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Aminergic signal transduction in invertebrates : focus on tyramine and octopamine receptors

14 Aminergic signal transduction in invertebrates: Focus on tyramine and octopamine receptors

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Abstract

Electro-chemical signal transduction is the basis of communication between neurons and their target cells. An important group of neuroactive substances that are released by action potentials from neurons are the biogenic amines. These are small organic molecules that bind to specific receptors located in the target cell membrane. Once activated these receptors cause changes in the intracellular concentration of second messengers, i.e. cyclic nucleotides, phospho-inositides, or Ca^{2+}, leading to slow but long-lasting cellular responses. Biochemical, pharmacological, physiological, and molecular biological approaches have unequivocally shown that biogenic amines are important regulators of cellular function in both vertebrates and invertebrates. In this review, we will concentrate on the properties of two biogenic amines and their receptors that were originally identified in invertebrates: tyramine and octopamine.

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Introduction

Some of the most fascinating properties of every neuron are to register, to evaluate, and to transmit information on a very fast time scale. Communication or, in molecular terms, signal transduction between neurons allows an organism to react to various external stimuli. Therefore, it is not surprising that changes in neural signaling efficacy can have a drastic impact on many physiological and behavioral processes including learning and memory. Most neurons in the central nervous system (CNS) use chemical synapses to convey information onto target organs or cells. An action potential stimulates the release of small organic compounds by exocytosis from specialized nerve terminals. These molecules may act as neurotransmitters, neuromodulators, or neurohormones.

Neurotransmitters can mediate fast responses of the target cell. This usually occurs when neurotransmitters bind to receptors that form ion channels in the plasma membrane (ionotropic receptors). Well-known examples for fast-acting neurotransmitters are acetylcholine and glutamate, which cause the excitation of neurons, whereas glycine and γ-aminobutyric acid (GABA) cause the inhibition of neurons. One group of neuroactive substances that primarily act by binding to membrane proteins that themselves do not form ion channels are the biogenic amines. These molecules are derived from amino acids by biochemical modification. In vertebrates, five compounds are considered as classical biogenic amines: dopamine, norepinephrine, epinephrine, serotonin (5-hydroxytryptamine, 5-HT), and histamine. Whereas dopamine, 5-HT, and histamine are also present in invertebrates, norepinephrine and epinephrine seem to be functionally substituted by two structurally related compounds: p-tyramine and octopamine [1, 2, 3]. Very low levels of p-tyramine and octopamine and of tryptamine and β-phenylethylamine have also been identified in mammals. In the mammalian brain, these substances account for less than 1% of all biogenic amines. Because of their low abundance, they are collectively called "trace amines" and have long been thought of as metabolic by-products only [4, 5]. However, the level of trace amines has been found to be altered in various human disorders (including depression, hepatic encephalopathy, hypertension, Parkinsonism, phenylketonuria, and schizophrenia) suggesting that these substances are physiologically relevant [6].

With a few exceptions, viz., the 5-HT$_3$ receptor channel in vertebrates [7] and histamine-gated ion channels in invertebrates [8, 9], all known biogenic amine receptors belong to the superfamily of G protein-coupled receptors (GPCRs) [10, 11]. Since the activation of GPCRs usually leads to metabolic changes in the target cell, they are also called metabotropic receptors. Ligand binding to GPCRs induces conformational changes of the protein and thereby activates the receptor. Activated GPCRs usually transmit the signal to intracellular trimeric GTP-binding (G) proteins. The activated G proteins can either alter the opening probability of ion channels or the activity of specific target enzymes. Physiologically, the activation or inhibition of these enzymes leads to changes in the intracellular concentration of cyclic nucleotides (cAMP, cGMP), inositol-1,4,5-trisphosphate (IP$_3$), diacylglycerol (DAG), or arachidonic acid metabolites (eicosanoids). Subsequently, the activity of second messenger-dependent enzymes changes thus causing modification of the functional properties of various cytosolic, membrane-bound, and nuclear proteins.

In this review, we will describe the properties of tyramine and octopamine receptors that initially were thought to be almost exclusively expressed in invertebrates. The recent isolation of mammalian trace amine receptor genes [12, 13], however, strongly suggests a role for tyramine and other trace amines as bona fide neurotransmitters also in vertebrates. We are going to compare the properties of known tyramine and octopamine receptors on the biochemical, pharmacological, molecular biological, and cellular signaling level. The interested reader will find other information on biogenic amines and their respective receptor systems not covered by this article in a number of recent and more comprehensive reviews [3, 11, 14 - 20].

Biosynthesis of tyramine and octopamine

The precursor of the phenolamines tyramine and octopamine is the essential amino acid L-tyrosine. Direct decarboxylation of tyrosine by tyrosine decarboxylase generates the phenolamine tyramine. For a long time, tyramine was considered to be solely an intermediate reaction product of the synthesis of octopamine. This assumption was based on the finding that octopamine is derived from tyramine by hydroxylation on the β-carbon of the side chain (Fig. 1). This reaction is catalyzed by the enzyme tyramine
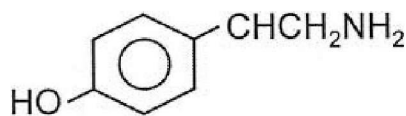
tyramine
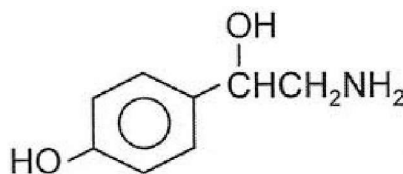
octopamine
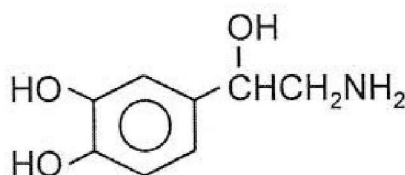
norepinephrine

Figure 1. Structural formulae of p-tyramine, octopamine, and norepinephrine.

β-hydroxylase, an enzyme the gene of which has been cloned in *Drosophila* [21]. The phenolamine octopamine and the catecholamine norepinephrine share substantial structural similarity (Fig. 1). They only differ in a hydroxyl group in the *meta* position of the benzoyl moiety of norepinephrine. Therefore, it is assumed that the norepinephrine/ epinephrine system of vertebrates is functionally substituted by the tyraminergic/ octopaminergic system in invertebrates [1, 2, 3].

Molecular properties of tyramine and octopamine receptors

Biogenic amines such as tyramine and octopamine bind to receptor proteins that are derived from a superfamily of related genes. Collectively these receptors are called GPCRs. Biogenic amine receptors are members of a receptor subfamily, of which rhodopsin was the founder. Based on recent crystal structure data [22, 23], hydropathy analyses of the deduced amino acid sequences, and phylogenetic comparisons [10, 24 - 27], GPCRs share the common motif of seven transmembrane (TM) domains (Fig. 2). The N-terminus is located extracellularly and the C-terminus is located intracellularly. The N-terminus is the target for post-translational modification and often contains consensus sequence motives for N-linked glycosylation [28, 29]. The membrane-spanning regions are linked by three extracellular loops (EL) that alternate with three intracellular loops (IL). Cysteine residues in the cytoplasmic tail of the polypeptides are the targets of post-translational palmitoylation. Insertion of these fatty acids into the plasma membrane will create a fourth intracellular loop (IL4) and is assumed to stabilize the structure of these receptors [30].
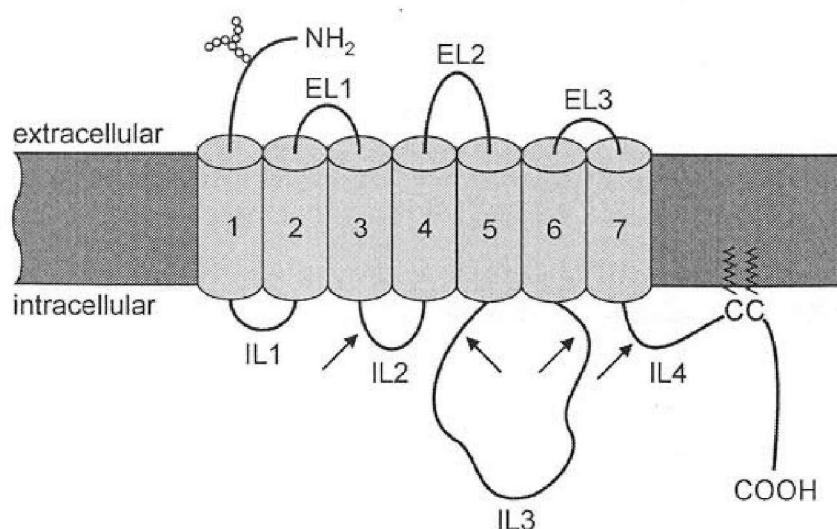
Figure 2. Topography of a biogenic amine receptor. The polypeptide spans the membrane seven times. The transmembrane regions (TM 1-7) are depicted as cylinders. The N-terminus (NH_2) is located extracellularly and often contains glycosylated residues (o). The C-terminus (COOH) is located intracellularly. The membrane spanning regions are linked by three extracellular loops (EL) that alternate with three intracellular loops (IL). When the protein is post-translationally palmitoylated at cysteine residues (C) in the cytoplasmic tail, a fourth intracellular loop (IL4) is formed. Arrows point to receptor segments involved in G protein binding.

Activation of the receptor occurs by the binding of a biogenic amine. This interaction takes place in a binding pocket formed by the TM regions in the plane of the membrane [31]. Specific residues in different TM segments interact with functional groups of the biogenic amines. Tyramine and octopamine receptors contain highly conserved amino acid residues originally identified as ligand-binding partners in other biogenic amine receptors. In particular, an aspartic acid residue in TM3, serine residues in TM5, and a phenylalanine residue in TM6 that have been shown to determine the ligand-binding properties of many biogenic amine receptors [26, 29] are also present in tyramine and octopamine receptor sequences. Once the ligand is tightly bound to its receptor, the conformation of the receptor will change [32]. This structural change is usually transferred to trimeric G proteins [27, 33]. Residues of IL2, 3, and 4 in close vicinity to the plasma membrane determine the specificity and efficacy of G protein activation (Fig. 2). Once activated, G protein α- and βγ-subunits regulate the activities of a structurally diverse group of effector molecules. These include enzymes participating in the synthesis and degradation of intracellular second messengers and ion-selective channels. Adenylyl cyclases often become activated or inhibited leading to changes in the intracellular concentration of cyclic AMP ([cAMP]$_i$). Another important pathway leads to the activation of phospholipase C (PLC). This enzyme generates the second messengers IP$_3$ and DAG, which cause Ca^{2+} release from intracellular stores and activation of protein kinase C (PKC), respectively. Receptor-mediated signaling can be turned off by the phosphorylation of serine and threonine residues in the C-terminus and IL3 of the receptor protein and by β-arrestin binding [34 - 37]. In addition to these classical signaling pathways, some recent observations strongly suggest that GPCRs also transduce signals by G protein-independent mechanisms (for reviews, see: [38, 39]).

The first tyramine receptor cDNA was independently cloned by two groups in 1990 from *Drosophila melanogaster* [40, 41]. Since then, a large number of related genes from various invertebrates and, recently, also from mammalian species have been cloned or deposited in sequence databases. We have used the *Drosophila* (DmTYR [41]) and *Apis mellifera* (AmTYR1 [42]) tyramine receptor sequences as "baits" to search the GenBank database for homologous genes. In addition, we also used the sequence of an octopamine receptor from *Drosophila* (OAMB [43]) to identify octopamine receptor genes. The results of these screens are summarized in Table 1 and in a dendrogram (Fig. 3) to illustrate the phylogenetic relationship. For convenience, we have included a dopamine receptor from *Drosophila* (DmDOP1 [51]), a serotonin receptor from *Drosophila* (Dm5HT7 [52]), and the human α_{2B}-adrenergic receptor (hA2B [53]) in these trees.

Based on these analyses, tyramine and octopamine receptors have been placed into separate clades within the biogenic amine receptor subfamily of GPCRs. Both groups are separated by the serotonin (Dm5HT7) and the α-adrenergic receptor (hA2B) sequences. Notably, two mammalian trace amine receptor sequences are only distantly related to all other sequences analyzed (Fig. 3). Although a considerable number of tyramine/ octopamine receptor sequences can be obtained from the databases, so far only a few of these genes are functionally expressed and characterized in terms of their pharmacology and second messenger coupling (for a review, see: [11]).

Table 1. Search of NCBI database with Amtyr1, Dmtyr, and OAMB receptor sequences for homologous genes and proteins
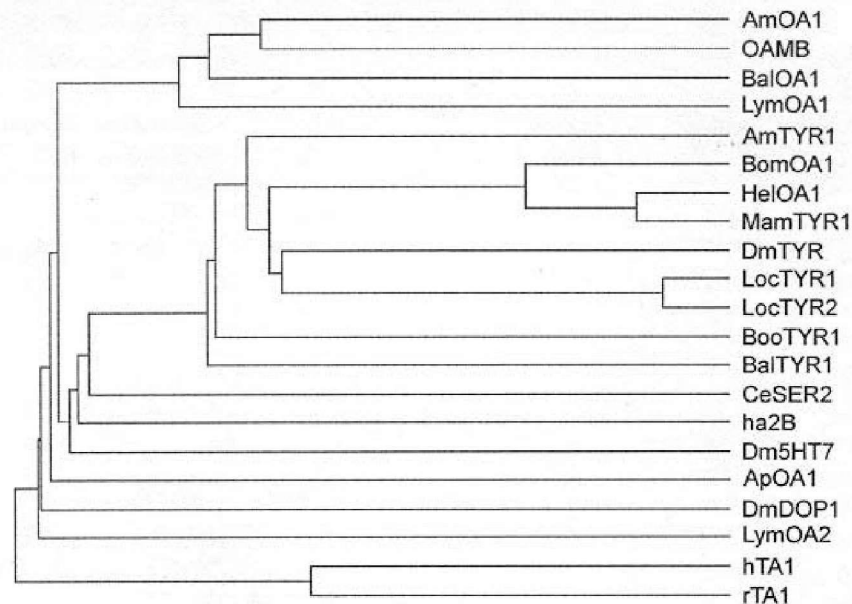
query	identified gene	accession number nucleic acid	accession number amino acid	e-value nucleic acid	e-value amino acid	reference
Dmtyr	*Drosophila melanogaster* Dmtyr	X54794	S12004	0.0	0.0	[40, 41]
	Bombyx mori BomOA1	X95607	Q17232		e-111	[44]
	Locusta migratoria Loctyr2	X69521	Q25322	2e-48	3e-70	[45]
	Locusta migratoria Loctyr1	X69520	Q25321	2e-43	3e-70	[45]
	Heliothis virescens HelOA1	X95606	Q25188		1e-68	[44]
	Balanus amphitrite Baltyr2	D78363	Q93127		8e-56	[46]
	Balanus amphitrite Baltyr1	D85547	Q93126		3e-31	[47]
	Boophilus microplus Bootyr1	AJ010743	CAA90335	3e-23		[48]
	Lymnea stagnalis LymOA1	U62771	AAC61296		3e-23	[49]
	Lymnea stagnalis LymOA2	U62770	AAC16969		2e-22	[50]
	Apis mellifera Amtyr1	AJ245824	CAB76374	2e-15		[42]
	Mamestra brassicae Mamtyr	AF343878	AAK14402	2e-11		
Amtyr1	*Apis mellifera* Amtyr1	AJ245824	CAB76374	0.0		[42]
	Balanus amphitrite Baltyr2	D78363	Q93127		8e-94	[46]
	Locusta migratoria Loctyr2	X69521	Q25322	1e-19	2e-77	[45]
	Locusta migratoria Loctyr1	X69520	Q25321	1e-17	3e-77	[45]
	Drosophila melanogaster Dmtyr	X54794	P22270	2e-15	5e-75	[40, 41]
	Bombyx mori BomOA1	X95607	Q17232		5e-74	[44]
	Heliothis virescens HelOA1	X95606	Q25188		2e-73	[44]
	Balanus amphitrite Baltyr1	D85547	Q93126		3e-36	[47]
	Lymnea stagnalis LymOA2	U62770	O01670		7e-28	[50]
	Boophilus microplus Bootyr1	AJ010743	CAA90335	8e-12		[48]
OAMB	*Drosophila melanogaster* OAMB	AF065443	AAC17442	0.0		[43]
	Balanus amphitrite Baltyr1	D78363	Q93126	6e-5	6e-50	[47]
	Lymnea stagnalis LymOA1	U62771	AAC61296	6e-5	4e-39	[49]
	Locusta migratoria Loctyr1	X69520	Q25321	0.25	2e-28	[45]
	Locusta migratoria Loctyr2	X69521	Q25322	0.25	2e-28	[45]
	Drosophila melanogaster Dmtyr	X54794	P22270		4e-28	[40, 41]
	Heliothis virescens HelOA1	X95606	Q25188		2e-27	[44]
	Bombyx mori BomOA1	X95607	Q17232		1e-26	[44]
	Balanus amphitrite Baltyr2	D78363	Q93127		6e-26	[46]
	Lymnea stagnalis LymOA2	U62770	O01670		2e-20	[50]

Functional properties of cloned tyramine and octopamine receptors

Functional characterization of cloned genes requires expression of the gene in heterologous expression systems. A variety of suitable expression vectors and established cell lines are available to perform these tests. Since different laboratories favor different expression systems, this diversity probably has an impact on the results obtained. In Table 2, we have summarized some of the characteristic features described for heterologously expressed tyramine and octopamine receptors.

What conclusions can be drawn from the above-mentioned experimental data? Most of the heterologously expressed tyramine receptor genes from invertebrate species encode GPCRs that inhibit cAMP production in cells. Therefore, the activated receptors probably bind to and stimulate G_i-type G proteins [27, 59, 60]. Since this coupling occurs in various cell lines from vertebrates (e.g., HEK 293, NIH 3T3, CHO) and non-vertebrate (e.g., Sf9, *Drosophila* S2) origin (Table 2), tyramine receptor activation *in vivo* will probably also cause the attenuation of adenylyl cyclase activity. We have previously mentioned that the NH_2-terminal and COOH-terminal extremes of the IL3 of the receptors appear to play a prominent role in G protein interaction (Fig. 2). An amino acid sequence comparison of these regions for some tyramine receptors is depicted in Figure 4. The highest degree of sequence conservation is seen between receptors from

AmOA1
OAMB
BalOA1
LymOA1
AmTYR1
BomOA1
HelOA1
MamTYR1
DmTYR
LocTYR1
LocTYR2
BooTYR1
BalTYR1
CeSER2
ha2B
Dm5HT7
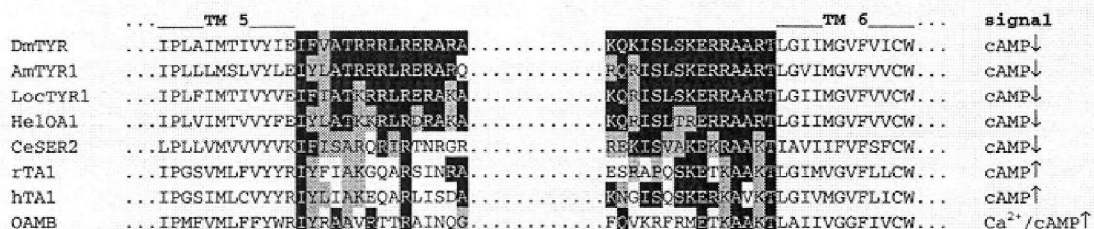ApOA1
DmDOP1
LymOA2
hTA1
rTA1

Figure 3. Dendrogram of biogenic amine receptor sequences. Sequence alignment was performed with the CLUSTAL program of PCGENE software (version 6.6; IntelliGenetic) and the complete amino acid sequence of each receptor. The receptor sequences followed by their GenBank accession numbers (#) are listed in the order illustrated: *Apis mellifera* octopamine (AmOA1, #CAD67999), *Drosophila melanogaster* octopamine (OAMB, #AAC17442), *Balanus amphitrite* octopamine (BalOA1, #Q93127), *Lymnea stagnalis* octopamine 1 (LymOA1, #AAC61296), *A. mellifera* tyramine (AmTYR1, #CAB76374), *Bombyx mori* "octopamine" (BomOA1, #Q17232), *Heliothis virescens* "octopamine" (HelOA1, #Q25188), *Mamestra brassicae* tyramine (MamTYR1, #AF343878), *D. melanogaster* tyramine (DmTYR, #P22270), *Locusta migratoria* tyramine (LocTYR1, #X69520; LocTYR2, #X69521), *Boophilus microplus* tyramine (BooTYR1, #AJ010743), *B. amphitrite* tyramine (BalTYR1, #Q93126), human α_{2B}-adrenergic (hA2B, #NP_000673), *D. melanogaster* serotonin (Dm5HT7, #P20905), *Aplysia kurodai* octopamine (ApOA1, #AAF28802), *D. melanogaster* dopamine (DmDOP1, #CAA54451), *L. stagnalis* octopamine 2 (LymOA2, #O01670), human trace amine receptor 1 (hTA1, #AAK71236), and rat trace amine receptor 1 (rTA1, #AAK71237).

Drosophila (DmTYR [41]), *Apis* (AmTYR1 [42]), *Locusta* (LocTYR1 [45]), and *Heliothis* (HelOA1 [44]). In these regions, $\geq 95\%$ of the residues are identical or conservatively substituted. A common characteristic of these receptors is that they attenuate adenylyl cyclase activity. A receptor recently cloned from *Caenorhabditis elegans* (CeSER2 [57]) also inhibits cAMP production in response to tyramine application. It shares ~80% sequence similarity with DmTYR. Sequence conservation, however, is less pronounced between those receptors that have been found to activate different intracellular signaling pathways. Both the rat and human trace amine receptors (rTA1 and hTA1 [12]) stimulate adenylyl cyclase, whereas the *Drosophila* octopamine receptor (OAMB [43]) causes an increase in $[Ca^{2+}]_i$ and $[cAMP]_i$. These receptors share only 43% - 63% of amino acid residues with DmTYR (Fig. 4) supporting the assumption that the amino acid residues flanking TM5 and TM6 largely determine the functional coupling properties of the receptors.

Table 2. Intracellular signaling pathways activated by heterologously expressed tyramine and octopamine receptors.

receptor	species	expression system	intracellular signalling	agonist preference	reference
AmTYR1	*Apis mellifera*	HEK 293	cAMP$\downarrow$	tyr > oct	[42]
DmTYR	*Drosophila melanogaster*	NIH 3T3	cAMP$\downarrow$	tyr > oct	[41]
		CHO-K1	cAMP$\downarrow$	tyr > oct	[54]
			Ca$^{2+}\uparrow$	oct $\geq$ tyr	
		Xenopus oocyte	Ca$^{2+}\uparrow$	oct ~ tyr	[55]
LocTYR1	*Locusta migratoria*	Sf9, S2	cAMP$\downarrow$	tyr > oct	[45]
		MEL-C88L	Ca$^{2+}\uparrow$ and cAMP$\downarrow$	tyr > oct	[56]
CeSER2	*Cenorhabditis elegans*	HEK 293	cAMP$\downarrow$	tyr > oct	[57]
rTA1	rat	*Xenopus* oocyte	cAMP$\uparrow$/Cl$^-$ current	tyr > oct	[12]
		HEK 293	cAMP$\uparrow$	tyr > oct	[13]
hTA1	human	*Xenopus* oocyte	cAMP$\uparrow$/Cl$^-$ current	tyr > oct	[12]
		COS-7	cAMP$\uparrow$	tyr > oct	
OAMB	*Drosophila melanogaster*	HEK 293, S2	cAMP$\uparrow$ and Ca$^{2+}\uparrow$	oct > tyr	[43]
LymOA1	*Lymnea stagnalis*	HEK 293	cAMP$\uparrow$ and Ca$^{2+}\uparrow$	oct > tyr	[49]
LymOA2	*Lymnea stagnalis*	HEK 293	Cl$^-$ current$\uparrow$	oct > tyr	[50]
ApOA1	*Aplysia californica*	HEK 293, CHO	cAMP$\uparrow$	oct > tyr	[58]
		Xenopus oocyte	cAMP$\uparrow$/Cl$^-$ current	tyr > oct	
HelOA1	*Heliothis virescens*	LLC-PK$_1$	cAMP$\downarrow$	oct > tyr	[44]

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         ..._____TM 5_____                      _____TM 6_____ ...   signal
DmTYR    ...IPLAIMTIVYIEIFVATRRRLRERARA..........KQKISLSKERRAARTLGIIMGVFVICW...   cAMP↓
AmTYR1   ...IPLLLMSLVYLEIYLATRRRLRERARQ..........RQRISLSKERRAARTLGVIMGVFVVCW...   cAMP↓
LocTYR1  ...IPLFIMTIVYVEIFIATKRRLRERAKA..........KQRISLSKERRAARTLGIIMGVFVVCW...   cAMP↓
HelOA1   ...IPLVIMTVVYFEIYLATKKRLRDRARA..........KQRISLTRERRAARTLGIIMGVFVVCW...   cAMP↓
CeSER2   ...LPLLVMVVVYVKIFISARQRFRTNRGR..........REKISVAKEKRAAKTIAVIIFVFSFCW...   cAMP↓
rTA1     ...IPGSVMLFVYYRIYFIAKGQARSINRA..........ESRAPQSKETRAAKTLGIMVGVFLLCW...   cAMP↑
hTA1     ...IPGSIMLCVYYRIYLIAKEQARLISDA..........KNGISQSKERKAVKTLGIVMGVFLICW...   cAMP↑
OAMB     ...IPMFVMLFFYWRIYRAAVETTRAINQG..........FQVKRFRMETKAAKTLAIIVGGFIVCW...   Ca²⁺/cAMP↑
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Figure 4. Sequence alignment of residues flanking TM5 and TM6 in tyramine receptors. The alignment shows part of the TM5 and TM6 segments. Fifteen amino acid residues flanking each of these TMs in the intracellular loop (IL3) are displayed. Identical residues in IL3 are given as white letters against black, conservative substitutions are highlighted in gray. The alignment was performed with tyramine receptor sequences from: *Drosophila* (DmTYR, #P22270), *Apis* (AmTYR1, #CAB76374), *Locusta* (LocTYR1, #X69520), *Heliothis* (HelOA1, #Q25188), *Caenorhabditis* (CeSER2 [57]), rat (rTA1, #AAK71237), human (hTA1, #AAK71236), and an octopamine receptor from *Drosophila* (OAMB, #AAC17442). The signaling pathways activated by the receptors are given on the right.

The recent cloning of mammalian GPCRs that are activated by trace amines [12, 13] adds at least two additional members to the tyramine receptor family, rat trace amine receptor 1 (rTA1) and human trace amine receptor 1 (hTA1). Both are more potently activated by tyramine than by octopamine [12, 13]. The signaling properties of the receptors were tested after injection of receptor mRNAs, together with mRNA encoding a cAMP-responsive Cl^- channel (CFTR), into *Xenopus* oocytes. Receptor activation by trace amines (tyramine and β-phenylethylamine) resulted in a Cl^- inward current. This result suggests that the mammalian TA1 receptors cause the stimulation of adenylyl cyclase through G_s-type G proteins. The increase in $[cAMP]_i$ leads to CFTR activation and Cl^- conductance. In comparison with invertebrate sequences, both rTA1 and hTA1 receptors contain more variant residues in IL3 (Fig. 4). This might be the reason for their different signaling properties.

What information can be drawn from the expression studies of octopamine receptor genes? The octopaminergic system of invertebrate species has attracted much more interest than the tyraminergic signaling system, and the physiological role of octopamine has been studied *in vivo* extensively (for recent reviews, see: [3, 11, 61]. Pharmacologically, four different types of octopamine receptor have been distinguished. They are associated either with an increase in $[Ca^{2+}]_i$ (OCT-1 receptor) or with the activation of adenylyl cyclases (OCT-2A, OCT-2B, and OCT-3 receptors) [1, 3, 62 - 67]. Molecular cloning of octopamine receptors has lagged behind the molecular identification of other biogenic amine receptors. The first *Drosophila* gene cloned and claimed to encode an octopamine receptor [40] was convincingly shown to encode a tyramine receptor (DmTYR, see above and [41]). The DmTYR receptor attenuates cAMP production. Related receptor genes have been cloned from moths, *Heliothis virescens* and *Bombyx mori* (HelOA1 and BomOA1 [44]). The heterologously expressed HelOA1 receptor causes inhibition of adenylyl cyclase activity when stimulated with octopamine, whereas no significant effect is observed with tyramine [44]. This result does not agree with the known signaling properties of native octopamine receptors. So far, only increases in $[cAMP]_i$ and/or $[Ca]_i$ have been described for octopamine receptors *in vivo* [68], in intact organ preparations [62-64], in membrane preparations of various tissues [42, 69 - 72], and in various insect cell lines [73 - 77]. Interestingly, the receptors of both moths share significant sequence identity with tyramine receptors. Residues involved in G protein binding are almost identical between HelOA1, BomOA1, DmTYR, AmTYR1, and LocTYR1 (Fig. 4). One thus might consider re-evaluating the pharmacological properties of the *Heliothis* receptor with respect to agonist affinity and efficacy.

In the meantime, however, one receptor from *Drosophila* (OAMB [43]), one from the sea slug *Aplysia californica* (ApOA1 [58]), and one from the pond snail *Lymnea stagnalis* (LymOA1 [49]) have been found to be specifically activated by octopamine. The signaling properties of these receptors largely confirm what is known from native receptors. The receptor cloned from *Aplysia* exclusively stimulates adenylyl cyclase activity [58]. Two cellular signals are produced by both the *Drosophila* and the *Lymnea* receptors. When activated by octopamine, they cause an elevation of $[cAMP]_i$ and $[Ca^{2+}]_i$ [43, 49]. The promiscuous coupling to both intracellular signaling pathways might be attributable to the expression system chosen, and it remains to be tested to which signaling pathway these two receptors couple *in vivo*. Our own results obtained

for an octopamine receptor from *Apis mellifera* (AmOA1 [78]) show that this receptor specifically activates the IP_3/Ca^{2+} pathway. Since AmOA1 is orthologous to LymOA1 and OAMB, one might assume these receptors form the native OCT-1 receptor class that is known to cause Ca^{2+} elevation in cells [1, 3, 67].

Meanwhile, a second octopamine receptor (LymOA2) cDNA has been cloned from *Lymnaea stagnalis* [50]. This receptor seems to couple to an atypical signaling pathway. Stimulation of heterologously expressed LymOA2 with octopamine leads to changes in the membrane conductance of the cells. It has been proposed that the receptor activates a voltage-independent Cl^- current by a mechanism that probably involves protein phosphorylation [50]. Whether stimulation of LymOA2 in *Lymnaea* neurons *in vivo* also affect Cl^- conductances remains to be investigated.

Physiological functions of tyraminergic and octopaminergic systems *in vivo*

In comparison with vertebrates in which only trace amounts of tyramine and octopamine have been detected, both phenolamines are present in high concentrations in the CNS and periphery of invertebrates [1, 5, 79, 80]. It was assumed for a long time that tyramine only serves as a biochemical precursor of octopamine and thus might not have any significant neuroactive function. However, molecular cloning and the functional characterization of specific tyramine receptors from various invertebrate species [41, 42, 45, 57] and recently also from rat and human [12, 13] call for a re-evaluation of the primary assumptions. Nevertheless, the physiological role of the tyraminergic system *in vivo* needs to be examined by further studies. Only a few reports have been published addressing the functional role of tyramine. Tyramine has been shown to alter the behavioral sensitivity of *Drosophila* to cocaine [81]. Exposure of flies to cocaine leads to an increase in tyrosine decarboxylase activity, which parallels the development of sensitization. In addition, tyramine causes an increase in chloride conductance across the *Drosophila* Malpighian tubule [82]. Physiological effects specific for tyramine have also been reported for other insects. In the cockroach, tyramine stimulates trehalose metabolism in isolated fat bodies [83]. In addition to fulfilling important criteria as a transmitter candidate in the locust CNS [84], tyramine inhibits the contraction of locust visceral muscles [85] and, after repeated injections, tyramine even reduces locust viability [86].

Analysis of biogenic amine signaling systems in invertebrates suffers from the lack of receptor mutants. This situation has recently been overcome. A tyramine receptor mutant with reduced receptor expression levels has been identified in *Drosophila* [87]. The *hono* mutant displays deficits in olfactory responses. Furthermore, tyramine fails to inhibit excitatory junctional potentials at larval body-wall muscles in the mutant. Tyramine is considered to be well-suited to act as a neuromodulator in the olfactory system and at the neuromuscular junction [87, 88]. However, tyramine and octopamine are structurally very similar, and tyramine receptors have been shown to be activated by octopamine, although at significantly higher concentrations, in pharmacological experiments and *vice versa* [41, 42, 43]. One could assume that some of the effects attributed to the missing tyramine receptors might be caused by promiscuously activated octopamine receptors. How could this take place? In *hono* flies, tyramine synthesis is not

impaired. The endogenous binding partner of tyramine, the receptor encoded by the *hono* gene, however, is almost absent. When tyramine is released upon activation of tyraminergic neurons, this might lead to an unusually high concentration of this amine. Since its ordinary binding partner is absent, tyramine instead might bind to octopamine receptors. These octopamine receptors are coupled to different intracellular signaling pathways from tyramine receptors. Rather than inhibiting cAMP production, a common property of tyramine receptors, octopamine receptors cause the production of cAMP or IP$_3$ and subsequent Ca^{2+} release. Because of these unusual actions of tyramine, the highly controlled neuronal circuits thus affected will be disturbed and cause the observed impairments. This interpretation is however speculative and needs to be tested by experimental approaches.

In contrast to tyramine, the physiological roles of octopamine have been studied in great detail in a number of invertebrate species. Octopamine can act as a neurotransmitter, a neuromodulator, and a neurohormone (for recent reviews, see: [3, 14, 15, 89]). Specific effects of octopamine have been described for both the CNS and peripheral organs. In insects, octopamine is referred to as a "flight or fight" hormone or a "sympathetic" circulation hormone [1, 2, 3, 5, 67, 79]. How can one address the role of a neuroactive substance when receptor mutants are missing? In a straightforward approach, Monastirioti et al. [21] have used a *Drosophila* mutant that does not express the enzyme tyramine-ß-hydroxylase. This enzyme is necessary to convert tyramine to octopamine. Therefore, these flies cannot synthesize octopamine. Nevertheless, the animals exhibit almost normal behavior and life span. The only phenotypic change occurs in female flies. They are unable to deposit their eggs properly and are therefore sterile. Sterility is overcome, however, by feeding them octopamine [21]. Although these results suggest that octopamine is not necessary for the proper development and differentiation of the fly, it is currently unclear whether the loss of octopamine might have been functionally substituted by promiscuous binding of tyramine to octopamine receptors.

Octopamine plays an important role during insect flight. This has been intensively studied in locusts (for reviews, see: [61, 90]). Previous work has suggested that octopamine influences the energy metabolism of flight muscles. It increases the concentration of fructose-2,6-bisphosphate and stimulates glycolysis [91 - 94]. During long flight periods, the flight muscles switch to lipid metabolism as the energy source [93, 94]. A subpopulation of octopaminergic dorsal unpaired median (DUM) neurons, viz., those that exclusively innervate flight power muscles, are inhibited during short bouts of flight activity [95]. It is assumed that the decrease in octopaminergic neurotransmission switches off the glycolytic pathway and thereby turns on lipid metabolism [61, 95]. Another group of DUM neurons, which activate leg and other thoracic muscles, behave differently in that they are activated during flight activity [95]. Leg muscles lack the biochemical machinery for lipid metabolism. Therefore, glycolysis has to be maintained for energy production in these muscles even during flight [95].

Skeletal and visceral muscles are the main targets for the octopaminergic efferent DUM neurons. Modulation of neuromuscular transmission by bath application of octopamine and/or stimulation of individual DUM neurons has been studied in great detail (for recent reviews, see: [61, 96]). Octopamine reduces basic tension and causes an increase in twitch amplitudes and relaxation rates in skeletal muscles [97 - 101]. It modulates heart rate in *Drosophila* [102, 103] and inhibits myogenic rhythms of visceral

muscles [104-106]. Generally, the octopaminergic system of invertebrates, like the norepinephrine/epinephrine system of vertebrates, seems to adapt the animals to energy-demanding situations ("fight or flight") [3]. In addition, octopamine also influences the response characteristics of sensory organs, including mechanoreceptors [107 - 110] and pheromone receptors [111, 112].

In a variety of behavioral tests, the effects of various amines have been examined by bath application, feeding, or injection. Response thresholds and habituation rates of feeding responses in honeybees [113-115] and flies [116] and of visual responses in bees [117, 118] and locusts [89, 119, 120] are significantly reduced by octopamine. Furthermore, octopamine modulates antennal scanning in the bee [121]. In the honeybee sting response, octopamine reduces the rhythmic motor component of the reflex but potentiates sting extension [122]. Similar differential effects have also been observed in the lobster, where octopamine selectively enhances the activity of neurons and muscles responsible for generating the "extension posture", while diminishing the activity in those causing the antagonistic "flexion posture" [123-125]. Other behaviors that are induced or modulated by octopamine are pharyngeal pumping, locomotion, and egg-laying in *Caenorhabditis elegans* [126], firefly flashing [127], locomotory and grooming patterns in decapitated *Drosophila* [128], and nestmate recognition and the onset of foraging in honeybees [129-131].

One particular octopaminergic neuron, VUM_{mx1}, plays an important role in the reinforcement pathway during honeybee olfactory conditioning [132]. 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 [133]. It has been concluded that octopamine selectively mediates the reinforcing but not the sensitizing or response-releasing function of the sucrose reward [134]. Recently, Farooqui et al. [135] have used the RNA interference (RNAi) technique to disrupt the octopamine-mediated reinforcement pathway involved in honeybee olfactory learning. Injection of RNAi directed against a putative octopamine receptor gene into the antennal lobe resulted in 80% and 50% inhibition of acquisition and recall responses, respectively [135]. This is a very promising result because it shows that the RNAi technique is a suitable tool for studying impaired gene function(s) in a behaviorally well characterized organism that is, however, difficult to manipulate genetically. We expect that, in the near future, the application of RNAi techniques will shed more light on the functions of invertebrate octopamine and tyramine receptors, which may include the possible involvement in learning and memory as one particularly fascinating aspect.

Conclusion

The phenolamines tyramine and octopamine are important mediators and regulators of diverse physiological functions in invertebrates and probably also in vertebrates. A prerequisite to understanding the physiological role of the tyraminergic and octopaminergic signaling systems is the molecular characterization of the respective receptors. Once the receptor genes are cloned, heterologous expression should aid the identification of their downstream reaction partners. The application of molecular biological methods has greatly enhanced our knowledge of the receptor polypeptides.

From these studies and the analysis of completely sequenced genomes of the nematode *Caenorhabditis elegans* [136] and various insects (*Drosophila melanogaster* [17], *Anopheles gambiae* [137]), it has become evident that multiple tyramine and octopamine receptor subtypes exist in invertebrates. All receptors identified so far belong to the superfamily of GPCRs. Heterologously expressed receptors usually activate the same signaling pathways as native receptors *in vivo*. Nevertheless, several receptor subtypes still appear to be "missing" as not all of the pharmacological and signaling properties of octopamine and tyramine receptors described *in vivo* are covered by the cloned receptors. However, this gap will certainly soon be closed for some "model organisms" by detailed analysis of their completely sequenced genomes. Knowledge of the receptor sequences and the most probable signaling partners of the various receptors should then lead to the design of experimental approaches aimed at elucidating the functions of the receptors *in vivo*. For some organisms, RNAi may become a versatile technical tool, and we eagerly anticipate results indicating whether the concepts that have been extrapolated from heterologous systems also apply *in vivo*.

Acknowledgement

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