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Sex peptides and MIPs can activate the same G protein-coupled receptor

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ABSTRACT

In many animal species, copulation elicits a number of physiological and behavioral changes in the female partner. In *Drosophila melanogaster*, the main molecular effector of these physiological responses has been identified as sex peptide (SP). The sex peptide receptor (SPR) has been characterized and recently, its activation by *Drosophila* myoinhibiting peptides (MIPs)–in addition to SP–has been demonstrated. The myoinhibiting peptides are members of a conserved peptide family, also known as B-type allatostatins, which generally feature the C-terminal motif –WX_6Wamide.

1. Sex peptides

Sex peptide (SP, also known as Accessory gland peptide 70A), was characterized in 1988 as a factor synthesized in the *Drosophila melanogaster* male accessory glands and transferred to the female upon copulation. As a result, egg laying is increased and the female displays a loss of receptivity towards new mating partners. Sequencing of the SP-encoding cDNA resulted in the identification of the corresponding precursor, which contains the 36 amino acids (aa) long SP preceded by a 19 aa hydrophobic signal sequence (Chen et al., 1988). Analysis of the SP aa sequence from various *Drosophila* species revealed two regions of high similarity. The five most N-terminal aa (WEWPW) are identical and the C-terminal region, encoded by the second exon, shows strong conservation (Kubli, 2003). In insects other than *Drosophila* species, SP has not been identified (Kim et al., 2010; Poels et al., 2010; Yapici et al., 2008).

From a structural point of view it is interesting to note that the N-terminal region of SP contains a WPWN motif that forms type 1 β-turns and a central region rich in hydroxyprolines which assumes an extended and/or polyproline II-like conformation. The latter conformation refers to the rather open helical structure formed by consecutive Pro residues in which three residues constitute a full left-handed turn (Rath et al., 2005). The C-terminus of SP, including the loop formed by the disulfide bond, displays nascent helical and β-turn structures (Chen et al., 1988; Moehle et al., 2011).

A second SP-like factor, the ductus ejaculatorius peptide 99B (DUP99B), was not characterized until 2002. As its name implies, DUP99B is produced in the ejaculatory duct from a 54 aa precursor which gives rise to a 31 aa mature peptide. Structural analysis of DUP99B indicated that it is linear N-terminally and features a circular C-terminal part resulting from a disulfide bridge formation. In between the two bridge-forming Cys residues, 9 out of the 11 aa are identical between SP and DUP99B. Furthermore, DUP99B elicits the same post-mating responses as SP when injected into a female fly's haemolymph. The mature DUP99B features a pyroglutamic acid modification and the N-terminal region undergoes glycosylation, consisting of a difucosylated oligosaccharide structure. This latter modification decreases the dose of peptide required to induce ovulation, but this effect has not been observed in receptivity assays (Saudan et al., 2002).

The genes encoding SP and DUP99B have been cloned and sequenced from *D. melanogaster* and both display an intron insertion at the same site. The highly similar C-terminal parts of the peptides are encoded by the second exon, whereas the first encodes the signal sequence and the N-terminal part of the peptide. It has been proposed that both genes have arisen by gene duplication. This is supported by the aa sequence similarities in not only the C-terminal regions of the peptides, but also in the signal sequences (Saudan et al., 2002).

The temporal expression profiles as determined for *D. melanogaster* reveal striking similarities for both the *sp* and the *dup99B* gene. During development little or no transcript can be detected until the late pupal stages and expression levels are by far the highest in male adults (Poels et al., 2010). In contrast, the spatial distribution is different for both peptide precursor mRNAs. *SP* transcript can only be detected in the male accessory gland, whereas *dup99B* expression is limited to the upper ductus ejaculatorius and the

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cardia of both male and female flies (Poels et al., 2010; Saudan et al., 2002).

2. Myoinhibiting peptides

The myoinhibiting peptides–also known as B-type allatostatins (AST-B) or in Lepidoptera, prothoracicostatic peptides (PTSP)–are a family of short peptides characterized by a C-terminal -WX_{6}-Wamide sequence. The first identified member of this peptide family was the myoinhibiting peptide from *Locusta migratoria* (Schoofs et al., 1991). This *Lom*-MIP was purified from an extract of brains, corpora cardiaca, corpora allata and suboesophageal ganglia. It was determined to repress spontaneous contractions of the hindgut and the oviduct in *L. migratoria*, as well as the hindgut of *Leucophaea maderae*. These peptides were termed B-type allatostatins after the discovery of the four peptide family members (*Grb*-AST1–4) from the cricket, *Gryllus bimaculatus*, which are capable of inhibiting the synthesis of juvenile hormone by the corpora allata in this species (Lorenz et al., 1995).

In the fruit fly, the *myoinhibitory peptide* (*mip*) gene encodes a 211 aa precursor in two exons, spaced by an intron. This prepro-hormone starts with a signal sequence and gives rise to five MIPs which are flanked by dibasic or tribasic cleavage sites (Vanden Broeck, 2001; Williamson et al., 2001). MIPs have been identified and/or detected in various insect species including in *B. mori*, in which the precursor gives rise to eight peptides (Yamanaka et al., 2010). In *Rhodnius prolixus,* the *mip* gene has four exons and encodes a precursor that holds 12 MIPs. Interestingly, three of these peptides feature the typical -WX_{6}Wamide C-terminus while the other nine contain –WX_{7}Wamide (Lange et al., 2012; Ons et al., 2011).

MIPs have also been identified in aphids and ticks (Christie, 2008a,b). In *Ixodes scapularis*, the 132 aa MIP precursor likely gives rise to three peptides, of which two show the hallmarks for C-terminal amidation (Simo et al., 2009b). Also in Crustacea, MIPs have been found and with five exons, the *AstB* gene is rather complex in *Daphnia pulex* (Dircksen et al., 2011). However, most of the crustacean MIPs have been identified in decapod species, such as *Cancer borealis*, *Homarus americanus* and *Marsupenaeus japonicus* (Fu et al., 2005, 2007; Gard et al., 2009; Ma et al., 2008, 2009, 2010). A MIP has also been found in the mollusc, *Aplysia californica* (Moroz et al., 2006).

In contrast to the strictly limited expression profile of the sex peptides, the MIP-encoding transcript is much more widely distributed. In *D. melanogaster*, expression of the precursor mRNA is found throughout all developmental stages and is by far most abundant in the central nervous system (CNS). These data correspond with results from immunohistochemistry, which point to MIP presence in the CNS of *D. melanogaster* and *Aedes aegypti* (Kim et al., 2010; Poels et al., 2010). Immunohistochemistry in combination with Gal4 enhancers has allowed for the identification of MIP expression sites in the central complex of the *D. melanogaster* brain. Immunoreactivity appears in the ventral, central and dorsal layers, with the latter showing strong labeling (Kahsai and Winther, 2011). The antennal lobes of *D. melanogaster* contain 10–15 MIP-positive interneurons each, which likely correspond to two cell populations (Carlsson et al., 2010). Also, a novel MIP-producing neuron was identified which has cell bodies anterior to the base of the optic lobe medulla. These neurons are closely associated with branches of clock neurons, suggesting that MIP has a role in the circadian clock (Kolodziejczyk and Nassel, 2011). Immunohistological studies on non-neuronal tissues of *D. melanogaster* have revealed additional sites of strong MIP immunoreactivity in the larval midgut in the region before and in between the copper cells. Also, a group of cells in the posterior part of the larval midgut shows MIP immunoreactivity. In contrast, in the adult midgut MIP immunoreactivity is hard to pinpoint (Veenstra, 2009).

In *R. prolixus*, a large number of MIP immunoreactive neurons are present in the brain and the mesothoracic ganglion mass. Within the brain, the neurons show processes associated with the optic, antennal and medial lobe. Further MIP immunoreactivity can be detected in the suboesophageal ganglion and prothoracic ganglion. Processes showing MIP immunoreactivity are also associated with the oesophagus, salivary gland, posterior midgut and anterior hindgut. Interestingly, in the reproductive tissues, MIPs are detected in processes on the oviduct and male accessory glands (Lange et al., 2012).

Recent data from the cockroach *Leucophaea maderae* demonstrated the wide distribution of MIP immunoreactivity in the brain and using mass spectrometry, the presence of five MIPs in the accessory medulla and the corpora cardiaca was established. Colocalization of MIP and pigment dispersing factor was found in a number of neurons which may point to a role of MIPs in the circadian clock of this species, as has also been suggested for *D. melanogaster* (Schulze et al., 2012).

Using *in situ* hybridization, the transcript for the *B. mori* PTSP has been mapped to the central nervous system with the most intense signals in a pair of brain medial neurosecretory cells, but also strong signals from the interneuron 704 and epiproctodeal glands. In the brain, the expression is detected during the feeding period, but decreases before the time of each ecdysis. Transcript levels in the terminal ganglia, however, remained constant during the fourth and fifth instar stages. In the epiproctodeal glands, the expression is strong in pharate fifth instar larvae and just after ecdysis. It is at a low level during the first five days of the fifth instar stage, then rises. These data are also supported by immunohistological studies in *B. mori* and *Manduca sexta* (Davis et al., 2003; Yamanaka et al., 2010).

Furthermore, in *L. migratoria*, the presence of the peptide has been traced to approximately 12 brain neurons, including those innervating the glandular cells of the corpora cardiaca and the corpora allata. MIP-positive cell bodies can also be found in the suboesophageal ganglion, the metathoracic ganglion and the abdominal ganglia and MIP processes are associated with the heart, the oviduct, and the hindgut (Schoofs et al., 1996).

For the tick species *Ixodes scapularis* and *Rhipicephalus appendiculatus*, MIP is detected in the synganglion, neurons of the protocerebrum, palpal lobe, cheliceral lobe and the pedal lobes 2 and 3 (and 4 for *I. scapularis*) and on the ventral side, the opistosomal lobe and a number of small neurons in *I. scapularis*. Furthermore, the salivary gland in the tick is innervated by MIP-positive projections (Simo et al., 2009a,b).

In decapods, MIPs are widely distributed throughout the CNS as indicated by mass spectrometry analyses. In these animals, MIPs likely serve as both auto- or paracrine factors and as circulating hormones (Christie et al., 2010). Immunostaining revealed the distribution of this peptide throughout the stomatogastric nervous system of the Jonah crab, *Cancer borealis*. Immunoreactivity was detected in neurons and neuropil in the commissural ganglia, in somata in the oesophageal ganglion, in fibers in the stomatogastric nerve and in neuropilar processes in the stomatogastric ganglion (Szabo et al., 2011).

3. Biological actions of sex peptides

The biological effects initially attributed to SP have been witnessed both in mated female flies and in virgin females that were injected with SP. First, mated females will actively reject new males that present themselves as additional mating partners. Second, the mated female displays a strongly increased egg laying (Chen et al., 1988).

Furthermore, SP induces the progression of oocytes to stage 10, likely mediated by juvenile hormone (JH) as SP is known to have