Restifo and White, 1990), norepinephrine and epinephrine have not been unequivocally identified in *Drosophila* (Wright, 1987) although low concentrations have been detected in some other insect species (Brown and Nestler, 1985; Evans, 1980).

In addition to the biosynthesis of dopamine, invertebrates use an alternative biochemical pathway to generate the phenolamines tyramine and octopamine from tyrosine (see Fig. 2). In a first step, tyrosine is decarboxylated to tyramine by tyrosine decarboxylase (TDC; Livingstone and Tempel, 1983). Similar to the conversion of dopamine to norepinephrine, tyramine can also be hydroxylated on the β-carbon of the side chain. This reaction is catalyzed by tyramine β-hydroxylase (TβH) and generates octopamine (Monastirioti et al., 1996). Since octopamine and norepinephrine are chemically very similar, though not identical substances, it has been suggested that the noradrenergic/adrenergic system of vertebrates is functionally substituted by the tyraminergic/octopaminergic system in invertebrates (Evans, 1985, 1993; Roeder, 1999).

Serotonin Is Derived From Tryptophan

In both vertebrates and invertebrates, identical biochemical pathways exist to synthesize the indolamine 5-hydroxytryptamine (5-HT, serotonin) from L-tryptophan (see Fig. 3). In the first and rate-limiting reaction, a hydroxyl group is added to the indole ring in the 5′-position by tryptophan hydroxylase (TRH; Livingstone and Tempel, 1983; Neckameyer and White, 1992). Once synthesized, 5-hydroxytryptophan is decarboxylated by DDC to serotonin (Hirsh and Davidson, 1981; Livingstone and Tempel, 1983). Since DDC also participates in the decarboxylation of L-DOPA to dopamine (see Fig. 1), a defect or loss of function of this enzyme will simultaneously result in a severely impaired production of both dopamine and serotonin. In *Drosophila*, DDC null mutants cause the death of the animal in early developmental stages (Wright, 1987).

Histamine Is Derived From Histidine

A single decarboxylation step converts L-histidine to histamine (see Fig. 4). The reaction is

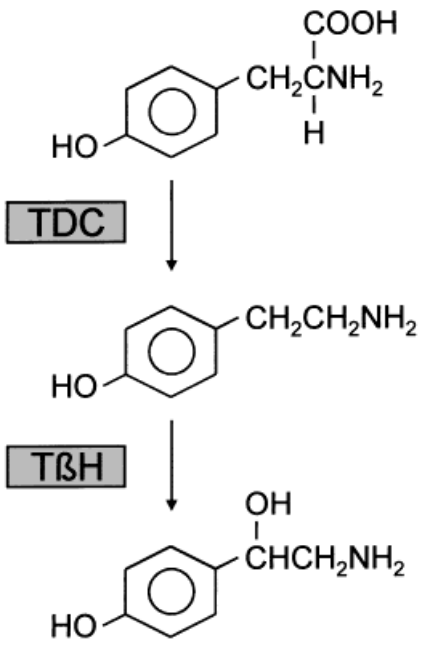

Fig. 2. Biosynthesis of phenolamines. Tyrosine is decarboxylated by tyrosine decarboxylase (TDC) to tyramine which is hydroxylated by tyramine β-hydroxylase (TβH) to octopamine.

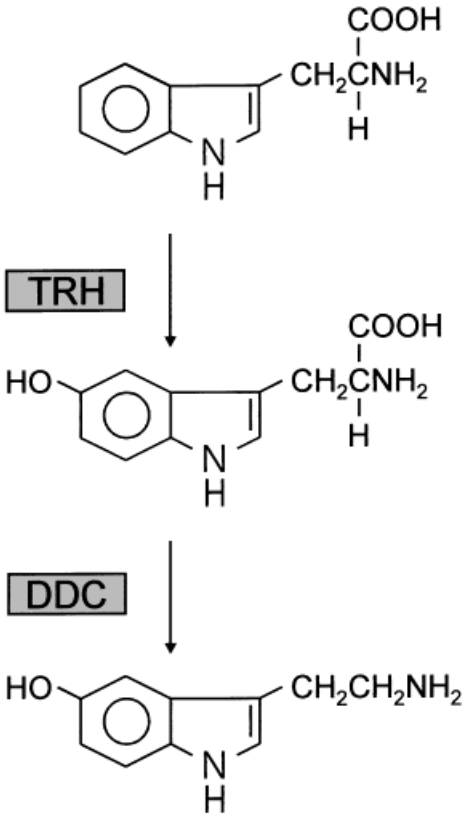

Fig. 3. Biosynthesis of the indolamine serotonin. L-tryptophan is hydroxylated by tryptophan hydroxylase (TRH) to 5-hydroxytryptophan, which is decarboxylated by DOPA decarboxylase (DDC) to serotonin.

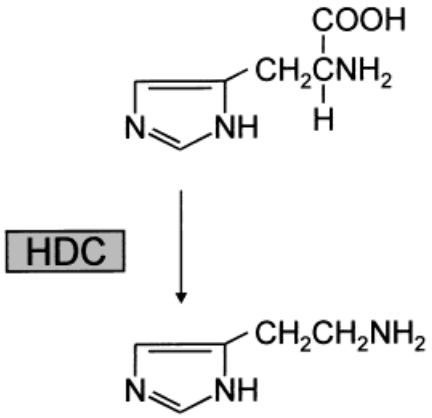

Fig. 4. Biosynthesis of histamine. L-histidine is decarboxylated by histidine decarboxylase (HDC) to histamine.

mediated by the enzyme histidine decarboxylase (HDC; Burg et al., 1993). Histamine has been shown to be the major neurotransmitter released from invertebrate photoreceptors (Hardie, 1989; for reviews see: Nässel, 1991, 1999). In vertebrates, histamine is considered one of the most important mediators of allergy and inflammation. In the vertebrate CNS, however, histamine is synthesized from a small population of neurons located in the posterior hypothalamus. These neurons project to most cerebral areas and have been implicated in hormonal secretion, cardiovascular control, thermoregulation, and memory functions (Schwartz et al., 1991).

STRUCTURAL PROPERTIES OF BIOGENIC AMINE RECEPTORS

Biogenic amine receptors belong predominantly to the superfamily of GPCRs. Within this gene family, biogenic amine receptors fall into the largest subfamily, i.e., rhodopsin-like receptors. All members of this group are integral membrane proteins. Based on crystal structure data (Palczewski et al., 2000) as well as hydropathy profile analyses and phylogenetic comparisons (Baldwin, 1994; Vernier et al., 1995; Baldwin et al., 1997; Valdenaire and Vernier, 1997), these receptors share the common motif of seven transmembrane (TM) domains (see Fig. 5). As type II membrane proteins, the N-terminus is located extracellularly and the C-terminus is located intracellularly. The N-terminus often contains consensus sequence motifs for N-linked glycosylation (Probst et al., 1992; Strader et al., 1995). The membrane-spanning re-

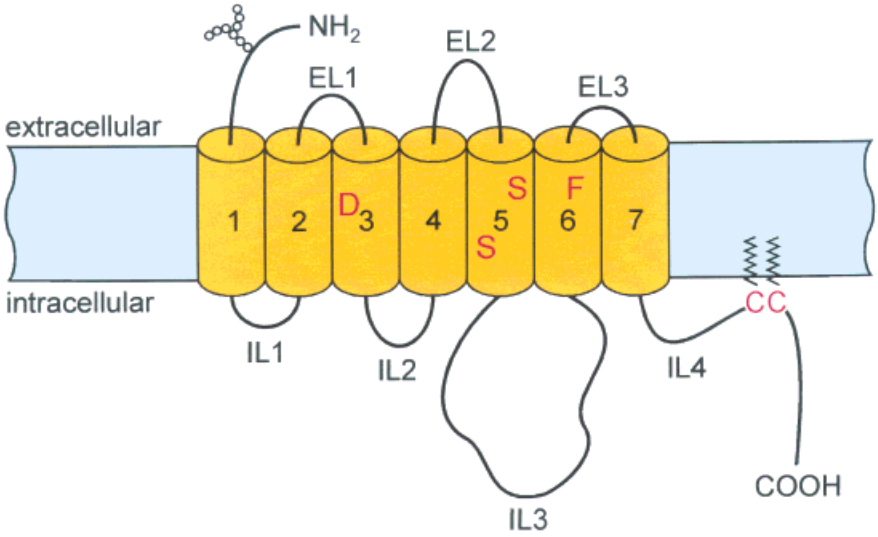

Fig. 5. Topography of a biogenic amine receptor. The polypeptide spans the membrane seven times. These transmembrane regions (TM 1–7) are depicted as cylinders. The N-terminus (NH_2) is located extracellularly and often contains glycosylated residues (small open circles). The C-terminus (COOH) is located intracellularly. The membrane spanning regions are linked by three extracellular loops (EL) that alternate with three intracellular loops (IL). Residues that participate in ligand binding are indicated in grey: an aspartic acid residue (D) in TM3, serine residues (S) in TM5, and a phenylalanine residue (F) in TM6. When the protein is posttranslationally palmitoylated at cysteine residues (C) in the cytoplasmic tail, a fourth intracellular loop (IL4) will be formed.

gions are linked by three extracellular loops (EL) that alternate with three intracellular loops (IL). A pair of cysteine residues in EL2 and TM3 are believed to form a disulfide bridge that contributes to the structural stability and binding properties of these receptors (Noda et al., 1994). Additional cysteine residues in the cytoplasmic tail of the polypeptides are the target of posttranslational palmitoylation. Insertion of these fatty acids into the plasma membrane will create a fourth intracellular loop (IL4) and further stabilize the structure of these receptors (Jin et al., 2000). Activation of the receptors occurs by binding of specific biogenic amines. The interaction between the ligand and its receptor takes place in a binding pocket formed by the TM regions in the plane of the membrane. Specific residues in different TM segments interact with functional groups of the biogenic amines. In particular, an aspartic acid residue (D) in TM3, serine residues (S) in TM5, and a phenylalanine residue (F) in TM6 were shown to determine the ligand binding properties of biogenic amine receptors (Strader et al., 1995; Valdenaire and Vernier, 1997). Once the ligand is tightly bound to its receptor, the receptor's conformation will change. This structural change is then transferred to trimeric G proteins. Residues in close vicinity to the plasma membrane of IL2, 3, and 4 determine the specificity and efficacy of G protein-activation. Receptor mediated signaling, however, will finally be turned off by phosphorylation of serine and threonine residues in the C-terminus and IL3 (Chuang et al., 1996; Palczewski, 1997).

SIGNALING PATHWAYS ACTIVATED BY BIOGENIC AMINE RECEPTORS

A common feature of GPCR activation is the subsequent change of intracellular messenger concentrations. Depending on which type of GPCR is activated, a change in the intracellular concentration of cAMP ($[cAMP]_i$) and/or Ca^{2+} ($[Ca^{2+}]_i$) is most likely to take place. Since these are the most commonly found cellular responses to biogenic amine treatments, they are used to functionally classify receptor subtypes. As a result of GPCR activation, $[cAMP]_i$ can change in two directions: $[cAMP]_i$ levels are either elevated or decreased (see Fig. 6). The cellular response strictly relies on the specificity of interaction between the receptor and the G protein (Gudermann et al., 1996, 1997). When the receptor binds to a G_s-type protein, the activated $G_{\alpha s}$ subunit will interact with

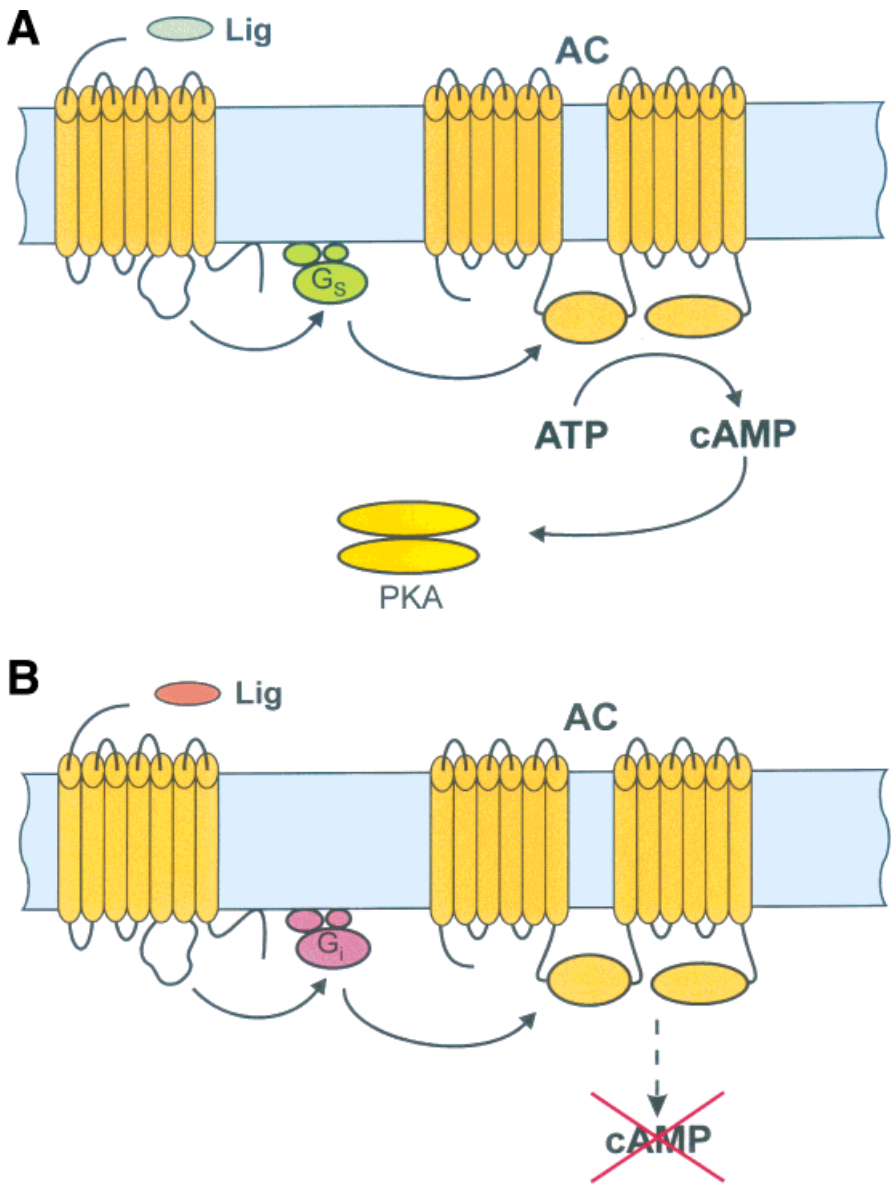

Fig. 6. Biogenic amine receptors coupled to intracellular cAMP signaling pathways. **A:** Biogenic amine receptors are activated by binding of agonists (ligand, Lig). The ligand-bound receptor then activates a stimulatory G protein (G_s), which leads to an increase in the enzymatic activity of adenylyl cyclase (AC). Adenylyl cyclase catalyzes the conversion of ATP to cAMP. As the intracellular concentration of cAMP increases, cAMP-dependent protein kinase (PKA) is activated and phosphorylates different target proteins on serine and threonine residues. **B:** Several biogenic amine receptors are known to inhibit AC activity via inhibitory G proteins (G_i).

adenylyl cyclase (AC) in the plasma membrane. This leads to an increase of cyclase activity and production of cAMP from ATP. The rise in $[cAMP]_i$ will then activate cAMP-dependent protein kinase (protein kinase A, PKA). Phosphorylation of serine and/or threonine residues by PKA modifies the properties of various substrate molecules including cytosolic proteins, ligand-gated and voltage-dependent ion channels, as well as transcription factors, such as CREB, CREM, and ATF-1 (De Cesare et al., 1999). Several biogenic amine receptors are also known to inhibit adenylyl cyclase activity. This effect is mediated by interaction of the receptor with inhibitory G proteins (G_i). Interaction of adenylyl cyclase with activated $G_{\alpha i}$ subunits most likely competes with binding of activated $G_{\alpha s}$ subunits and thereby interferes with cyclase activation.

Another pathway that is activated by several biogenic amine receptors results in a rise of $[Ca^{2+}]_i$ (see Fig. 7). Here, the amine-activated receptor binds to G proteins of the $G_{q/o}$ family (Gudermann et al., 1996, 1997). The activated $G_{\alpha q/o}$ subunits bind to and stimulate phospholipase C (PLC) activity (Rhee and Bae, 1997). The enzyme hydrolyzes a membrane-bound substrate, phosphatidylinositol 4,5-bisphosphate. Cleavage gives rise to two second messengers, IP_3 and DAG. The IP_3 freely diffuses and binds to specific IP_3 receptors on the membrane of intracellular Ca^{2+} stores (endoplasmic reticulum). These receptors are second messenger-gated Ca^{2+} channels. Therefore, after binding of IP_3, the channel pore is opened and Ca^{2+} is released into the cytoplasm. Ca^{2+} ions play a pivotal role in the regulation of many cellular functions by directly controlling enzymatic or ion channel activities. Furthermore, Ca^{2+} can also bind to members of the large family of Ca^{2+}-binding proteins (calmodulin, calbindin, calretinin, etc.) that modulate the activation properties of many effector proteins by protein-protein interaction. Since PLC not only generates IP_3 but also DAG, receptor coupling to $G_{q/o}$ proteins might activate a second signaling pathway in addition to Ca^{2+} release. In contrast to IP_3, DAG remains associated with the membrane, where it activates protein kinase C (PKC). Full enzymatic activity of PKC, however, requires the presence of DAG and Ca^{2+} as well as association of the kinase with the membrane. Similar to PKA (see above), PKC

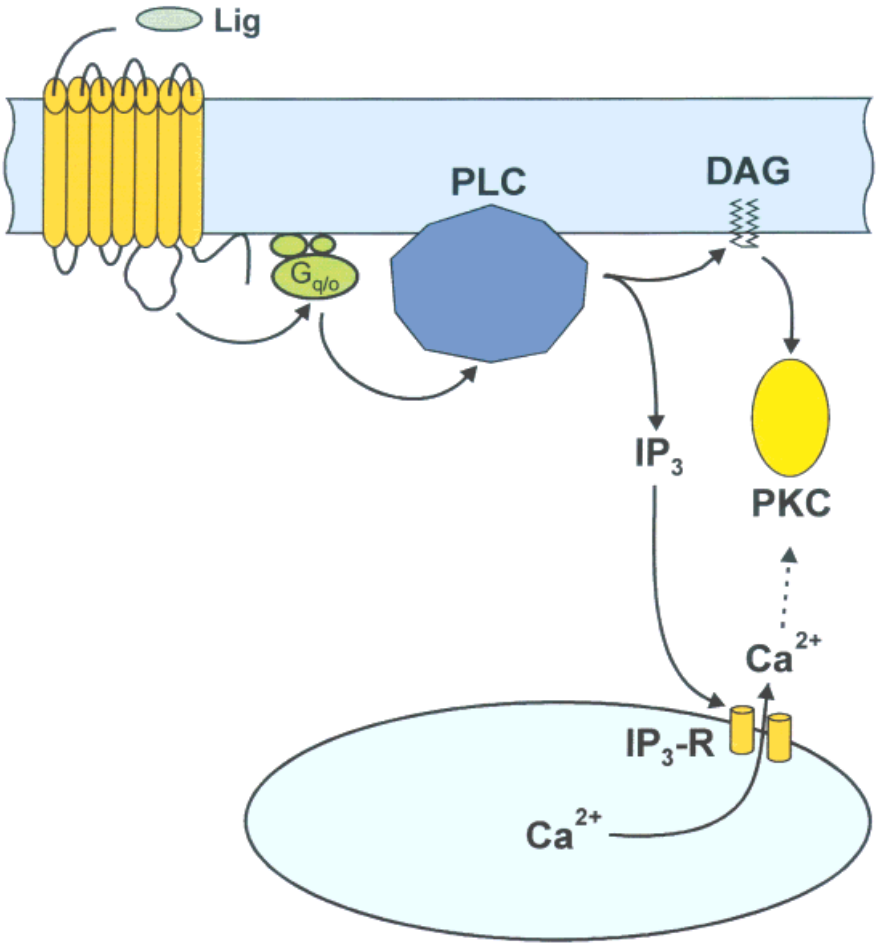

Fig. 7. Biogenic amine receptors coupled to intracellular IP_3/DAG signaling pathways. Agonist (ligand, Lig)-bound biogenic amine receptors activate G proteins of the $G_{q/o}$ family ($G_{q/o}$) that regulate the enzymatic activity of phospholipase C (PLC). This enzyme hydrolyzes phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Binding of IP_3 to specific receptors (IP_3-R) that form ion channels in the membrane of the endoplasmic reticulum (ER) causes release of Ca^{2+} into the cytoplasm. Both the increase in intracellular Ca^{2+} and the membrane-bound DAG activate protein kinase C (PKC) that phosphorylates different target proteins.

phosphorylates a variety of proteins on serine and threonine residues, which alters the functional properties of these proteins. In summary, GPCR activation generates graded cellular responses depending on the second messenger pathways involved. The different intracellular messenger pathways may also be activated in parallel within the same cell when the respective receptors and coupling partners are present. Such co-activation events potentially lead to either amplifications or diminishments of the cellular responses and provide a cellular basis for "coincidence detection."

DOPAMINE RECEPTORS

The catecholamine dopamine is present in relatively high concentrations in the insect CNS (Mercer et al., 1983; Brown and Nestler, 1985; Fuchs et al., 1989; Harris and Woodring, 1992; Taylor et al., 1992; Kirchhof et al., 1999; Wagener-Hulme et al., 1999; Schulz and Robinson, 1999). Dopaminergic neurons possess widefield arborizations that extend to most areas of the brain suggesting that dopamine plays an important role as a neurotransmitter and/or neuromodulator in insects (Budnik and White, 1988; Nässel and Elekes, 1992; Schäfer and Rehder, 1989; Schürmann et al., 1989; Blenau et al., 1999).

During *Drosophila* development, dopamine levels show four discrete peaks. These coincide with larval molts, pupariation, and adult emergence (Martínez-Ramírez et al., 1992). The analysis of *Drosophila* mutants also suggests a role for dopamine in the terminal differentiation of the nervous system as well as in learning behavior. Mutants that lack the enzyme DDC (*Ddc*, see Biogenic Amines Derived From Tyrosine and Serotonin Is Derived From Tryptophan) and, therefore, are devoid of the biogenic amines dopamine and serotonin, die as early embryos (Tempel et al., 1984). In addition to these null alleles, less severe *Ddc* mutants exist that only have a reduced enzymatic activity of DDC. The greater the impact on DDC activity in these mutants, the worse their performance is in associative learning tests (Tempel et al., 1984). It was also shown that the threshold concentration to sucrose, which elicits the proboscis extension response, is raised ~6-fold in the mutants (Tempel et al., 1984). The learning defect of *Ddc* mutants, however, could not be reproduced in another independent investigation (Tully, 1987). Nevertheless, during larval development *Ddc* mutants show an aberrant pattern of neuronal arborization (Budnik et al., 1989). The extent of branching can be partially restored to wild-type levels by feeding the mutants with dopamine. Inactivation of TH (see Biogenic Amines Derived From Tyrosine) during development results in akinesia, developmental retardation, and decreased fertility of the flies (Neckameyer, 1996). In addition, dopamine-depleted adult males show impaired habituation in experience-dependent courtship paradigms (Neckameyer, 1998).

In the honeybee, the physiological role of different biogenic amines has been examined by behavioral and electrophysiological studies of olfactory brain centers (for a review see: Bicker and Menzel, 1989). Injection of dopamine into the α-lobe of the mushroom bodies, important brain structures for higher order olfactory information processing, alters the electrical responses to olfactory stimuli (Mercer and Erber, 1983) and reduces proboscis extension responses to water vapor (Blenau and Erber, 1998). Dopamine also affects the retrieval of olfactory memories (Mercer and Menzel, 1982; Macmillan and Mercer, 1987; Michelsen, 1988). Since the experimental approach used in these studies may have evoked non-specific responses, an alternative strategy was used by Menzel et al. (1999). Amine levels were first depleted with reserpine. This caused a significant impairment of motor-output patterns. Subsequent injection of dopamine into the brain, restored the reserpine-induced defects in motor patterns, but had no effect on either sensitization or conditioning (Menzel et al., 1999).

In vertebrates, dopamine binds to two subfamilies of dopamine receptors: D1- and D2-(like) receptors (Kebabian and Calne, 1979). These receptors belong to the family of GPCRs and possess different pharmacological and biochemical properties. In humans, D1- and D5-receptors constitute the D1-subfamily and activate adenylyl cyclase, whereas members of the D2-subfamily, i.e., the D2-, D3-, and D4-receptors, either inhibit adenylyl cyclase or couple to different intracellular second messenger systems (for reviews see: Jackson and Westlind-Danielsson, 1994; Missale et al., 1998; Vallone et al., 2000). Benzazepines like R(+)-SCH 23390 bind to D1-like receptors with high

affinity but not to D2-like receptors. In contrast, butyrophenones, like spiperone, bind with high affinity to D2-like but not to D1-like receptors.

Drosophila DmDOP1- and the Honeybee AmDOP1-Receptor

Application of low stringency hybridization protocols led to the cloning of the first dopamine receptor genes from *Drosophila* (Dm*dop1*, Gotzes et al., 1994; *d*DA1, Sugamori et al., 1995). Both genes encode almost identical polypeptides except for a 126–amino acid extension of the N-terminus found only in DmDOP1. This difference most likely is caused by strain-specific nucleotide substitutions and insertions in the *d*DA1-gene, which was cloned from a different *Drosophila* wild-type strain than the Dm*dop1*-gene (Gotzes and Baumann, 1996). Therefore, we will refer to both genes as Dm*dop1*.

Within the putative TMs, DmDOP1 displays high sequence similarity to human dopamine D1- (53.1%) and D5-receptors (51.8%). Amino acid residues that interact with ligands in vertebrate dopamine receptors (Missale et al., 1998) are well conserved in DmDOP1. Functional expression of the Dm*dop1*-gene in human embryonic kidney (HEK 293) cells specifically elicited cAMP production after dopamine application (Gotzes et al., 1994). This response was also evoked by dopamine D1-receptor agonists, e.g., SKF 38393 (Gotzes et al., 1994) and 6,7-ADTN (Sugamori et al., 1995). In contrast to dopamine, stimulation of Dm*dop1*-transfected HEK 293 (Gotzes et al., 1994), COS-7, and *Spodoptera frugiperda* (Sf)9 cells (Sugamori et al., 1995) with the benzazepine SKF 38393 gave rise to only a small increase in $[cAMP]_i$. The production of cAMP was inhibited by dopaminergic antagonists such as S(+)-butaclamol and *cis*(Z)-flupentixol. Most notably, the benzazepine R(+)-SCH 23390, a typical vertebrate D1-receptor antagonist (see above), neither bound with high affinity to DmDOP1 nor was it as potent as butaclamol or flupentixol in inhibiting dopamine-induced cAMP elevation (Gotzes et al., 1994; Sugamori et al., 1995).

The expression pattern of the Dm*dop1*-gene was examined by in situ hybridizations and Northern blot analyses. Receptor mRNA is expressed as a maternal transcript and restricted to apical regions of the cortical peripheral cytoplasm in the embryo (Sugamori et al., 1995). In adult flies, the gene is widely expressed in the CNS (Gotzes et al., 1994). The expression pattern of the receptor gene agrees well with the widespread distribution of dopaminergic nerve fibers determined immunohistochemically.

A homologous gene to Dm*dop1* was recently cloned from honeybee (*Apis mellifera*) brain (Am*dop1*, Blenau et al., 1998). Within the putative TM regions, the deduced amino acid sequence of Am*dop1* (AmDOP1) shares ~75% similarity with vertebrate D1-like receptors and ~93% similarity with DmDOP1. Therefore, the honeybee AmDOP1-receptor most likely is the orthologue of the *Drosophila* DmDOP1-receptor. The pharmacological profile of the heterologously expressed AmDOP1-receptor (Blenau et al., 1998) is very similar to that determined from [^{3}H]LSD binding studies to membrane preparations of honeybee brains (Table 2; Blenau et al., 1995a). [^{3}H]LSD specifically binds to AmDOP1 with a K_D ~5 nM. Of all biogenic amines tested, dopamine was the most potent competitor (K_i = 56 nM). In addition, several synthetic dopaminergic agonists and antagonists also potently displaced [^{3}H]LSD from its binding site on the AmDOP1-receptor (Table 2). Similar to the *Drosophila* DmDOP1-receptor, benzazepines, i.e., SCH 23390 and SKF 38393, which bind to mammalian D1-like receptors with nanomolar affinity, were 200 to >1,000-fold less potent at the AmDOP1-receptor. When either dopamine or 6,7-ADTN was applied to AmDOP1-expressing HEK 293 cells, an increase in $[cAMP]_i$ was observed (Blenau et al., 1998), suggesting that AmDOP1 also belongs to the dopamine D1-like receptor family.

In situ hybridization to tissue sections of adult honeybee brain revealed that Am*dop1* mRNA is expressed in many neurons of the CNS, including neurons of the optic lobes, intrinsic mushroom body neurons, neurons of the antennal lobes, and neurons of the suboesophageal ganglion (Blenau et al., 1998). This widespread distribution suggests that the AmDOP1-receptor is a likely candidate for the processing of higher order sensory information.

Drosophila DAMB/DopR99B-Receptor and the Honeybee AmBAR6-Receptor

A second dopamine receptor gene was cloned from *Drosophila* using polymerase chain reaction

TABLE 2. Pharmacological Properties of Dopamine Receptors in *Apis mellifera

	Apis AmDOP1 in HEK 293: [^{3}H]LSD (K_i [nM])	*Apis* brain homogenate: dopamine-sensitive [^{3}H]LSD binding site (K_i [nM])	*Apis* brain homogenate: [^{3}H]SCH 23390 binding site (K_i [nM])
Biogenic amines			
Dopamine	56	22	30,800
L(–)-Norepinephrine	3,100	—	—
Serotonin	3,600	7,000	548,000
Tyramine	9,900	—	≥1,000,000
DL-Octopamine	110,000	—	892,000
Dopamine receptor agonists			
R(+)-Lisuride	4.3	4.7	—
6,7-ADTN	93	78	—
R(+)-SKF 38393	4,200	—	3,200
Dopamine receptor antagonists			
Chlorpromazine	15	48	208
ciz(*Z*)-Flupentixol	17	150	218
Spiperone	64	—	25,400
S(+)-Butaclamol	77	89	13,800
R(+)-SCH 23390	250	—	9.5
Haloperidol	390	—	—
S(–)-SCH 23388	440	—	—
trans(E)-Flupentixol	650	3,600	—
R(–)-Butaclamol	42,000	>100,000	—

Values for AmDOP1 expressed in HEK 293 cells are from Blenau et al. (1998). Values for the dopamine-sensitive [^{3}H]LSD binding site of the honeybee are from Blenau et al. (1995a) and for the honeybee [^{3}H]SCH 23390 binding site are from Kokay and Mercer (1996). Note the rather low affinity for the AmDOP1-receptor of the benzazepines R(+)-SKF 38393 and R(+)-SCH 23390 and the low affinity of the putative endogenous agonist dopamine for the [^{3}H]SCH 23390 binding site in membrane homogenate of honeybee brains.

(PCR) and single-strand conformation analysis (DAMB, Han et al., 1996; DopR99B, Feng et al., 1996). DAMB and DopR99B encode identical polypeptides except for 27 amino acids at the C-terminus. The difference most likely is caused by incomplete splicing of the DopR99B transcript (Feng et al., 1996; Han et al., 1996). The DAMB/DopR99 cDNA and deduced amino acid sequence, however, clearly differ from the *Drosophila* DmDOP1-receptor (Gotzes et al., 1994).

Functional expression of DAMB in *Drosophila* S2 and HEK 293 cells resulted in an increase in $[cAMP]_i$ upon dopamine application. Since this response was blocked by *cis*(Z)-flupentixol, DAMB is considered an additional member of the subfamily of D1-like dopamine receptors in *Drosophila* (Han et al., 1996). Agonist stimulation of DopR99B expressed in *Xenopus* oocytes led to an increase in $[Ca^{2+}]_i$ as monitored by Ca^{2+}-dependent chloride channel activity (Feng et al., 1996; Reale et al., 1997a). In addition to the calcium response, dopamine application also induced an increase in $[cAMP]_i$ in DopR99B-expressing oocytes (Feng et al., 1996). These results suggest that the DAMB/DopR99B-receptor activates different intracellular signaling pathways in parallel when expressed in *Xenopus* oocytes. Synthetic dopamine receptor-antagonists blocked both cellular responses with a similar rank order of potency (Feng et al., 1996; Reale et al., 1997a). In contrast, the rank order of potency of a range of synthetic receptor agonists that activated the different signaling pathways was not identical (Reale et al., 1997a).

DAMB/DopR99B mRNA was detected in heads as well as in legs and/or antennae by Northern blotting, suggesting that this receptor might be functional in both the central and peripheral nervous system (Han et al., 1996; Feng et al., 1996). In situ hybridization showed that DAMB transcripts are preferentially expressed in the perikarya of intrinsic mushroom body cells. Signals were almost absent in other parts of the brain, thoracic, and abdominal ganglia as well as in other tissues (Han et al., 1996). Staining of tissue sections with a polyclonal antibody also showed that the protein was preferentially expressed in the mushroom bodies. Strong labeling was found in the α- and β-lobes while the pedunculi and γ-lobes were less intensely stained. In con-

trast, immunoreactivity was absent in the calyces, which house the dendrites of intrinsic mushroom body neurons. The staining pattern of the DAMB-receptor is very similar to the expression pattern of a Ca^{2+}/calmodulin-regulated adenylyl cyclase encoded by the *rutabaga*-gene (Han et al., 1992). It has been suggested that this enzyme serves as a coincidence detector during conditioning in *Drosophila* and integrates cellular signals mediated by GPCR activation and/or the Ca^{2+}/calmodulin complex (Davis, 1993). Therefore, the co-localization of DAMB and the *rutabaga*-gene product in axons of mushroom body neurons as well as the ability of DAMB to activate adenylyl cyclase make this receptor an attractive candidate to mediate the effects of reinforcers during associative conditioning (Han et al., 1996).

Recently, a number of partial cDNA clones that probably code for biogenic amine receptors have been isolated from the honeybee by library scanning (Ebert et al., 1998). One of these fragments served to isolate a full-length cDNA clone (Am*BAR6*; Kokay et al., 1999; Humphries et al., unpublished data). The deduced amino acid sequence of Am*BAR6* (AmBAR6) shares ~70% identity with DAMB suggesting that it is the honeybee orthologue of the *Drosophila* dopamine receptor. Whole-mount in situ hybridization to worker honeybees and drones revealed that the expression pattern of Am*BAR6* is restricted to the mushroom bodies but differs between large and small diameter Kenyon (= intrinsic mushroom body) cells (Humphries and Ebert, 1998). In both sexes, Am*BAR6* mRNA is highly expressed in small-diameter Kenyon cells whereas expression levels in larger diameter Kenyon cells were variable and increased with the age of the worker bee (Humphries and Ebert, 1998). Whether AmBAR6 is a member of the D1-like dopamine receptor subfamily still awaits pharmacological characterization as well as identification of its intracellular transduction pathway.

Putative D2-Like Dopamine Receptors

Although dopamine D2-like receptors have not yet been cloned from *Drosophila* or other insects, pharmacological investigations suggest that they exist (Davis and Pitman, 1991; Granger et al., 1996; Yellman et al., 1997; Andretic and Hirsh, 2000). Receptors that display a D2-like pharmacology were identified in the honeybee. High affinity binding of [^{3}H]spiperone (K_D ~0.1 nM) was observed in brain homogenates (Kokay and Mercer, 1996). In addition to the pronounced pharmacological similarity with mammalian D2-like receptors, the honeybee [^{3}H]spiperone binding site also exhibits a phenolaminergic component (Kokay and Mercer, 1996). Therefore, it was assumed that the ligand will bind to other, most likely tyramine and/or octopamine receptors, as well. Incubation of brain sections with the radioligand showed that the binding sites are concentrated in the α- and β-lobes and calyces of the mushroom bodies (Kokay et al., 1998). In primary cultures of antennal lobe neurons, spiperone binding sites were also described (Kirchhof and Mercer, 1997; Kokay et al., 1999). A cDNA fragment (Am*BAR3*; Ebert et al., 1998) has recently been identified that is very similar to human dopamine D2-receptors and has led to the cloning of a full-length cDNA for a putative dopamine D2-like receptor from the honeybee (Kokay et al., 1999).

In summary, the dopamine receptors cloned from insects display almost unique pharmacological properties that set them apart from vertebrate receptors. The functional coupling of individual receptors to certain intracellular messenger systems is an alternative way to classify receptor subtypes. At present, two D1-like receptors have been characterized in *Drosophila* (DmDOP1, DAMB) and the honeybee (AmDOP1, AmBAR6). Whether additional subtypes are expressed should soon be answered with the availability of the complete genomic sequence of *Drosophila* (Adams et al., 2000). The presence of D2-like dopamine receptors in insects still has to be confirmed by additional cloning efforts and functional characterization of receptor candidates in both *Drosophila* and the honeybee.

RECEPTORS FOR TYRAMINE AND OCTOPAMINE

High concentrations of the phenolamines tyramine and octopamine are found in insect nervous tissue, whereas only trace amounts, if any, have been detected in vertebrate brains (for reviews see: Axelrod and Saavedra, 1977; David and Coulon, 1985). Little is known about the physiological role of tyramine in insects. It was assumed

that tyramine might only serve as biochemical precursor of octopamine rather than being a neuroactive substance itself. However, with the molecular cloning (see below) of specific tyramine receptors, and recent reports that attribute a role to tyramine in cocaine sensitization in *Drosophila* (McClung and Hirsh, 1999), a new picture is emerging.

In contrast to tyramine, the physiological role of octopamine has been thoroughly studied in a number of invertebrate species. Octopamine has been shown to act as a neurotransmitter, neuromodulator, and neurohormone, and modulates/regulates various behavioral patterns in insects (for reviews see: David and Coulon, 1985; Bicker and Menzel, 1989; Erber et al., 1993; Roeder, 1999). Often, octopamine is considered as a "fight or flight" hormone in insects and its physiological functions are compared with those of norepinephrine and epinephrine in vertebrates (Evans, 1993; Roeder, 1999).

In *Drosophila*, interneurons and efferent neurons constitute the octopaminergic neuronal population (Monastirioti et al., 1995; Monastirioti, 1999). In the larval CNS, all octopamine immunoreactive somata are localized in the midline of the ventral ganglion while in the adult CNS immunoreactivity is observed in clusters of both unpaired and bilateral neurons (Monastirioti et al., 1995; Monastirioti, 1999). When flies were fed with formamidines that probably bind to octopamine receptors they displayed impaired learning after classical conditioning (Dudai et al., 1987). Null mutations for the enzyme TβH, which is essential for the synthesis of octopamine (see Biogenic Amines Derived From Tyrosine), have been generated by P element insertion (Monastirioti et al., 1996). Interestingly, the animals are viable and do not show severe phenotypic alterations. Female flies, however, are sterile because they cannot deposit their eggs but sterility is rescued by feeding them with octopamine (Monastirioti et al., 1996). Although these results suggest that octopamine might not be as important for development and differentiation as dopamine (see Dopamine Receptors) it is currently unclear whether or not the loss of octopamine might have been functionally substituted by promiscuous binding of tyramine to octopamine receptors. The mutants that are unable to produce octopamine, however, will certainly help to unravel the contribution of octopamine in *Drosophila* learning and memory.

In the honeybee brain, five clusters of ~100 octopamine-immunoreactive somata were identified (Kreissl et al., 1994). Varicose octopaminergic fibers invade all parts of the brain and the suboesophageal ganglion except the pedunculi of the mushroom bodies and large parts of the α- and β-lobes (Kreissl et al., 1994). It has been shown that octopamine modulates many physiological functions such as the proboscis extension response (Mercer and Menzel, 1982; Braun and Bicker, 1992), sting response (Burrell and Smith, 1995), juvenile hormone release from the corpora allata (Rachinsky, 1994; Kaatz et al., 1994), and the discrimination of nestmates from unrelated bees (Robinson et al., 1999). Injection of octopamine into different areas of the CNS enhances neural activity and facilitates motor-reflexes (Erber et al., 1993; Erber and Kloppenburg, 1995; Kloppenburg and Erber, 1995; Pribbenow and Erber, 1996). One particular octopaminergic neuron, VUM_{mx1}, plays an important role in the reinforcement pathway during honeybee olfactory conditioning (Hammer, 1993). The VUM_{mx1} neuron depolarizes in response to the presentation of sucrose rewards to antennae and proboscis. Current injection into the VUM_{mx1} neuron or octopamine injection into either the antennal lobe or the calyces of the mushroom bodies can substitute for the sucrose reward during olfactory conditioning (Hammer and Menzel, 1998). It was concluded that octopamine is involved in selectively mediating the reinforcing but not the sensitizing or response-releasing function of the sucrose reward (Menzel et al., 1999).

Numerous pharmacological studies have been performed in locusts in order to classify octopamine receptor classes. According to their pharmacological properties and intracellular signaling pathways, four different classes were identified: OCT-1, OCT-2A, OCT-2B, and OCT-3 (for reviews see: Evans and Robb, 1993; Roeder, 1999). Activation of OCT-1 receptors induces an increase in $[Ca^{2+}]_i$, whereas activation of OCT-2A, OCT-2B, or OCT-3 receptors stimulates adenylyl cyclase and leads to increases in $[cAMP]_i$. In comparison to the locust, only a few studies were performed in *Drosophila* and the honeybee to determine the pharmacological properties of octopamine receptors (Table 3; Dudai and Zvi, 1984a; Degen et al., 2000). In *Drosophila* head homogenates, octopamine is a potent stimulator of

TABLE 3. Pharmacological properties of tyramine and octopamine receptors in *Drosophila melanogaster* and *Apis mellifera*

	Drosophila DmTYR in CHO-K1: [^{3}H]yohimbine (K_i[nM])	*Drosophila* DmTYR in COS-7: [^{3}H]yohimbine (K_i[nM])	*Drosophila* head homogenate: [^{3}H]yohimbine binding site (K_i[nM])	*Drosophila* head homogenate: [^{3}H]octopamine binding site (EC_{50}[nM])	*Apis* brain homogenate: [^{3}H]NC 5Z binding site (K_i[nM])
Biogenic amines					
Tyramine	1,400	1,200	380	300	51.4
DL-Synephrine	10,800	20,000	—	30	34.4
DL-Octopamine	129,200	40,000	62,100	6	13.4
Dopamine	137,900	50,000	—	5,000	—
(–)-Epinephrine	139,000	70,000	—	1,000	—
L(–)-Norepinephrine	—	150,000	—	700	—
Serotonin	75,000	175,000	—	20,000	—
Other ligands					
Yohimbine	6.2	5.5	4.6	4,000	—
Chlorpromazine	180	25	—	300	553
Phentolamine	2,200	85	350	20	48.7
Mianserin	1,200	100	—	—	0.73
Cyproheptadine	2,600	175	—	—	—
Metoclopramide	4,600	—	—	—	812
Clonidine	21,000	15,000	—	20	—

Values for DmTYR expressed in CHO-K1 cells are from Arakawa et al. (1990) and from Robb et al. (1994). Values for DmTYR expressed in COS-7 cells are from Saudou et al. (1990). Values for the *Drosophila* [^{3}H]yohimbine binding site are from Robb et al. (1994) and values for the *Drosophila* [^{3}H]octopamine binding site are from Dudai and Zvi (1984a). Values for the honeybee [^{3}H]NC-5Z binding site are from Degen et al. (2000). Note the higher affinity of tyramine compared to octopamine for the cloned DmTYR-receptor and the [^{3}H]yohimbine binding site in *Drosophila* head homogenates. This is in contrast to the nanomolar affinity of octopamine for native octopamine receptor binding sites.

adenylyl cyclase activity (Uzzan and Dudai, 1982). Interestingly, simultaneous application of both tyramine and octopamine reduces the effect of octopamine. This observation suggests that tyramine activates specific tyramine receptors that inhibit adenylyl cyclase and thereby reduce the stimulatory effect of octopamine (Uzzan and Dudai, 1982). In membrane homogenates of honeybee brains, octopamine also stimulates cAMP production (Blenau et al., 1996). In addition, injections of octopamine into the antennal lobe of the honeybee, evoke a rapid and transient activation of PKA (Hildebrandt and Müller, 1995). The effects of tyramine were also tested on membrane preparations from honeybee brain. When tyramine is applied at high concentrations, it activates adenylyl cyclase (EC_{50} of ~2.2 μM) but at low concentrations (0.1–1 μM) it attenuates forskolin-stimulated cAMP production (Blenau et al., 1996, 2000). Taken together, the results indicate that both octopamine and tyramine mediate their effects by binding to different members of the GPCR family.

The distribution of binding sites for [^{3}H]octopamine in tissue sections of honeybee brain has been analyzed with autoradiographic methods (for a review see Erber et al., 1993). Specific and high labeling densities were detected in the mushroom bodies, especially in the pedunculus and in the α- and β-lobes. Interestingly, these brain regions are not innervated by octopaminergic neurons (Erber et al., 1993; Kreissl et al., 1994). Phentolamine displaced ~93% of [^{3}H]octopamine binding in all brain areas except the mushroom bodies (~70% displacement). These results suggested that octopamine receptors in the mushroom bodies may be pharmacologically different from those in the rest of the brain (Erber et al., 1993).

Drosophila DmTYR- and the Honeybee AmTYR1-Receptor

A gene encoding the first member of the tyramine/octopamine receptor family from *Drosophila* (Dm*oct/tyr*) was independently cloned by two groups (Arakawa et al., 1990; Saudou et al., 1990). The deduced amino acid sequence of DmOCT/TYR is highly homologous to mammalian α_2-adrenergic receptors (Arakawa et al., 1990). The functional coupling to intracellular sig-

naling pathways was examined after heterologous expression of the gene in either Chinese hamster ovary (CHO-K1; Arakawa et al., 1990) or COS-7 cells (Saudou et al., 1990). Application of tyramine or octopamine attenuated forskolin-stimulated adenylyl cyclase activity (Arakawa et al. 1990; Saudou et al., 1990). The affinity of tyramine to the receptor is ~12 times higher than that of octopamine (Table 3; Saudou et al., 1990; Robb et al., 1994). This led to the suggestion that the gene most likely encodes a functional tyramine receptor (Saudou et al., 1990). In subsequent biochemical studies, it was shown that the receptor mediates both inhibition of adenylyl cyclase activity and elevation of $[Ca^{2+}]_i$ (Robb et al., 1994). Tyramine is about two orders of magnitude more potent than octopamine in inhibiting forskolin-induced cAMP accumulation, whereas octopamine is slightly more potent in elevating $[Ca^{2+}]_i$ (Robb et al., 1994). Interestingly, when expressed in *Xenopus* oocytes, stimulation of DmOCT/TYR with either ligand led to an increase of $[Ca^{2+}]_i$ but failed to inhibit adenylyl cyclase activity (Reale et al., 1997b). Thus, this *Drosophila* receptor displays "agonist-specific coupling to different second messenger systems," which seems to depend on the specific supply of G proteins provided by the different cell types used for heterologous expression (Robb et al., 1994; Reale et al., 1997b).

Recently, a *Drosophila* mutant (*hono*) was identified that shows impaired olfactory avoidance behavior to repellents (Kutsukake et al., 2000). This strain is a β-Gal enhancer-trap line generated using a *P*-element mutagenesis approach aimed at identifying novel olfactory mutants. Standard cloning techniques revealed that the *P*-element is located in the promoter region of the Dm*oct/tyr*-gene and causes reduced expression of the Dm*oct/tyr* mRNA (Kutsukake et al., 2000). In addition to the reduced olfactory sensitivity, the mutation also reduced the electrical responses to tyramine at the neuromuscular junction in the larval body-wall whereas responses to octopamine remained normal (Kutsukake et al., 2000). Since *hono* is the first biogenic amine receptor mutant identified in invertebrates and the effects caused by the *hono* mutant are specifically correlated with impaired functions of tyramine, the Dm*oct/tyr*-gene should be considered as a "true" tyramine receptor gene (Dm*tyr*).

The expression pattern of the *hono*-gene was examined by staining for β-Gal positive cells. In addition to the antennae, many areas of the adult brain and thoracic ganglion were stained (Kutsukake et al., 2000). This result agrees well with the mRNA expression pattern that was independently determined by in situ hybridization to tissue sections of wild-type flies (Hannan and Hall, 1996). In third instar larvae of the *hono* mutant, β-Gal staining was detected in the olfactory organ (dorsal organ), in the CNS, and along the morphogenetic furrow of the eye-antennal disc. The results strongly suggest that the DmTYR-receptor is important in various aspects of olfactory information processing (Kutsukake et al., 2000).

An orthologue of the *Drosophila* tyramine receptor gene was cloned from *Apis mellifera* (Am*tyr1*; Blenau et al., 2000). Pronounced sequence conservation of 83.2 and 78.2% was observed between the deduced amino acid sequence of Am*tyr1* and a tyramine receptor cloned from *Locusta migratoria* (LocTYR; Vanden Broeck et al., 1995) and an octopamine receptor cloned from *Heliothis virescens* (K50Hel = HelOCT; von Nickisch-Rosenegk et al., 1996), respectively. Multiple alignments of invertebrate receptor sequences showed that AmTYR1, DmTYR, LocTYR, and HelOCT form a distinct group within the biogenic amine receptor family (see Fig. 8). When stably expressed in HEK 293 cells, the AmTYR1-receptor attenuated forskolin-induced cAMP production after stimulation with tyramine in a dose-dependent manner. The EC_{50} for tyramine was ~130 nM. Octopamine also reduced cAMP production in the transfected cell line but was both less potent (EC_{50} ~3 μM) and less efficacious than tyramine (Blenau et al., 2000). Similar to the *Drosophila* Dm*tyr*-gene (Hannan and Hall, 1996; Kutsukake et al., 2000), Am*tyr1* mRNA is abundantly expressed in many neurons of the honeybee brain, including neurons of the optic lobes, mushroom body intrinsic neurons, and neurons of the deutocerebrum. Whether the honeybee AmTYR1-receptor participates in olfactory signaling as has been demonstrated for the *Drosophila* DmTYR-receptor is currently unknown.

Drosophila OAMB- and the Honeybee AmOCT1- (= AmBAR1-) Receptor

A cDNA encoding an octopamine receptor (OAMB) was isolated from a *Drosophila* head specific library (Han et al., 1998). The deduced amino

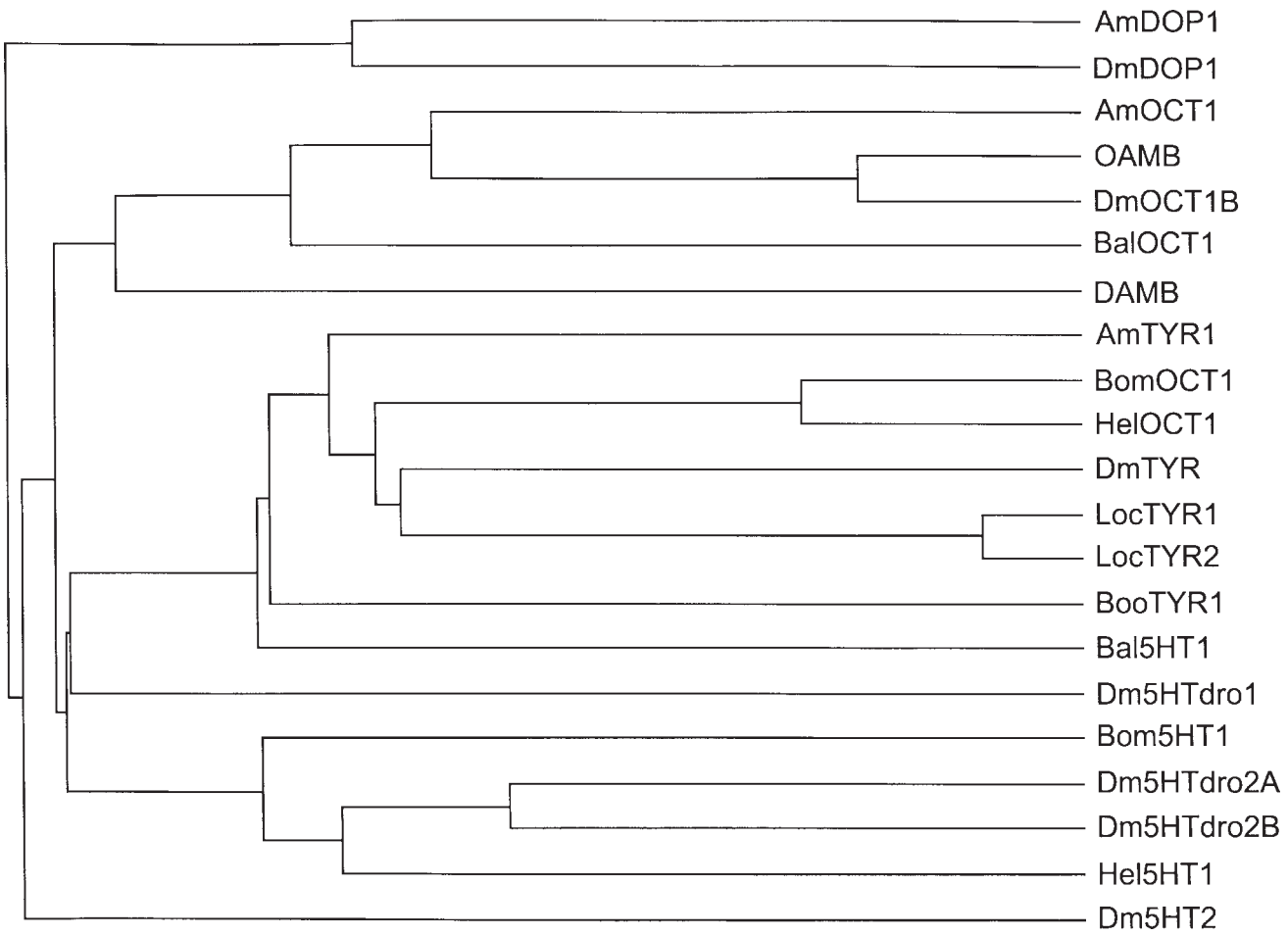

Fig. 8. Dendrogram of arthropod biogenic amine receptors. Sequence alignment was done with the CLUSTAL program of PCGENE software (version 6.6; IntelliGenetic), using the complete amino acid sequence of each receptor. The receptor sequences followed by their GenBank/EMBL accession numbers (#) are listed in the order illustrated: *Apis mellifera* dopamine D1 (AmDOP1, #Y13429), *Drosophila melanogaster* dopamine D1 (DmDOP1, #X77234), *A. mellifera* octopamine (AmOCT1), *D. melanogaster* octopamine (OAMB, #AF065443; DmOCT1B, #AJ007617), *Balanus amphitrite* (barnacle) putative octopamine (BalOCT1, #D78363), *D. melanogaster* dopamine (DAMB, #U61264), *A. mellifera* tyramine (AmTYR1, #AJ245824), *Bombyx mori* octopamine (BomOCT1, #Q17232), *Heliothis virescens* octopamine (HelOCT1, Q25188), *D. melanogaster* tyramine (DmTYR, #M60789), *Locusta migratoria* tyramine (LocTYR1, #Q25321; LocTYR2, #Q25322), *Boophilus microplus* (cattle tick) putative tyramine (BooTYR1, #AJ010743), *B. amphitrite* putative serotonin (Bal5HT1, #D83547), *D. melanogaster* serotonin (Dm5HTdro1, #P20905), *B. mori* putative serotonin (Bom5HT1, #Q25414), *D. melanogaster* serotonin (Dm5HTdro2A, #Z11489; Dm5HTdro2B, #Z11490), *H. virescens* putative serotonin (Hel5HT1, X95605), and *D. melanogaster* serotonin (Dm5HT2, X81835).

acid sequence is very similar to those of mammalian adrenergic as well as invertebrate tyramine and dopamine receptors. One unique feature of OAMB is the extension of EL2. This loop usually contains ~20 amino acids but in OAMB consists of 130 residues. The functional significance of this extension is still unknown (Han et al., 1998). Expression of the OAMB-gene in either *Drosophila* S2 or HEK 293 cells led to an increase in $[cAMP]_i$. The EC_{50} for octopamine was $\sim 1.9 \times 10^{-7}$ M (Han et al., 1998). Tyramine was much less effective, strongly suggesting that the gene encodes a functional octopamine receptor. In addition to cAMP production, octopamine also induced an increase in $[Ca^{2+}]_i$ in OAMB expressing cells although only a relatively high concentration of octopamine (10^{-5} M) was tested (Han et al., 1998). Since octopamine receptors were originally classified according to their coupling to either $[cAMP]_i$ or to $[Ca^{2+}]_i$ (see above), the OAMB-receptor is difficult to ascribe to any one of the existing receptor classes (Han et al., 1998).

The tissue distribution of the OAMB mRNA and protein were examined by in situ hybridization and immunohistochemistry (Han et al., 1998). OAMB transcripts were detected in perikarya of intrinsic mushroom body neurons, and in two clusters of cells in the anterior brain cortex in proximity to the mushroom body lobes. In addition, cells scattered throughout the central brain and medulla were stained. Immunoreactivity was observed in the calyces, pedunculi, α-, β-, and γ-lobes of the mushroom bodies, and in the ellipsoid body of the central complex (Han et al., 1998). Since OAMB can activate adenylyl cyclase and is preferentially expressed in the mushroom bodies, it was suggested that this receptor could participate in olfactory learning in *Drosophila* (Han et al., 1998).

In the honeybee, the octopaminergic VUM_{mx1} neuron is involved in olfactory conditioning (Hammer, 1993) and it is assumed that specific octopamine receptors modulate the biochemical signaling cascades within the olfactory pathway. To date, sequence information on only one octopamine receptor gene has emerged (Am*BAR1*; Ebert et al., 1998; Am*oct1*, Grohmann et al., 2000). The deduced amino acid sequence displays high similarity to the OAMB-gene from *Drosophila*. When expressed in HEK 293 cells, AmOCT1 activation leads to an increase in $[Ca^{2+}]_i$ and $[cAMP]_i$ (Grohmann et al., 2000). In contrast to OAMB, submicromolar concentrations of octopamine are sufficient to induce the Ca^{2+} response whereas micromolar or higher concentrations are necessary to activate adenylyl cyclase. In situ hybridization to honeybee brain sections showed that the gene is expressed in some perikarya of intrinsic mushroom body neurons, in somata of the antennal and optic lobes, and in somata of the suboesophageal ganglion (Grohmann et al., 2000).

Tyramine and octopamine receptor genes have also been cloned from *Locusta migratoria* (GenBank accession nos. Q25322, Q25321; Vanden Broeck et al., 1995) as well as *Bombyx mori* and *Heliothis virescens* (Q17323 and Q25188; von Nickisch-Rosenegk et al., 1996). The physiological role of these receptors still awaits identification.

SEROTONIN RECEPTORS

Serotonin (see Serotonin Is Derived From Tryptophan) serves as a neurotransmitter or neuromodulator in most animal species. Serotonin regulates or modulates a wide variety of behaviors such as aggression in lobsters, feeding and learning in snails, locomotion in lampreys, as well as sleep, appetite, and mood in mammals (for reviews see Bicker and Menzel, 1989; Hen, 1992; Boess and Martin, 1994).

In *Drosophila*, as in most arthropods, the majority of serotonergic neurons are interneurons in the brain and in the ventral nerve cord (Vallés and White, 1988). The total number of these neurons is small (~100 cells in *Drosophila*) but they send projections to most parts of the nervous system. Serotonergic innervation is also found in the pharyngeal muscles as well as in the ring gland, the endocrine organ of the larvae (Vallés and White, 1988), suggesting that serotonin modulates feeding behavior and neuroendocrine activity of *Drosophila*. During *Drosophila* development, a peak in serotonin concentration precisely coincides with the onset of germband extension in gastrulating embryos. The peak of serotonin synthesis strictly depends on the maternal deposition of biopterins into the egg and the zygotic synthesis of TRH and DDC (see Serotonin Is Derived From Tryptophan; Colas et al., 1999a). Mutants with impaired serotonin synthesis are embryonic lethal and/or display abnormal gastrulation movements and cuticular defects (Colas et al., 1999a).

The distribution of serotonin-immunoreactivity has been intensively studied in the brain of adult (Schürmann and Klemm, 1984; Nässel et al., 1985; Schäfer and Bicker, 1986; Rehder et al., 1987) and pupal worker honeybees (Seidel and Bicker, 1996). A small set of ~75 cell bodies gives rise to neuronal branches that arborize into most parts of the brain neuropil. The adult set of serotonergic neurons consists of both persistent embryonic neurons and neurons that differentiate during pupal metamorphosis. Behavioral experiments have shown that serotonin often functionally antagonizes the effects of octopamine and reduces or blocks rhythmic behavior and reflexes (Erber et al., 1993; Erber and Kloppenburg, 1995; Kloppenburg and Erber, 1995; Pribbenow and Erber, 1996; Blenau and Erber, 1998). Serotonin also has profound effects on learning and memory in impairing acquisition and retrieval of learned behaviors (for reviews see Bicker and Menzel, 1989; Menzel et al., 1994).

In vertebrates, serotonin binds to and activates different types of membrane receptors. From 14 receptor genes cloned, one forms an ion channel (5-HT_3). All other genes encode GPCRs. Five receptors inhibit cAMP production (5-$HT_{1A/B/D/E/F}$), three receptors induce cAMP production (5-$HT_{4/6/7}$), three receptors lead to an increase in $[Ca^{2+}]_i$ (5-$HT_{2A/B/C}$), and for two receptors (5-$HT_{5A/B}$) the intracellular signaling pathways have not yet been determined (Hoyer and Martin, 1997). Besides their functional coupling characteristics, serotonin receptors were classified according to their binding properties to specific (synthetic) compounds (for a review see: Boess and Martin, 1994).

Serotonin-sensitive binding sites have also been characterized in *Drosophila* head homogenates (Table 4; Dudai and Zvi, 1982, 1984b) and in membrane preparations of honeybee brains (Table 4; Blenau et al., 1995b). Autoradiographic studies in the honeybee showed that receptor density was low in the antennal lobes but high in the calyces of the mushroom bodies (for a review see: Erber et al., 1993). Similar to the different signaling pathways described for vertebrate serotonin receptors, it was shown in membrane homogenates of honeybee brains that serotonin not only stimulates cAMP production but also inhibits forskolin-induced cAMP production (Blenau et al., 1996). Molecular cloning and functional characterization of serotonin receptors from *Drosophila* revealed that serotonin also operates through different signaling pathways in the fly (Hen, 1992).

Drosophila Dm5HTdro1-Receptor

Using a homology screening approach, the first *Drosophila* serotonin receptor gene (Dm5HTdro1; Witz et al., 1990) was cloned from a head specific cDNA library. The deduced amino acid sequence shares considerable sequence similarity with mammalian 5-HT_7-receptors (Gerhardt and van Heerikhuizen, 1997). A hydropathy profile analysis revealed an interesting feature in the N-terminus of Dm5HTdro1. In addition to the seven TMs, an eighth hydrophobic stretch was identified (Witz et al., 1990). This segment is theoretically long enough to span the membrane as a TM but could also serve as an internal signal sequence that is cleaved off during maturation of the protein. However, an additional TM segment would perturb the transmembrane architecture of the receptor and cause exposure of usually intracellular loops to the external surface. Such an orientation would render the receptor non-functional. This, however, is not the case as has been demonstrated using heterologous expression of the Dm5HTdro1-gene in either mouse fibroblasts (NIH 3T3 cells; Witz et al., 1990) or insect Sf9 cells (Obosi et al., 1996). In both cell lines, application of serotonin or receptor agonists led to an increase in $[cAMP]_i$. Stimulation of adenylyl cyclase is a common feature of vertebrate 5-$HT_{4/6/7}$-receptors. Therefore, Dm5HTdro1 not only shares sequence similarity to this group of vertebrate serotonin receptors, but also activates the same intracellular signaling pathway. However, as has already been shown for other invertebrate receptors, the pharmacological properties of Dm5HTdro1 differ quite significantly from those of vertebrate 5-$HT_{4/6/7}$-receptors (Table 4; Saudou et al., 1992). Particularly striking is the low affinity of 8-OH-DPAT for the *Drosophila* receptor (Table 4).

Another sequence motif in the N-terminus of Dm5HTdro1 provides a clue to a possible physiological role for this receptor in the fly. A stretch of Ser-Gly repeats is located between the newly identified eighth hydrophobic domain and the first putative TM. Repetitive stretches of Ser-Gly or Thr-Gly motives have been implicated as attachment sites for posttranslational modifications with glycosaminoglycans. Similar motives were already identified in biological clock genes like *period* in *Drosophila* or *frequency* in *Neurospora*. It was assumed, therefore, that Dm5HTdro1 might also be linked to glycosaminoglycans (Witz et al., 1990). A function of this modification could be to localize the receptor in a specialized compartment or subdomain of the cell.

Drosophila Dm5HTdro2A- and Dm5HTdro2B-Receptors

Molecular cloning of two additional, closely related serotonin receptor genes again was achieved by an homology based screening approach (Saudou et al., 1992). The deduced amino acid sequences of Dm5HTdro2A- and Dm5HTdro2B-receptors share 84.3% sequence homology. Both genes encode GPCRs that also share sequence similarity with mammalian 5-HT_{1A}-receptors (Gerhardt and van Heerikhuizen, 1997). When the *Drosophila*

TABLE 4. Pharmacological Properties of Serotonin Receptors in *Drosophila melanogaster* and *Apis mellifera*

	Drosophila Dm5HTdro1 in COS-7: [^{125}I]LSD binding (K_i[nM])	*Drosophila* Dm5HTdro2A in COS-7: [^{125}I]LSD binding (K_i[nM])	*Drosophila* Dm5HTdro2B in COS-7: [^{125}I]LSD binding (K_i[nM])	*Drosophila* Dm5HT2 in COS-1: [^{125}I]DOI binding (K_i[nM])	*Drosophila* head homogenate: [^{3}H]serotonin binding site (IC_{50}[nM])	*Apis* brain homogenate: serotonin-sensitive [^{3}H]LSD binding site (K_i[nM])
Biogenic amines						
Serotonin	1,600	16,000	2,100	15	1.4 130	2.6
Tryptamine	—	—	—	1,580	700	130
5-Methoxytryptamine	—	—	—	5,010	700	43
Tyramine	>200,000	>200,000	>200,000	≥100,000	80,000	>>10,000
DL-Octopamine	>200,000	>200,000	>200,000	≥100,000	—	>>10,000
Dopamine	>200,000	>200,000	>200,000	≥100,000	200,000	47,000
L(–)-Norepinephrine	>200,000	>200,000	>200,000	—	—	>>10,000
Histamine	>200,000	>200,000	>200,000	≥100,000	—	>>10,000
Other ligands						
LSD	0.44	0.26	0.31	—	—	0.89
Dihydroergocryptine	13	11	3.8	—	—	—
S(+)-Butaclamol	32	330	64	—	—	>10,000
R(–)-Butaclamol	31,000	14,000	30,000	—	—	>>10,000
Prazosin	9,800	250	180	—	—	—
Methysergide	1,200	1,400	720	79	40	22
Yohimbine	32,000	18,000	9,800	398	50,000	—
Mianserin	—	—	—	1,995	—	1,500
8-OH-DPAT	106,000	43,000	27,000	12,600	—	2,300

Values for Dm5HTdro1, Dm5HTdro2A, and Dm5HTdro2B are from Saudou et al. (1992). Values for Dm5HT2 are from Colas et al. (1995). Values for the *Drosophila* [^{3}H]serotonin binding site are from Dudai and Zvi (1984b) and values for the serotonin-sensitive [^{3}H]LSD binding site of the honeybee are from Blenau et al. (1995b). Note the low affinity of 8-OH-DPAT for the *Drosophila* Dm5HTdro1-receptor.

genes were mapped on the chromosomes, the loci were located within the same chromosomal band. This suggested that the genes most likely arose by duplication of a common ancestor gene. In addition to their sequence homology to vertebrate 5-HT_1-receptors, both *Drosophila* receptors also share the intracellular transduction pathway with their vertebrate counterparts. Application of serotonin to heterologously expressed receptors decreased forskolin-stimulated cAMP production with an EC_{50} of ~3×10^{-8} M and ~1.8×10^{-8} M for Dm5HTdro2A and Dm5HTdro2B, respectively. In addition to the inhibition of adenylyl cyclase, both receptors moderately activate PLC in response to serotonin (Saudou et al., 1992). The specificity of these receptors for serotonin was confirmed by direct radioligand binding assays (Table 4; Saudou et al., 1992). Serotonin efficiently displaced the radioligand [^{125}I]LSD from both receptors whereas dopamine, octopamine, tyramine, and histamine were ≥100-fold less efficient. The expression patterns of the genes were examined by Northern blotting and in situ hybridization (Saudou et al., 1992). Transcripts were detected in RNA preparations from embryos, larvae, and adult heads. Expression in the embryo was restricted to the CNS. The two receptors, however, were expressed in distinct cell populations. While Dm5HTdro2A is predominantly found in VUM neurons, cells that are involved in axonal guidance during commissure formation in embryonic development (Klämbt et al., 1991), the Dm5HTdro2B most likely is expressed in motor neurons. Although the subcellular distribution of the receptor proteins has not yet been examined, it was suggested that both receptors could control motor activity in the fly (Saudou et al., 1992).

Drosophila Dm5HT2-Receptor

Isolation of the Dm5HT2-receptor gene again was achieved by a homology screening approach (Colas et al., 1995). When compared to vertebrate serotonin receptors, the Dm5HT2 amino acid sequence displayed striking similarity to 5-HT_2-receptors (Gerhardt and van Heerikhuizen, 1997). Activation of these receptors causes an increase in $[Ca^{2+}]_i$. The transduction mechanism of Dm5HT2 has not yet been reported. In comparison to the deduced amino acid sequences of mammalian 5-HT_2-receptors, the only difference in Dm5HT2 is the longer size of the N-terminus. A partial deletion of the N-terminus of Dm5HT2 does not alter its pharmacological properties but significantly increases its expression pattern in mammalian cell lines (Colas et al., 1997). Therefore, it is likely that the long N-terminus interferes with proper processing/maturation of the receptor in heterologous expression systems. The pharmacological properties of the wild-type Dm5HT2-receptor were determined after expression of the gene in COS-1 cells (Table 4; Colas et al., 1995, 1997). Binding studies showed that the pharmacological profile of the receptor, in addition to its striking sequence similarity, correlated well with that of mammalian 5-HT_2-receptors, but not with any other cloned serotonin receptor (Colas et al., 1995, 1997).

The expression pattern of the Dm5HT2-gene was examined by in situ hybridization and quantitative RT-PCR (Colas et al., 1995). The gene is expressed during embryogenesis as well as in the larval and adult CNS. Interestingly, the mRNA is already found after 3 h of embryonic development. In the embryo, the mRNA is present in seven evenly spaced transverse stripes along the antero-posterior axis. This pattern is very similar to that of pair-rule genes. Comparison with expression patterns of different pair-rule genes finally showed that Dm5HT2 is co-expressed with *fushi-tarazu* in the even-numbered parasegments (Colas et al., 1995). It is most likely that the Dm5HT2-receptor is necessary for proper germband extension. Mutants that do not express Dm5HT2 are embryonic lethal. They show a significant delay in germband extension, which results in uncoupling of ectodermal elongation from endoderm and mesoderm invaginations (Colas et al., 1999b).

A serotonin receptor gene was also cloned from *Bombyx mori* (Q17239; von Nickisch-Rosenegk et al., 1996). All cloned insect serotonin receptors are members of the GPCR family. A serotonin-gated ion channel comparable to the mammalian 5-HT_3-receptor has not been described in insects, so far. While amino acid sequences and transduction mechanisms are often well conserved between insect and mammalian receptors, the pharmacological properties often differ considerably and do not allow insect receptors to be classified according to existing mammalian schemes (Saudou et al., 1992; Blenau et al., 1995b).

HISTAMINE RECEPTORS

Histamine has been established as the major neurotransmitter that is released from insect photoreceptor cells in response to illumination (for reviews see: Nässel, 1991, 1999). In *Drosophila,* histamine-like immunoreactivity was also detected in a small number (18–24) of neurons within the brain (Nässel et al., 1990; Sarthy, 1991; Pollak and Hofbauer, 1991; Nässel and Elekes, 1992). Many of these neurons have extensive bilateral arborizations that innervate distinct regions of neuropil. It has also been shown in *Drosophila* that almost all mechanosensory neurons of imaginal hair sensilla contain histamine (Buchner et al, 1993). The brain of the honeybee contains about 150 histaminergic neurons (Bornhauser and Meyer, 1997). The axons of these neurons innervate most parts of the protocerebrum except the mushroom bodies (Bornhauser and Meyer, 1997). Photoreceptor fibers terminating either in the lamina or in the medulla as well as axons emanating from ocellar photoreceptors also contain histamine (Bornhauser and Meyer, 1997).

Histamine synthesis has been experimentally impaired by mutations in the gene encoding HDC (see Histamine Is Derived From Histidine; Burg et al., 1993). Although the mutants (*hdc*) are devoid of histamine, they are viable. This suggests that histamine does not have a vital function during development or in the adult. However, HDC null mutants are blind (Burg et al., 1993). This is most likely due to non-existing synaptic transmission between photoreceptor terminals and postsynaptic monopolar cells in the lamina or medulla. In addition to its role in the visual system, analysis of several other *hdc* alleles suggested that histamine is important for mechanosensory transduction as well (Melzig et al., 1996). It was shown recently that a histamine-selective uptake mechanism can restore the wild-type functions of photo- and mechanoreceptors in *hdc* mutants (Melzig et al., 1998). Besides release from receptor-neurons, histamine is also important for communication between interneurons. In the cricket *Gryllus bimaculatus*, histamine has also been shown to inhibit an auditory interneuron within the prothoracic ganglion (ON1; Skiebe et al., 1990). Therefore, histamine should be considered as a sensory transmitter as well as a modulator of interneurons in insects.

So far, three histamine receptor genes have been cloned from vertebrates. They all belong to the family of GPCRs. Histamine H_1-receptors lead to an increase in $[Ca^{2+}]_i$, whereas histamine H_2-receptors activate and histamine H_3-receptors inhibit adenylyl cyclase (Hill et al., 1997). Selective agonists and antagonists are available that allow these different receptor subtypes to be differentiated pharmacologically.

No invertebrate histamine receptor homologues have yet been cloned. However, binding studies with different radioligands, as well as attempts to purify receptors from membrane preparations, have already been performed in the locust (Elias et al., 1984; Roeder, 1990; Roeder et al., 1993, 1995). At present, it is not clear whether invertebrate histamine receptors belong to the family of GPCRs. Since electrophysiological investigations have shown that histamine released from photoreceptors activates chloride currents in postsynaptic monopolar cells (Hardie, 1987, 1989; Skingsley et al, 1995), one could speculate that invertebrates possess (only) histamine-gated ion channels. Nevertheless, ongoing and already completed genome projects will certainly assist us to answer this question in the near future.

CONCLUSIONS

Biogenic amines are important mediators and/or regulators of neuronal signaling in the central and peripheral nervous system of insects. A prerequisite to understanding the physiological role of biogenic amine signaling systems is the molecular characterization of biogenic amine receptors and their downstream reaction partners. The application of molecular biological methods has greatly enhanced our knowledge of the receptor polypeptides. As in vertebrates, multiple receptor subtypes mediate the actions of biogenic amines in insects. All biogenic amine receptors identified so far in invertebrates belong to the superfamily of GPCRs and activation of heterologously expressed receptor polypeptides generally activate the same intracellular signaling pathways as native receptors do in vivo. However, several receptor subtypes still appear to be “missing” as not all of the pharmacological and signaling properties of biogenic amine receptors described in vivo are covered by the receptors cloned so far.

However, the availability of complete genomic sequences (*Drosophila melanogaster*; Adams et al., 2000) and ongoing molecular research (*Apis mellifera*; Maleszka, 2000) will allow such gaps to be filled in the near future. Combining the experimental advantages of both insect species, i.e., honeybees to study social behavior and the neuronal and biochemical basis of learning and memory (Menzel and Müller, 1996, Meller and Davis, 1996, Hammer, 1997), and *Drosophila* with its genetic potential, will certainly improve our understanding of the behavioral, developmental, and physiological role of individual biogenic amine-regulated transduction pathways.

ACKNOWLEDGMENTS

This work was supported by grants from the German Research Foundation awarded to Drs. J. Erber (Er 79/4-1, 4-2), A. Baumann (Ba 1541/2-1, 2-2), and W. Blenau (Bl 469/1), as well as an Otago Research Grant awarded to Dr. A. R. Mercer (ORG MFZ B00). W. Blenau is supported by a Feodor Lynen Research Fellowship from the Alexander von Humboldt-Foundation. We are grateful for the continuous support of J. Erber (TU Berlin, Dept. of Ecology and Biology, Germany) with whom this work was initiated. For helpful suggestions on the manuscript we thank Drs. A. R. Mercer, J. Erber, and J. A. Mustard, as well as L. Grohmann and R. Scheiner.

LITERATURE CITED

Adams MD, Celniker SE, Holt RA et al. 2000. The genome sequence of *Drosophila melanogaster*. Science 287:2185–2195.

Andretic R, Hirsh J. 2000. Circadian modulation of dopamine receptor responsiveness in *Drosophila melanogaster*. Proc Natl Acad Sci USA 97:1873–1878.

Arakawa S, Gocayne JD, McCombie WR, Urquhart DA, Hall LM, Fraser CM, Venter JC. 1990. Cloning, localization, and permanent expression of a Drosophila octopamine receptor. Neuron 2:343–354.

Axelrod J, Saavedra JM. 1977. Octopamine. Nature 265: 501–504.

Baldwin JM. 1994. Structure and function of receptors coupled to G proteins. Curr Opin Cell Biol 6:180–190.

Baldwin JM, Schertler GFX, Unger VM. 1997. An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. J Mol Biol 272:144–164.

Bicker G, Menzel R. 1989. Chemical codes for the control of behaviour in arthropods. Nature 337:33–39.

Blenau W, Erber J. 1998. Behavioural pharmacology of dopamine, serotonin and putative aminergic ligands in the mushroom bodies of the honeybee (*Apis mellifera*). Behav Brain Res 96:115–124.

Blenau W, May T, Erber J. 1995a. Characterization of a dopamine-sensitive [^{3}H]LSD binding site in honeybee (*Apis mellifera*) brain. Comp Biochem Physiol 110C: 197–205.

Blenau W, May T, Erber J. 1995b. Characterization of [^{3}H]LSD binding to a serotonin-sensitive site in honeybee (*Apis mellifera*) brain. Comp Biochem Physiol 112B:377–384.

Blenau W, May T, Erber J. 1996. The in vitro effects of biogenic amines on adenylate cyclase activity in the brain of the honeybee (*Apis mellifera* L.). In: Elsner N, Schnitzler H-U, editors. Göttingen Neurobiology Report 1996. Stuttgart: Thieme. p 548.

Blenau W, Erber J, Baumann A. 1998. Characterization of a dopamine D1 receptor from *Apis mellifera*: cloning, functional expression, pharmacology, and mRNA localization in the brain. J Neurochem 70:15–23.

Blenau W, Schmidt M, Faensen D, Schürmann F-W. 1999. Neurons with dopamine-like immunoreactivity target mushroom body Kenyon cell somata in the brain of some hymenopteran insects. Int J Insect Morphol Embryol 28:203–210.

Blenau W, Balfanz S, Baumann A. 2000. Am*tyr*1: characterization of a gene from honeybee (*Apis mellifera*) brain encoding a functional tyramine receptor. J Neurochem 74:900–908.

Boess FG, Martin IL. 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317.

Bornhauser BC, Meyer EP. 1997. Histamine-like immunoreactivity in the visual system and brain of an orthopteran and a hymenopteran insect. Cell Tissue Res 287:211–221.

Braun G, Bicker G. 1992. Habituation of an appetitive reflex in the honeybee. J Neurophysiol 67:588–598.

Brown CS, Nestler C. 1985. Catecholamines and indolalkylamines. In: Kerkut GA and Gilbert LI, editors. Comprehensive insect physiology, biochemistry, and pharmacology, vol 11: pharmacology. Oxford: Pergamon Press. p 435–497.

Buchner E, Buchner S, Burg MG, Hofbauer A, Pak WL, Pollack I. 1993. Histamine is a major mechanosensory neurotransmitter candidate in *Drosophila melanogaster*. Cell Tissue Res 273:119–125.

Budnik V, White K. 1988. Catecholamine-containing neurons

in *Drosophila melanogaster*: Distribution and development. J Comp Neurol 268:400–413.

Budnik V, Wu C-F, White K. 1989. Altered branching of serotonin-containing neurons in *Drosophila* mutants unable to synthesize serotonin and dopamine. J Neurosci 9:2866–2877.

Burg MG, Sarthy PV, Koliantz G, Pak WL. 1993. Genetic and molecular identification of a *Drosophila* histidine decarboxylase gene required in photoreceptor transmitter synthesis. EMBO J 12:911–919.

Burrell BD, Smith BH. 1995. Modulation of the honey bee (*Apis mellifera*) sting response by octopamine. J Insect Physiol 41:671–680.

Chuang TT, Iacovelli L, Sallese M, De Blasi A. 1996. G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. Trends Pharmacol Sci 17:416–421.

Clapham DE, Neer EJ. 1997. G protein βγ subunits. Annu Rev Pharmacol Toxicol 37:167–203.

Colas J-F, Launay J-M, Kellermann O, Rosay P, Maroteaux L. 1995. *Drosophila* 5-HT_2 serotonin receptor: coexpression with fushi-tarazu during segmentation. Proc Natl Acad Sci USA 92:5441–5445.

Colas J-F, Choi D-S, Launay J-M, Maroteaux L. 1997. Evolutionary conservation of the 5-HT2B receptors. Ann NY Acad Sci 812:149–153.

Colas J-F, Launay J-M, Maroteaux L. 1999a. Maternal and zygotic control of serotonin biosynthesis are both necessary for *Drosophila* germband extension. Mech Dev 87:67–76.

Colas J-F, Launay J-M, Vonesch J-L, Hickel, P, Maroteaux L. 1999b. Serotonin synchronises convergent extension of ectoderm with morphogenetic gastrulation movements in *Drosophila*. Mech Dev 87:77–91.

David J-C, Coulon J-F. 1985. Octopamine in invertebrates and vertebrates. A review. Prog Neurobiol 24:141–185.

Davis JPL, Pitman RM. 1991. Characterization of receptors mediating the actions of dopamine on an identified inhibitory motoneurone of the cockroach. J Exp Biol 155:203–217.

Davis RL. 1993. Mushroom bodies and Drosophila learning. Neuron 11: 1–14.

De Cesare D, Fimia GM, Sassone-Corsi P. 1999. Signaling routes to CREM and CREB: plasticity in transcriptional activation. Trends Biochem Sci 24:281–285.

Degen J, Gewecke M, Roeder, T. 2000. Octopamine receptors of the honey bee and locust nervous system: pharmacological similarities between homologous receptors of distantly related species. Br J Pharmacol 130:587–594.

Dudai Y, Zvi S. 1982. Aminergic receptors in *Drosophila melanogaster:* Properties of [^{3}H]dihydroergocryptine binding sites. J Neurochem 38:1551–1558.

Dudai Y, Zvi S. 1984a. High-affinity [^{3}H]octopamine-binding sites in *Drosophila melanogaster*: Interaction with ligands and relationship to octopamine receptors. Comp Biochem Physiol 77C:145–151.

Dudai Y, Zvi S. 1984b. [^{3}H]Serotonin binds to two classes of sites in *Drosophila* head homogenate. Comp Biochem Physiol 77C:305–309.

Dudai Y, Buxbaum J, Corfas G, Ofarim M. 1987. Formamidines interact with *Drosophila* octopamine receptors, alter the flies' behavior and reduce their learning ability. J Comp Physiol 161A:739–746.

Ebert PR, Rowland JE, Toma DP. 1998. Isolation of seven unique biogenic amine receptor clones from the honey bee by library scanning. Insect Mol Biol 7:151–162.

Elias MS, Lummis SCR, Evans PD. 1984. [^{3}H]Mepyramine binding sites in the optic lobe of the locust: autoradiographic and pharmacological studies. Brain Res 294: 359–362.

Erber J, Kloppenburg P. 1995. The modulatory effects of serotonin and octopamine in the visual system of the honey bee (*Apis mellifera* L.). I. Behavioral analysis of the motion-sensitive antennal reflex. J Comp Physiol 176A:111–118.

Erber J, Kloppenburg P, Scheidler A. 1993. Neuromodulation by serotonin and octopamine in the honeybee: behaviour, neuroanatomy and electrophysiology. Experientia 49:1073–1083.

Evans PD. 1980. Biogenic amines in the insect nervous system. Adv Insect Physiol 15:317–473.

Evans PD. 1985. Octopamine. In: Kerkut GA, Gilbert LI, editors: Comprehensive insect physiology, biochemistry, and pharmacology, vol 11: pharmacology. Oxford: Pergamon Press. p 499–530.

Evans PD. 1993. Molecular studies on insect octopamine receptors. In: Pichon Y, editor: Comparative molecular neurobiology. Basel: Birkhäuser. p 286–296.

Evans PD, Robb S. 1993. Octopamine receptor subtypes and their modes of action. Neurochem Res 18:869–874.

Feng G, Hannan F, Reale V, Hon YY, Kousky CT, Evans PD, Hall LM. 1996. Cloning and functional characterization of a novel dopamine receptor from *Drosophila melanogaster*. J Neurosci 16: 3925–3933.

Fuchs E, Dustmann JH, Stadler H, Schürmann FW. 1989. Neuroactive compounds in the brain of the honeybee during imaginal life. Comp Biochem Physiol 92C: 337–342.

Gerhardt CC, van Heerikhuizen H. 1997. Functional characteristics of heterologously expressed 5-HT receptors. Eur J Pharmacol 334:1–23.

Gotzes F, Baumann A. 1996. Functional properties of *Drosophila* dopamine D1-receptors are not altered by the size of the N-terminus. Biochem Biophys Res Commun 222:121–126.

Gotzes F, Balfanz S, Baumann A. 1994. Primary structure and functional characterization of a *Drosophila* dopamine receptor with high homology to human $D_{1/5}$ receptors. Recept Chan 2:131–141.

Granger NA, Sturgis SL, Ebersohl R, Geng C, Sparks TC. 1996. Dopaminergic control of corpora allata activity in the larval tobacco hornworm, *Manduca sexta*. Arch Insect Biochem Physiol 32:449–466.

Grohmann L, Blenau W, Meadows B, Ebert PR, Baumann A. 2000. Characterisation of an octopamine receptor from *Apis mellifera* (AmOCT1): cloning, functional expression and mRNA localisation in the brain. Eur J Neurosci 12(Suppl 11):94.

Gudermann T, Kalkbrenner F, Schultz G. 1996. Diversity and selectivity of receptor-G protein interaction. Annu Rev Pharmacol Toxicol 36:429–459.

Gudermann T, Schöneberg T, Schultz G. 1997. Functional and structural complexity of signal transduction via G-protein-coupled receptors. Annu Rev Neurosci 20:399–427.

Hammer M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. Nature 366:59–63.

Hammer M. 1997. The neural basis of associative reward learning in honeybees. Trends Neurosci 20:245–252.

Hammer M, Menzel R. 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. Learn Mem 5:146–156.

Han K-A, Millar NS, Grotewiel MS, Davis RL. 1996. DAMB, a novel dopamine receptor expressed specifically in Drosophila mushroom bodies. Neuron 16:1127–1135.

Han K-A, Millar NS, Davis RL. 1998. A novel octopamine receptor with preferential expression in *Drosophila* mushroom bodies. J Neurosci 18:3650–3658.

Han P-L, Levin LR, Reed RR, Davis RL. 1992. Preferential expression of the Drosophila *rutabaga* gene in mushroom bodies, neural centers for learning in insects. Neuron 9: 619–627.

Hannan F, Hall LM. 1996. Temporal and spatial expression patterns of two G-protein coupled receptors in *Drosophila melanogaster*. Invert Neurosci 2:71–83.

Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG. 1996. Goodman and Gilmans's the pharmacological basis of therapeutics. New York: McGraw-Hill.

Hardie RC. 1987. Is histamine a neurotransmitter in insect photoreceptors? J Comp Physiol 161A:201–213.

Hardie RC. 1989. A histamine-activated chloride channel involved in neurotransmission at a photoreceptor synapse. Nature 339:704–706.

Harris JW, Woodring J. 1992. Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (*Apis mellifera* L.) brain. J Insect Physiol 38: 29–35.

Hen R. 1992. Of mice and flies: commonalities among 5-HT receptors. Trends Pharmacol Sci 13:160–165.

Hildebrandt H, Müller U. 1995. Octopamine mediates rapid stimulation of protein kinase A in the antennal lobe of honeybees. J Neurobiol 27:44–50.

Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, Haas HL. 1997. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 49:253–278.

Hille B. 1994. Modulation of ion-channel function by G-protein-coupled receptors. Trends Neurosci 17:531–536.

Hirsh J, Davidson N. 1981. Isolation and characterization of the dopa decarboxylase gene of *Drosophila melanogaster*. Mol Cell Biol 1:475–485.

Hoyer D, Martin G. 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology 36:419–428.

Humphries M, Ebert PR. 1998. A unique D1-like dopamine receptor gene in honey bees shows age related expression in mushroom body intrinsic cells. In: Schwarz MP, Hogendoorn K, editors: Proceedings of the XIII International Congress of IUSSI. Adelaide: Flinders University Press. p 216.

Jackson DM, Westlind-Danielsson A. 1994. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 64:291–369.

Jin H, George SR, Bouvier M, O'Dowd BF. 2000. Palmitoylation of G protein-coupled receptors. In: Benovic JL, editor. Regulation of G protein-coupled receptor function and expression. New York: Wiley-Liss. p 93–117.

Kaatz H, Eichmüller S, Kreissl S. 1994. Stimulatory effect of octopamine on juvenile hormone biosynthesis in honey bees (*Apis mellifera*): physiological and immunocytochemical evidence. J Insect Physiol 40:865–872.

Kebabian JW, Calne DB. 1979. Multiple receptors for dopamine. Nature 277:93–96.

Kirchhof BS, Mercer AR. 1997. Antennal lobe neurons of the honey bee, *Apis mellifera*, express a D2-like dopamine receptor in vitro. J Comp Neurol 383:189–198.

Kirchhof BS, Homberg U, Mercer AR. 1999. Development of dopamine-immunoreactive neurons associated with the antennal lobes of the honey bee, *Apis mellifera*. J Comp Neurol 411:643–653.

Klämbt C, Jacobs JR, Goodman CS. 1991. The midline of the Drosophila central nervous system: a model for the genetic analysis of cell fate, cell migration, and growth cone guidance. Cell 64:801–815.

Kloppenburg P, Erber J. 1995. The modulatory effects of serotonin and octopamine in the visual system of the honey bee (*Apis mellifera* L.) II. Electrophysiological analysis of motion-sensitive neurons in the lobula. J Comp Physiol 176A:119–129.

Kokay IC, Mercer, AR. 1996. Characterisation of dopamine receptors in insect (*Apis mellifera*) brain. Brain Res 706:47–56.

Kokay I, McEwan J, Mercer A. 1998. Autoradiographic localisation of [^{3}H]-SCH23390 and [^{3}H]-spiperone binding sites in honey bee brain. J Comp Neurol 394:29–37.

Kokay IC, Ebert PR, Kirchhof BS, Mercer AR. 1999. Distribution of dopamine receptors and dopamine receptor homologs in the brain of the honey bee, *Apis mellifera* L. Microsc Res Tech 44:179–189.

Kreissl S, Eichmüller S, Bicker G, Rapus J, Eckert M. 1994. Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. J Comp Neurol 348:583–595.

Kutsukake M, Komatsu A, Yamamoto D, Ishiwa-Chigusa S. 2000. A *tyramine receptor* gene mutation causes a defective olfactory behavior in *Drosophila melanogaster*. Gene 245:31–42.

Livingstone MS, Tempel BL. 1983. Genetic dissection of monoamine neurotransmitter synthesis in *Drosophila*. Nature 303:67–70.

Macmillan CS, Mercer AR. 1987. An investigation of the role of dopamine in the antennal lobes of the honeybee, *Apis mellifera*. J Comp Physiol 160A:359–366.

Maleszka R. 2000. Molecules to behaviour in the honeybee: the emergence of comparative neurogenomics. Trends Neurosci 23:513–514.

Martínez-Ramírez AC, Ferré J, Silva FJ. 1992. Catecholamines in *Drosophila melanogaster*: DOPA and dopamine accumulation during development. Insect Biochem Mol Biol 22:491–494.

McClung C, Hirsh J. 1999. The trace amine tyramine is essential for sensitization to cocaine in *Drosophila*. Curr Biol 9:853–860.

Meller VH, Davis RL. 1996. Biochemistry of insect learning: lessons from bees and flies. Insect Biochem Mol Biol 26: 327–335.

Melzig J, Buchner S, Weibel F, Wolf R, Burg M, Pak WL, Buchner E. 1996. Genetic depletion of histamine from the nervous system of *Drosophila* eliminates specific visual and mechanosensory behavior. J Comp Physiol 179A:763–773.

Melzig J, Burg M, Gruhn M, Pak WL, Buchner E. 1998. Selective histamine uptake rescues photo- and mechanoreceptor function of histidine decarboxylase-deficient *Drosophila* mutant. J Neurosci 18:7160–7166.

Menzel R, Müller U. 1996. Learning and memory in honeybees: from behavior to neural substrates. Annu Rev Neurosci 19:379–404.

Menzel R, Durst C, Erber J, Eichmüller S, Hammer M, Hildebrandt H, Mauelshagen J, Müller U, Rosenboom H, Rybak J, Schäfer S, Scheidler A. 1994. The mushroom bodies in the honeybee: From molecules to behaviour. In: Schildberger K, Elsner N, editors: Neural basis of behavioural adaptations. Stuttgart: Fischer. p 81–102.

Menzel R, Heyne A, Kinzel C, Gerber B, Fiala A. 1999. Pharmacological dissociation between the reinforcing, sensitizing, and response-releasing functions of reward in honeybee classical conditioning. Behav Neurosci 113: 744–754.

Mercer AR, Erber J. 1983. The effects of amines on evoked potentials recorded in the mushroom bodies of the bee brain. J Comp Physiol 151A:469–476.

Mercer AR, Menzel R. 1982. The effects of biogenic amines on conditioned and unconditioned responses to olfactory stimuli in the honeybee *Apis mellifera*. J Comp Physiol 145A:363–368.

Mercer AR, Mobbs PG, Davenport AP, Evans PD. 1983. Biogenic amines in the brain of the honeybee, *Apis mellifera*. Cell Tissue Res 234:655–677.

Michelsen DB. 1988. Catecholamines affect storage and retrieval of conditioned odour stimuli in honey bees. Comp Biochem Physiol 91C:479–482.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. 1998. Dopamine receptors: from structure to function. Physiol Rev 78:189–225.

Monastirioti M. 1999. Biogenic amine systems in the fruit fly *Drosophila melanogaster*. Microsc Res Tech 45:106–121.

Monastirioti M, Gorczyca M, Eckert M, Rapus J, White K, Budnik V. 1995. Octopamine immunoreactivity in the fruit fly *Drosophila melanogaster*. J Comp Neurol 356:275–287.

Monastirioti M, Linn CE, White K. 1996. Characterization of *Drosophila tyramine β-hydroxylase* gene and isolation of mutant flies lacking octopamine. J Neurosci 16:3900–3911.

Nässel DR. 1991. Neurotransmitters and neuromodulators in the insect visual system. Prog Neurobiol 37:179–254.

Nässel DR. 1999. Histamine in the brain of insects: a review. Microsc Res Tech 44:121–136.

Nässel DR, Elekes K. 1992. Aminergic neurons in the brain

of blowflies and *Drosophila*: dopamine- and tyrosine hydroxylase-immunoreactive neurons and their relationship with putative histaminergic neurons. Cell Tissue Res 267:147–167.

Nässel DR, Meyer EP, Klemm N. 1985. Mapping and ultrastructure of serotonin-immunoreactive neurons in the optic lobes of three insect species. J Comp Neurol 232:190–204.

Nässel DR, Pirvola U, Panula P. 1990. Histamine-like immunoreactive neurons innervating putative neurohaemal areas and central neuropil in the thoraco-abdominal ganglia of the flies *Drosophila* and *Calliphora*. J Comp Neurol 297:525–536.

Neckameyer WS. 1996. Multiple roles for dopamine in *Drosophila* development. Dev Biol 176:209–219.

Neckameyer WS. 1998. Dopamine and mushroom bodies in *Drosophila*: experience-dependent and -independent aspects of sexual behavior. Learn Mem 5:157–165.

Neckameyer WS, Quinn WG. 1989. Isolation and characterization of the gene for Drosophila tyrosine hydroxylase. Neuron 2:1167–1175.

Neckameyer WS, White K. 1992. A single locus encodes both phenylalanine hydroxylase and tryptophane hydroxylase activities in *Drosophila*. J Biol Chem 267:4199–4206.

Noda K, Saad Y, Graham RM, Karnik SS. 1994. The high affinity state of the β_2-adrenergic receptor requires unique interaction between conserved and non-conserved extracellular loop cysteines. J Biol Chem 269:6743–6752.

Obosi LA, Schuette DG, Europe-Finner GN, Beadle DJ, Hen R, King LA, Bermudez I. 1996. Functional characterisation of the *Drosophila* 5-HT$_{dro1}$ and 5-HT$_{dro2B}$ serotonin receptors in insect cells: activation of a $G_{\alpha s}$-like protein by 5-HT$_{dro1}$ but lack of coupling to inhibitory G-proteins by 5-HT$_{dro2B}$. FEBS Lett 381:233–236.

Palczewski K. 1997. GTP-binding-protein-coupled receptor kinases. Two mechanistic models. Eur J Biochem 248:261–269.

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289:739–745.

Pollak I, Hofbauer A. 1991. Histamine-like immunoreactivity in the visual system and brain of *Drosophila melanogaster*. Cell Tissue Res 266:391–398.

Pribbenow B, Erber J. 1996. Modulation of antennal scanning in the honeybee by sucrose stimuli, serotonin, and octopamine: behavior and electrophysiology. Neurobiol Learn Mem 66:109–120.

Probst WC, Snyder LA, Schuster DI, Brosius J, Sealfon SC. 1992. Sequence alignment of the G-protein coupled receptor superfamily. DNA Cell Biol 11:1–20.

Rachinsky A. 1994. Octopamine and serotonin influence on corpora allata activity in honey bee (*Apis mellifera*) larvae. J Insect Physiol 40:549–554.

Reale V, Hannan F, Hall LM, Evans PD. 1997a. Agonist specific coupling of a cloned *Drosophila melanogaster* D1-like dopamine receptor to multiple second messenger pathways by synthetic agonists. J Neurosci 17:6545–6553.

Reale V, Hannan F, Midgley JM, Evans PD. 1997b. The expression of a cloned *Drosophila* octopamine/tyramine receptor in *Xenopus* oocytes. Brain Res 769:309–320.

Rehder V, Bicker G, Hammer M. 1987. Serotonin-immunoreactive neurons in the antennal lobes and suboesophageal ganglion of the honeybee. Cell Tissue Res 247:59–66.

Restifo LL, White K. 1990. Molecular and genetic approaches to neurotransmitter and neuromodulator systems in *Drosophila*. Adv Insect Physiol 22:115–219.

Rhee SG, Bae YS. 1997. Regulation of phosphoinositide-specific phospholipase C isozymes. J Biol Chem 272:15045–15048.

Robb S, Cheek TR, Hannan FL, Hall LM, Midgley JM, Evans PD. 1994. Agonist-specific coupling of a cloned *Drosophila* octopamine/tyramine receptor to multiple second messenger systems. EMBO J 13:1325–1330.

Robinson GE, Heuser LM, LeConte Y, Lenquette F, Hollingworth RM. 1999. Neurochemicals aid bee nestmate recognition. Nature 399:534–535.

Roeder T. 1990. Histamine H_1-receptor-like binding sites in the locust nervous tissue. Neurosci Lett 116:331–335.

Roeder T. 1999. Octopamine in invertebrates. Prog Neurobiol 59: 533–561.

Roeder T, Voßfeldt R, Gewecke M. 1993. Pharmacological characterization of the locust neuronal ^{3}H-mianserin binding site, a putative histamine receptor. Comp Biochem Physiol 106C:503–507.

Roeder T, Voßfeldt R, Gewecke M. 1995. The solubilized locust neuronal ^{3}H-mianserin binding site, a histamine H_1-like receptor molecule. Insect Biochem Mol Biol 25:1049–1054.

Sarthy PV. 1991. Histamine: a neurotransmitter candidate for *Drosophila* photoreceptors. J Neurochem 57:1757–1768.

Saudou F, Amlaiky N, Plassat J-L, Borrelli E, Hen R. 1990. Cloning and characterization of a *Drosophila* tyramine receptor. EMBO J 9:3611–3617.

Saudou F, Boschert U, Amlaiky N, Plassat J-L, Hen R. 1992. A family of *Drosophila* serotonin receptors with distinct

intracellular signalling properties and expression patterns. EMBO J 11:7–17.

Schäfer S, Bicker G. 1986. Common projection areas of 5-HT- and GABA-like immunoreactive fibers in the visual system of the honeybee. Brain Res 380:368–370.

Schäfer S, Rehder V. 1989. Dopamine-like immunoreactivity in the brain and suboesophageal ganglion of the honeybee. J Comp Neurol 280:43–58.

Schneider T, Igelmund P, Hescheler J. 1997. G protein interaction with K^+ and Ca^{2+} channels. Trends Pharmacol Sci 18:8–11.

Schulz DJ, Robinson GE. 1999. Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. J Comp Physiol 184A:481–488.

Schürmann FW, Klemm N. 1984. Serotonin-immunoreactive neurons in the brain of the honeybee. J Comp Neurol 225:570–580.

Schürmann FW, Elekes K, Geffard M. 1989. Dopamine-like immunoreactivity in the bee brain. Cell Tissue Res 256:399–410.

Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. 1991. Histaminergic transmission in the mammalian brain. Physiol Rev 71:1–51.

Seidel C, Bicker G. 1996. The developmental expression of serotonin-immunoreactivity in the brain of the pupal honeybee. Tissue Cell 28:663–672.

Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD. 1998. Basic neurochemistry: molecular, cellular, and medical aspects. Philadelphia: Lippincott Williams & Wilkins.

Skiebe P, Corrette BJ, Wiese K. 1990. Evidence that histamine is the inhibitory transmitter of the auditory interneuron ON1 of crickets. Neurosci Lett 116:361–366.

Skingsley DR, Laughlin SB, Hardie RC. 1995. Properties of histamine-activated chloride channels in the large monopolar cells of the dipteran compound eye: a comparative study. J Comp Physiol 176A:611–623.

Strader CD, Fong TM, Graziano MP, Tota MR. 1995. The family of G-protein-coupled receptors. FASEB J 9:745–754.

Sugamori KS, Demchyshyn LL, McConkey F, Forte MA, Niznik HB. 1995. A primordial dopamine D1-like adenylyl cyclase-linked receptor from *Drosophila melanogaster* displaying poor affinity for benzazepines. FEBS Lett 362:131–138.

Taylor DJ, Robinson GE, Logan BJ, Laverty R, Mercer AR. 1992. Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. J Comp Physiol 170A:715–721.

Tempel BL, Livingstone MS, Quinn WG. 1984. Mutations in the dopa decarboxylase gene affect learning in *Drosophila*. Proc Natl Acad Sci USA 81:3577–3581.

Tully T. 1987. *Drosophila* learning and memory revisited. Trends Neurosci 10:330–335.

Uzzan A, Dudai Y. 1982. Aminergic receptors in *Drosophila melanogaster*: responsiveness of adenylate cyclase to putative neurotransmitters. J Neurochem 38:1542–1550.

Valdenaire O, Vernier, P. 1997. G protein coupled receptors as modules of interacting proteins: A family meeting. Prog Drug Res 49:173–218.

Vallés AM, White K. 1988. Serotonin-containing neurons in *Drosophila melanogaster*: Development and distribution. J Comp Neurol 268:414–428.

Vallone D, Picetti R, Borrelli E. 2000. Structure and function of dopamine receptors. Neurosci Biobehav Rev 24:125–132.

Vanden Broeck J, Vulsteke V, Huybrechts R, De Loof A. 1995. Characterization of a cloned locust tyramine receptor cDNA by functional expression in permanently transformed *Drosophila* S2 cells. J Neurochem 64:2387–2395.

Vernier P, Cardinaud B, Valdenaire O, Philippe H, Vincent J-D. 1995. An evolutionary view of drug-receptor interaction: the bioamine receptor family. Trends Pharmacol Sci 16:375–381.

von Nickisch-Rosenegk E, Krieger J, Kubick S, Laage R, Strobel J, Strotmann J, Breer H. 1996. Cloning of biogenic amine receptors from moths (*Bombyx mori* and *Heliothis virescens*). Insect Biochem Mol Biol 26: 817–827.

Wagener-Hulme C, Kuehn JC, Schulz DJ, Robinson GE. 1999. Biogenic amines and division of labor in honey bee colonies. J Comp Physiol 184A:471–479.

Witz P, Amlaiky N, Plassat J-L, Maroteaux L, Borrelli E, Hen R. 1990. Cloning and characterization of a *Drosophila* serotonin receptor that activates adenylate cyclase. Proc Natl Acad Sci USA 87:8940–8944.

Wright TRF. 1987. The genetics of biogenic amine metabolism, sclerotization, and melanization in *Drosophila melanogaster*. Adv Genet 24:127–222.

Yellman C, Tao H, He B, Hirsh J. 1997. Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated *Drosophila*. Proc Natl Acad Sci USA 94:4131–4136.