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Review

Insect gonadotropic peptide hormones: some recent developments

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Abstract: Gonadotropic peptides are a new generation of peptide hormone regulators of insect reproduction. They have been isolated from ovaries, oviducts, or brains of insects. The subject of this paper is insect peptides that exert stimulatory or inhibitory effects on ovarian development and oocyte maturation. On the basis of the literature data and the results of our investigations, the structure and biological properties of different groups of peptides are presented. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: insect reproduction; gonadostimulatory peptides; gonadoinhibitory peptides; ovary development; oocyte growth; vitellogenesis regulators

INTRODUCTION

The subject of the present paper is gonadotropic peptide hormones from insects, the substances that regulate their reproduction. Factors isolated from gonads or insect brains that do not influence the reproduction process directly, but are inhibitors of proteolytic enzymes in the gut and affect the cyclic adenosine monophosphate (cAMP) biosynthesis in insect ovaries or control the activity of neuroendocrine glands of *corpora allata* (CA) also belong to this group of peptides. It should be emphasized that until recently the juvenile hormones (JHs), substances of isoprenoid character, were considered to be the insect gonadotropic hormones. In the last 15 years, considerable progress has been made in the field of isolation and identification of insect peptides. There has been particular emphasis on peptide hormones that stimulate or inhibit the development of insect ovaries and oocytes growth [1–3].

The insect female gonadotropic cycle is a process subject to nervous and hormonal control. Ovary functions are controlled by hormones of diverse chemical structures, including JHs, ecdysteroids, and peptide hormones. In many insects, JHs secreted by the CA stimulate the differentiation of follicular epithelium in ovaries and regulate vitellogenin synthesis in the fat body and accumulation of vitellins in the egg yolk [3]. Ecdysteroids are produced by the ovaries of many insects [4], but their role in ovarian development is not completely understood. In *Drosophila melanogaster*, ecdysteroids regulate vitellogenin biosynthesis and can have an antagonistic effect on oocyte progression and lead to apoptosis of egg chambers (Figure 1) [3].

Peptide gonadotropic factors present in insect ovaries and brains show different activities. They stimulate or inhibit ovary development and oocyte growth [1,2], affect vitellogenin biosynthesis in the fat body [5], influence the synthesis of trypsin and serine proteases in the gut [6,7], and regulate the ovarian biosynthesis of ecdysteroids and cAMP [1,8].

As regards their structure, insect gonadotropins are polypeptides (neuroparsins, parsins) or oligopeptides, like *Aea*-TMOF, *Neb*-TMOF, *Neb*-colloostatin, and *Led*-NPF (NPF – neuropeptide F). The search for such hormones is justified not only due to cognitive but also due to practical reasons so that gonadotropins may be used as ecologically safe insect population control agents. Studies of one of these peptides, *Aea*-TMOF, are at an advanced stage with respect to its use as a biorational insecticide against mosquito larvae and possibly other aquatic insect larvae [6,9].

Parsins and Neuroparsins

It has been supposed for many years that parsins and neuroparsins are the only insect gonadotropin hormones. This view changed in 1987, when Girardie *et al.* [10] discovered peptidic gonadotropic factors. These were neuroparsins A (NPA) (*Lom*-NPA I-IV [10,11] and *Scg*-NPA I-IV [11,12]), neuroparsins B (NPB) (*Lom*-NPB [10,11] and *Scg*-NPB [11,12]), and parsins (*Lom*-OMP [13] and *Scg*-OMP I-IV [12]). These factors had been isolated from the brain – cardiac cardiaca complex of the migratory locust *Locusta migratoria* and the desert locust *Schistocerca gregaria* (Figures 2 and 3).

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BIOGRAPHIES

Dr Mariola Kuczer is a member of Chemistry of Natural Products Team, Faculty of Chemistry, University of Wroclaw. She obtained her MSc in chemistry from Wroclaw University in 1992 and started work at the University as an assistant. She obtained the PhD degree in organic chemistry in 1998. She spent a year (1999–2000) as a postdoctoral fellow at the Laboratory for Development Physiology and Molecular Biology of the Zoological Institute of the Katholieke Universiteit, Leuven, Belgium. The main subjects of her studies are the synthesis and structure/function relationship investigations of insect peptides, such as proctolin and oostatic hormones. She is a co-author of 20 scientific papers published in international journals and in several conference presentations. As a research associate at the University, she handles lectures, seminars and laboratories in organic chemistry for students of chemistry and biology.

Grzegorz Rosinski was born in Kruchowo, Poland, in 1948. He received the MD degree in zoology in 1973 and PhD degree in animal physiology in 1981 at the Poznan University. His current research interests include animal physiology and biochemistry, imaging of physiological functions, insect neuroendocrinology, the development of new bioassays in insect physiology and biosignal acquisition and analysis. He is currently an associate professor in the Department of Animal Physiology and Development. He is a member of the Polish Entomological Society, and he has served on the editorial board of the journal *Pesticides*.

Konopinska Danuta is full professor and head of the Chemistry of Natural Products Group at the Faculty of Chemistry, University of Wroclaw. She graduated from the Pharmaceutical Faculty of the Medical University in Wroclaw and also obtained the masters degree. During 1966–1969 she worked as an assistant in the Department of Biochemistry of the Medical University in Wroclaw. In 1969 she moved to the Department of Organic Chemistry in the Faculty of Chemistry at the University of Wroclaw, from where she obtained the PhD (Doctor of Chemistry) degree in 1972. She obtained her DSc degree in 1978 and became a full professor in 1991. During 1973–1974, she worked as a postdoctoral fellow at the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences, Prague, in Dr Zaoral's group. In 1979–1980 and again in 1985 she was a scientific visitor to the Tufts University of Boston, USA. In 1988 she was at the Weitzmann Institute in Rehovot, Israel, and in 1992 at Northwestern University, Chicago. Her subjects of interest are biologically active peptides and amino acids. Her studies have comprised the synthesis and structure/function relationship investigations of insect neuropeptides, such as proctolin, hypertrehalozemic and allatotropic peptides, and other myotropic peptide factors as well as arthropod neurotoxic peptides. She has published 354 papers, which include original and review articles as well as patents (190) and short communications (164). In addition, she teaches at the University and conducts lectures and seminars for students of chemistry and biology in organic chemistry, biochemistry and chemistry of natural products.

Neuroparsin A I (NPA I), isolated from *L. migratoria*, is an 83-amino acid residue polypeptide cleaved from its peptide precursor *Lom*-NPP (NPP – neuroparsin precursor) consisting of 107 amino acid residues (Table 1) [14], and neuroparsin B is the remainder (Table 1). Neuroparsins A from *L. migratoria*, like neuroparsins A from *S. gregaria*, contain 78–83 amino acid residues and they show small differences in their peptide sequences (Table 1). On the other hand, neuroparsin B from *S. gregaria* contains 93 amino acid residues and it exhibits a partial structural similarity to neuroparsin B from *L. migratoria*. It is interesting from the structural point of view that all neuroparsins contain 12 Cys residues at the identical positions of the peptide chain. Moreover, *Lom*- and *Scg*-neuroparsins have the same *N*-terminal peptide fragment.

It follows from the *in vivo* biological studies [12,15] that all neuroparsins inhibit the JHs biosynthesis, which results in the blocking of ovarian development and vitellogenin biosynthesis in locust.

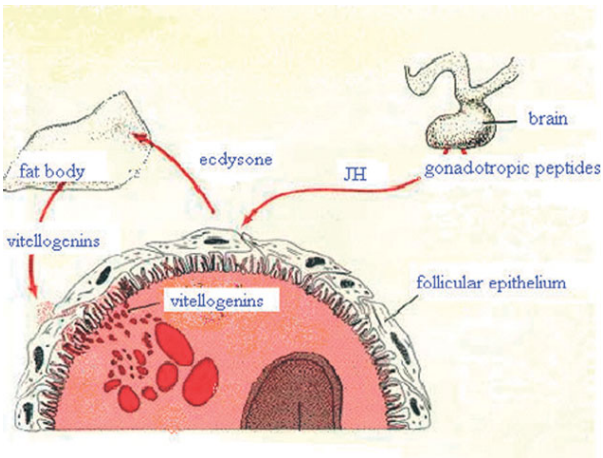

Figure 1 The female insect gonadotropic cycle.

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Figure 2 *Locusta migratioria*.

In a continuation of their studies, Girardie *et al.* [12,13] have isolated further peptide gonadotropins from the brain of locusts *L. migratoria* and *S. gregaria*,

Figure 3 *Schistocerca gregaria*.

parsins *Lom*-OMP [13] and *Scg*-OMP 1–3 [12] (OMP - ovary maturing parsin). *Lom*-OMP has been found in mature males, females, and larvae. It consists of 65 amino acid residues in two forms, which differ from each other by the presence of Ala or Ser at position 25. The sequence of parsin derived from *S. gregaria*, *Scg*-OMP 1, is similar to that of parsins *Lom*-OMP [12] (Table 1). *In vivo* biological studies have shown that parsins stimulate the growth of oocytes and induce vitellogenin synthesis in mature locust specimens only [16]. Furthermore, they stimulate the ecdysone production by insect follicular cells [17]. On the basis of the results of biological studies, it can be assumed that parsins are a physiological counterpart of the luteinizing hormone (LH) and follicle stimulating hormone (FSH) in mammals [18].

Table 1 Structure neoparsin and parsin

Peptide	Sequence	Ref.
Neuroparsin: *Lom*-NPP	MKATAALVAATLLLAVTLFHRAER**NPISRSCEGANCVVDLTRCEYGDVTDFFGRKVCAK-GPGDKCGGPYELHGKCGVGMDCRCGLCSGCSLHNLQCFFFEGGLPSSC**	6
Lom-NPA I	NPISRSCEGANCVVDLTRCEYGDVTDFFGRKVCAKGPGDKCGGPYELHGKCGVGMDCRC GLCSGCSL-HNLQCFFFEGGLPSSC	10
Lom-NPA II	[3–83]-*Lom*-NPA	10
Lom-NPA III	[4–83]-*Lom*-NPA	10
Lom-NPA IV	[5–83]-*Lom*-NPA	10
Lom-NPB	[6–83]-*Lom*-NPA	10
Scg-NPA I	NPISRSCEGANCVVDLTRCEYGEVTDFFGRKVCAKGPGDKCGGPYELHGKCGDGMDCRC-GVCSGCSMQSLECFFFEGAAPNSC	12
Scg-NPA II	[3–83]-*Scg*-NPA	12
Scg-NPA III	[4–83]-*Scg*-NPA	12
Scg-NPA IV	[5–83]-*Scg*-NPA	12
Scg-NPB	[6–83]-*Scg*-NPA	12
Parsin: *Lom*–OMP	YYEAPPDGRHLLLQPAPAAPVAPA(A or S)PASWPHQQRRQALDEFAAAAAAAADAQFQDEEE-DGGRRV	13
Scg-OMP 1	YYEAPPDGQRLLLQAAPAAAPAAPAAASWPHQQRRQAIDEFAWPHQQRRQAIDEFAAAAAAAA-DAQYQDEEEDGARRV	12
Scg-OMP 2	YYEAPPDGQRLLLQAAPAAAPAAASWPHQQRRQAIDEFAAAAAAAADAQYQDEEEDGARRV	12
Scg-OMP 3	QAAPAAAPAAPAAASWPHQQRRQAIDEFAAAAAAAADAQYQDEEEDGARRV	12

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TRYPSIN BIOSYNTHESIS MODULATING OOSTATIC FACTORS

Aea-TMOF

The second group of insect gonadotropins is made up of factors inhibiting oocyte maturation and trypsin biosynthesis; among them are decapeptide *Aea*-TMOF and hexapeptide *Neb*-TMOF. These peptides are interesting because they are shorter than neuroparsin and parsin.

Borovsky *et al.* [19,20] reported the discovery of the gonadotropic peptide *Aea*-TMOF in the ovaries of the malaria vector, the mosquito *Aedes aegypti* (Figure 4). It is a peptide of the sequence YDPAPPPPPP. It consists of 10 amino acid residues and contains as many as 7 Pro residues, with 6 of them located in a row at the *C*-terminus of the peptide chain. Biological studies showed that the peptide inhibits the oocyte maturation and trypsin biosynthesis in insects. Combining these biological properties, Borovsky *et al.* [21] called it *Aea*-TMOF (TMOF – trypsin biosynthesis modulating oostatic factor).

Aea-TMOF has become the subject of various studies. The purpose of these studies has been to explore the biological properties of the peptide, the structure–biological activity relationship, and its possible use in insect population control.

Biological tests have been carried out on female mosquitos fed with *Aea*-TMOF mixed with chicken blood. The influence of *Aea*-TMOF on the oocyte growth and trypsin biosynthesis has been estimated [20]. Analyzing the inhibition of oocyte growth, maturation, and trypsin biosynthesis, the authors found that it results from blocking of vitellogenin transport from haemolymph to the ovaries [9]. Further studies have shown that the peptide is produced by the epithelium ovary cells. Within 24–42 h, it is transported by haemolymph to the endothelial alimentary tract cells and then it gives a signal to inhibit trypsin biosynthesis [20]. This results in blocking of synthesis of vitellogenin, the protein that is essential for oocyte growth. It has also been found that after the mosquitos were fed isotopically labeled *Aea*-TMOF, 72 h later, 28% of the peptide was found in the ovaries [20], showing that after administration via the alimentary tract, *Aea*-TMOF retains oostatic and trypsin biosynthesis inhibition properties. Further investigations of the peptide have shown that it modulates the biosynthesis not only of trypsin but also of chymotrypsin-like enzymes [7].

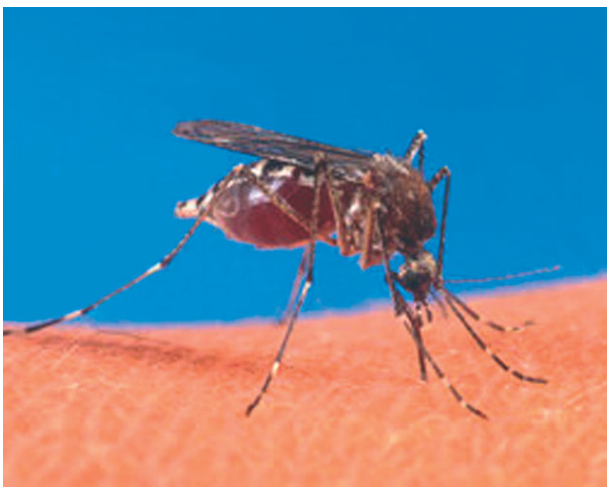

Figure 4 *Aedes aegypti.*

Studies on the structure–biological properties of *Aea*-TMOF have been taken up by Borovsky *et al.* [6,20] and Hlavacek *et al.* [22–27]. Oostatic and trypsin biosynthesis modulation effects of *Aea*-TMOF analogues have been evaluated *in vivo* on adult specimens of *A. aegypti* [19,20] and *Neobellieria bullata* [22–27] and their influence on larva *A. aegypti* development has been investigated [6]. Among synthetic analogues of *Aea*-TMOF, biological activity is exhibited by the pentapeptide fragment [1–5]-*Aea*-TMOF and the protein HIV-2S-ORF fragment, H-Trp-Arg-Pro-Gly-Pro-Pro-Pro-Pro-Pro-Pro-NH_2 [20]. In the case of tests performed on *N. bullata* [22–27], it has been found that the native peptide, like its analogues with a shortened sequence of the H-Tyr-Asp-Pro-Ala- $(Pro)_n$-OH (YDPA$(P)_n$) type, where $n = 1–5$, and analogues c-YDPAP and c-YDPA retain biological activity. Derivatives Tyr-Asp-Pro-ΨCH_2O-Ala-OH and Tyr-Asp-Pro-ΨCH_2O-Ala-Pro-OH are similarly active. In the test for larva *A. aegypti* growth, it turned out that oligopeptides: $(DPAR)_4$ and H-Tyr-Asp-Pro-Arg-Tyr- Asp-Pro-Arg-Tyr-Asp-Pro-Arg-Tyr-Asp-Pro-Arg-OH $((YDPAR)_4)$ are twice and four times more potent, respectively, than *Aea*-TMOF [6]. On the basis of these results it is difficult to draw a conclusion for *Aea*-TMOF regarding the structure–oostatic and structure–trypsin biosynthesis modulation function relationships.

Recently, during a search for a possibility of using *Aea*-TMOF as an ecologically safe insecticide, the synthesis of nonpeptide analogues of *Aea*-TMOF was carried out [28]. These analogues are carboxylic acid esters (Table 2) and they were designed as nonpeptide analogues of the *N*-terminal fragment of *Aea*-TMOF, Tyr-Asn-Pro-Ala-Pro.

In vivo biological studies have shown that three of these analogues (CHEA, PPHEN, and PHA) exhibit higher toxic activity toward mosquito larvae *Culex pipiens* than *Aea*-TMOF. They are similarly toxic towards further insect species, like pests of tobacco – *Heliothis virescen*, cotton – *Hlicovera zea*, and cockroach – *Blattela germanica*. Moreover, PHEA, like *Aea*-TMOF, shows antifeeding activity toward *H. virescen*. The results of these studies suggest that in this group of compounds substances with the insecticide activity have been found, which may have practical application.

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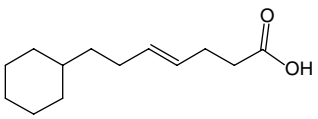
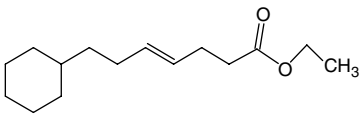
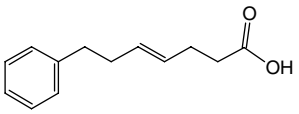
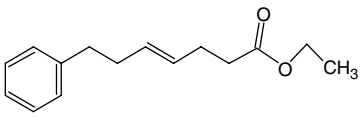
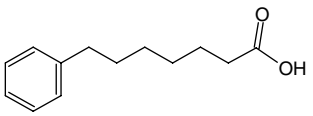
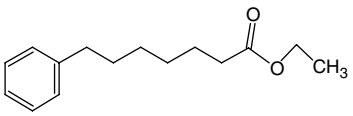
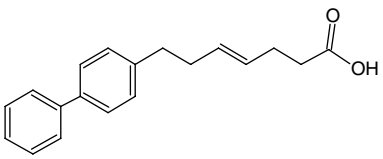
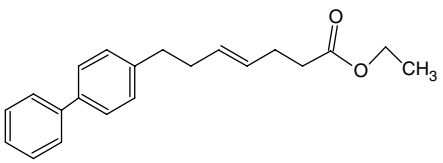
Table 2 Nonpeptide analogues of *Aea*-TMOF [28]

Structure	Abbreviation	Biological activity in relative to *Aea*-TMOF				
		Larvi of *C. pipiens*	Larvi of *H. virescens*	Larvi of *H. zea*	Cockroach of *B. germanica*	Larvi of *M. sexta*
	CHEA	+++	+++	+++	+	+++
	CHEN	+	nac	nac	nac	+
	PHEA	+	+++	+++	+++	+++
	PHEN	+++	nac	nac	nac	+
	PHA	+++	+++	+++	+++	+++
	PHN	+++	nac	nac	nac	+
	PPHEA	+++	nac	nac	nac	nac
	PPHEN	+++	nd	nd	nd	nac

+++, active; +, weak active; nac, non-active; nd, not done; CHEA, E-7-(cyclohexyl)hept-4-enoic acid; CHEN, ethyl E-7-(cyclohexyl)hept-4-enoate; PHA, 7-phenylheptanoic acid; PHEA, E-7-phenylhept-4-enoic acid; PHEN, ethyl E-7-phenylhept-4-enoate; PHN, ethyl 7-phenylheptanoate; PPHEA, E-7-((4-phenyl)phenyl)hept-4-enoic acid; PPHEN, ethyl E-7-((4-phenyl)phenyl)hept-4-enoate.

Neb-TMOF

In 1994, De Loof *et al.* isolated the hexapeptide NPTNLH (*Neb*-TMOF) from the ovaries of the flesh fly *N. bullata*, with the oostatic and trypsin biosynthesis inhibition in *N. bullata* properties similar to *Aea*-TMOF [29] (Figure 5). Like *Aea*-TMOF, *Neb*-TMOF is secreted at the last stage of vitellogenesis and it inhibits the biosynthesis of trypsin-like enzymes in the insect midgut cells. According to these authors, insufficient amount of trypsin evokes a deficiency of free amino acids in haemolymph, which are indispensable for vitellogenin production by the fat body, resulting in oocyte growth inhibition. Further studies have shown that *Neb*-TMOF also inhibits the biosynthesis of ecdysone, the vitellogenin synthesis inducing hormone of insect larvae [30–32]. It has been found recently that *Neb*-TMOF exerts a strong gonadoinhibiting effect

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on ovary development and oocyte maturation in beetle *Tenebrio molitor* (Figure 6) [33,34].

The stability of *Neb*-TMOF in the insect body was determined in *in vitro* tests on *N. bullata* by incubation of tritium-labelled *Neb*-TMOF in the hemolymph and the gut. It turned out that the peptide undergoes a fast enzymatic degradation in the haemolymph, whereas it is stable in the intestine [35]. Degradation of [^{3}H]-*Neb*-TMOF gives shorter fragments of its peptide chain. The main isolated products were the tetrapeptide H-Asn-Pro-Thr-Asn-OH (NPTN) and the dipeptides NP and LH.

In the literature, a lot of attention has been paid to the studies on the structure–biological function relationship of *Neb*-TMOF [36–40]. Its peptide chain has been modified at different positions to evaluate the influence of individual amino acid residues on its biological function. It follows from these studies that, among analogues of *Neb*-TMOF modified at position 1 of the peptide chain, only [Asp^1]- and [Ser^1]-*Neb*-TMOF show a similar activity to the native peptide [38]. In the case of analogues modified at position 4, [Thr^4]-, [Asp^4]-, [Glu^4]-, [D-Asn^4]-, and [Val^4]-*Neb*-TMOF, the inhibition of trypsin biosynthesis was comparable to *Neb*-TMOF [39]. On the other hand, replacing Asn^4 by such residues as Ser, D-Ser, Gln, or Asp(β-Cyc) gave analogues devoid of biological activity [39]. Among analogues of *Neb*-TMOF in which the *C*-terminal L-His residue at position 6 was substituted by other amino acid residues [36,37,39], several inhibited trypsin biosynthesis in *N. bullata*. These are [Lys^6]-, [Phe(4-NO_2)^6]-, [Phe(4-NH_2)^6]-, [Phe(4-OEt)^6]-, [Phe(4-OMe)^6]-, and [Phe(4-Cl)^6]-*Neb*-TMOF. In a series of *Neb*-TMOF analogues, it has been shown that the Asn residues at positions 1 and 4 of the peptide chain have a significant influence on the trypsin biosynthesis inhibition.

In further studies, new biological effects of *Neb*-TMOF and its analogues in vertebrates have been looked for. It turned out that *Neb*-TMOF and [Phg(4-Me_2N)^6]-*Neb*-TMOF exhibit a strong analgesic activity in the *in vivo* behavioral tests, compared to that of enkephalin [41]. This effect is due to the interaction of these peptides with the opiate receptors of the central nervous system of rats [41].

Figure 5 *Neobellieria bullata.*

OTHER GONADOINHIBITORY PEPTIDES

Neb-Colloostatin

Besides *Neb*-TMOF, De Loof *et al.* have isolated a peptide from the ovaries of the flesh fly *N. bullata* with oostatic properties, consisting of 19 amino acid residues of the sequence: SIVPLGLPVPIGPIVVGPR (*Neb*-colloostatin) [5]. It is another structurally characterized substance of the oostatic properties. It shows a structural homology with vertebrate and invertebrate collagen and, because of its oostatic properties, it has been called *Neb*-colloostatin. Looking at its amino acid sequence one can notice the presence of a large proportion of hydrophobic amino acid residues. Out of its 19 residues, 17 are amino acids like Ile (3), Val (4), Pro (5), and Gly (3). Moreover, in the peptide chain of colloostatin, there is a palindromic region between the third and ninth residues.

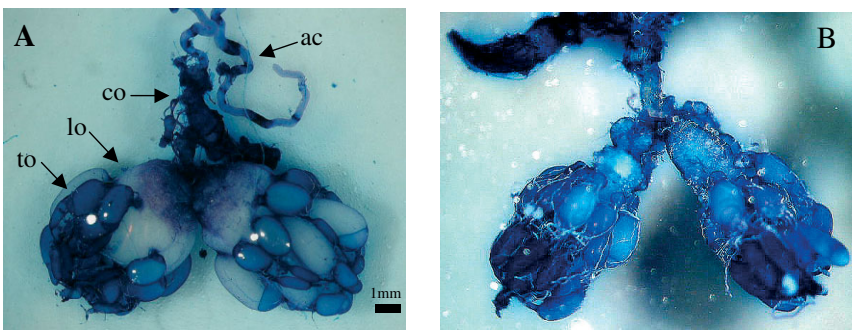

Figure 6 The ovaries of 4-day-old females of *T. molitor*. A-control, B-4 μg *Neb*-TMOF To-terminal oocyte, lo-lateral oviduct, co-common oviduct, ac-spermatheca with accessory gland.

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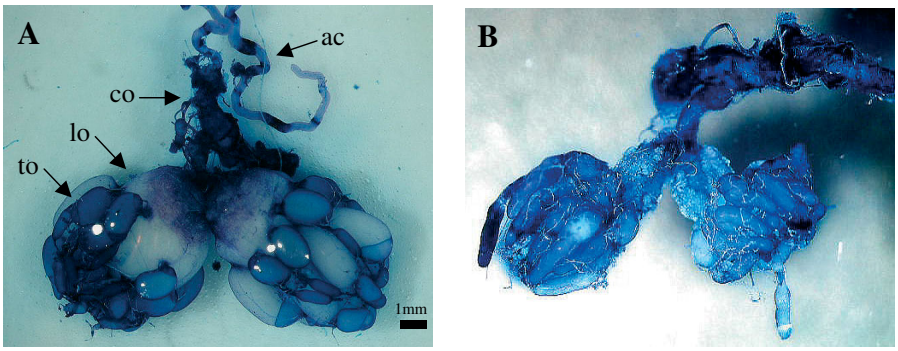

Figure 7 The ovaries of 4-day-old females of *T. molitor*. A-control, B-4 μg *Neb*-colloostatin To-terminal oocyte, lo-lateral oviduct, co-common oviduct, ac-spermatheca with accessory gland.

Neb-colloostatin exhibits a structural similarity to known vertebrate and invertebrate collagens. In the peptide chain of *Neb*-colloostatin, as in collagen, characteristic tripeptide amino acid sequences $(Gly\text{-}X_1\text{-}X_2)_n$ are present, where X_1 and X_2 are Pro and Hyp respectively, with Val instead of Gly at positions 3, 9, and 15. The largest structural analogy is observed between *Neb*-colloostatin and the 468–480 sequence of preprocollagen $\alpha 1$ (IV) present in the fruit fly *D. melanogaster* [5].

The biological properties of *Neb*-colloostatin consist in inhibition of ovarian development in the flesh fly *N. bullata* [5] and the mealworm *T. molitor* (Figure 7) [33]. It inhibits oocyte growth, reduces the number of eggs and their hatchability, and delays embryonic development in *T. molitor* [34]. Biochemical tests show that *Neb*-colloostatin does not inhibit trypsin and ecdysone biosynthesis, although it inhibits vitellogenin biosynthesis [5]. This means that the peptide controls the accumulation of yolk in the insect oocytes. The oostatic activity of *Neb*-colloostatin is not species-specific.

α-Chymotrypsin and Serine Proteases Inhibitors

Another set of peptides isolated from the desert locust *S. gregaria* ovaries is the five oligopeptides SGP-1-5 [42] (Table 3) as well as a 6-kDa peptide consisting of 30 amino acid residues: AYPAAHQGY-PAHVGYARVGYGGYPSYGYPA at *N*-terminal sequence [43]. Three SGP peptides: SGP-2, SGP-4, and SGP-5 are glycopeptides where the Tyr residue at position 9 or 10 of the peptide chain is glycosylated by fucose [42].

In vitro biological tests have shown that peptides SGP-1-5 are inhibitors of α-chymotrypsin and other serine proteases. Furthermore, it has been found that these inhibitors are present not only in the ovaries but also in the haemolymph and fat body of insects. Their influence on the oocyte maturation in insects has not been studied so far.

The 30-residue peptide isolated from the *S. gregaria* ovaries is characterized by a large number of hydrophobic residues, such as Gly, Pro, Ala, and Tyr, which make up more than 80% of its amino acid composition [43]. One should notice a repeating tripeptide motif in the chain of that peptide of the X_1-Tyr-X_2 type, where X_1 = Gly or Ala and X_2 = Pro, Gly, or Ala (Table 3), whose structure shows a large similarity to GPRP proteins, rich in glycine and proline, found in plants [51]. It is likely that this 6-kDa peptide is a fragment of a novel insect glycine and proline rich protein. The SGP-1-5 and 30-residue peptides are suggested in the literature as insect gonadotropins because they have been isolated from the insect ovaries.

OOCYTE GROWTH STIMULATING PEPTIDES

Led-NPF-1 and *Led*-NPF-2

A few years ago two neuropeptides: *Led*-NPF-1 with sequence ARGPQLRLRFamide and *Led*-NPF-2 with sequence APSLRLRFamide, were found in the potato beetle *Leptinotarsa decemlineata* brain (Figure 8) [47]. It follows from the biological tests that *Led*-NPF-1 is a strong oocytes- and ovaries-development stimulator in the locust *L. migratoria* [52] and *S. gregaria* [48]. The second peptide, *Led*-NPF-2, shows about 10-fold lower activity as compared to *Led*-NPF-1 [48,52].

Studies carried out on other insect species such as *N. bullata* [52] and *T. molitor* [34] have shown that neither peptide influences the egg-laying and

Figure 8 *Lymantria dispar*.

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Table 3 Other gonadotropic peptides

Peptide	Sequence	Ref.
Serine proteases inhibitors:		42
SGPI-1	EQECTPGQTKKQDCNTCNCTPTGVWACTRKGCPPH	
SGPI-2	EVTCEPGTTFKDKCNTCRCGSDGKSAACTLKACPQ fukose	42
SGPI-3	CTPGSRKYDGCNWCTCSSGGAWICTLKYCPPSSGGGLTFA	42
SGPI-4	SEGHCTPNTTFKKDCNTCSCDNDGTAAVCTLKACLS??? fukose	42
SGPI-5	EVNCTPGATFKNKCNTCRCGSNGRSASCTLMACPPGSY fukose	42
'30-residue peptide'	AYPAAHQGYPAHVGYARVGYGGYPSYGYPA	43
ODAIF:		44
Neb-ODAIF-1_{1-13}	NLLKPSQWISL	
Neb-ODAIF-1_{1-9}	NLLKPSQWI	44
Neb-ODAIF-2	SLKPSNWLTPSE	44
	LEQIYHL	44
Ecdysteroid biosynthesis stimulating	QTNVLEIRCKLYSGPAVQNTGECVHGAELNPCGKLSCLKGVGDKCGES	45
hormone: OEH	TAGIIMSGKCASGLMCCGGQCVGCKNGIC-DHRLCPPR	
LTE	ISDFDEYEPLNDADNNEVLDF	46
Oocytes development stimulating peptides:		
Led-NPF-1	ARGPQLRLRFa	47
Led-NPF-2	APSLRLRFa	
Accelerate the ovarian growth in insects:		
Scg-NPF	YSQVARPAFa	48
Insect cAMP biosynthesis stimulating	AGAEAEKLSGLSKYF	49
peptides:	AGAEAEKLSGLSKYFNGTTMAGRANVAKATYAVIGLIIAYNVMKPKKK	50

subsequent embryogenesis, indicating that they are species-specific. Owing to the structural similarity of *Led*-NPF-1 and 2 to the insect neuropeptides of the Phe-Met-Arg-Phe-NH_2 (FMRF)-amide family, they were evaluated in myotropic tests and their influence on the insect heart beat was investigated [33]. It turned out in preliminary studies, which are continuing, that both peptides significantly inhibit the heartbeat of two beetles, *T. molitor* and *Zophobas atratus*.

Scg-NPF

The other peptide *Scg*-NPF, YSQVARPRFamide, has been isolated from the brain of the locust *S. gregaria*. Preliminary biological studies indicate that, like *Led*-NPF-1, it accelerates the ovarian growth and increases the vitellogenin concentration in the locust haemolymph [48].

Insect cAMP Biosynthesis Stimulating Peptides

What physiological importance the stimulation of ovaries for cAMP production has for insects is not known so far. A 15-residue peptide, AGAEAEKLSGLSKYF, has been isolated from *N. bullata* brain, which stimulates cAMP production in insect ovaries [49], and a 48-peptide with the same *N*-terminal sequence has been isolated from the Malpighian tubules of the moth *Manduca sexta* [50], which stimulates cAMP production in cells (Table 3). The physiological significance of ovarian cAMP production in insects is observed and requires further study.

Ecdysteroid Biosynthesis Stimulating Hormones

OEH (ovary ecdysteroidogenic hormone). The gonadotropic hormone was isolated by Brown [45] from the mosquito *A. aegypti* brain (Table 3). This polypeptide is made up of 86 amino acid residues and is cleaved from a 149-residue preprohormone. Its structure resembles *Lom*-neuroparsin. Its biological activity consists of the *in vitro* stimulation of ovarian ecdysone biosynthesis in the insect ovaries and *in vivo* stimulation of vitellogenin biosynthesis [45].

LTE (lymantria testis ecdysiotropin). Interesting results have been presented by Wegener *et al.* [46], who have isolated a new peptide, LTE, from the male of the moth *Lymantria dispar's* brain (Figure 8). It consists of 21 amino acid residues: ISDFDEYEPLNDADNNEVLDF. Biological studies have shown that it stimulates ecdysone production in the testis. It is the only

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gonadotropin found and characterized so far in male insects.

ODAIF(ovary derived angiotensin converting enzyme interactive factor). In 2002, during a search for other peptide factors, four new oligopeptides, *Neb*-ODAIF (Table 3), were isolated from ovaries of the fly *N. bullata* [44]. These substances turned out to be substrates of angiotensin convertase (ACE, *angiotensin-converting enzyme*), an enzyme whose biological role is well known in mammals [53], but not in insects.

ALLATOTROPIC AND ALLATOSTATIC NEUROPEPTIDES

In many insects, neuropeptides play a key role in regulating the production of the JHs and ecdysteroids, which are considered to be the primary hormones affecting female reproduction, by acting separately or in coordination to stimulate oogenesis and vitellogenesis, depending on the insect group or species [1,3,54]. Two types of important neuropeptides control the production of JHs: allatotropins (ATs) and allatostatins (ASTs). The last group of peptides occur in three major structural forms, allatostatins A-, B- and C-type [55,56].

In insects where vitellogenesis is JH-dependent, allatotropin is probably an important initiator of reproduction. *In vitro* assays with adult CA of *M. sexta* allatotropin stimulate the production of JHI, II, and III [57]. This peptide also stimulates JH secretion *in vitro* by larval and adult CA of other lepidopteran species and the adults of the honeybee, *Apis mellifera* [58] and blowfly, *Phormia regina* [59]. In adult insects, ATs have demonstrated effects on other processes associated with reproduction. *L. migratoria* AT may play a role in oviposition, as suggested by its myotropic activity on the oviducts of locust and cockroach females, *Leucophaea maderae* [60]. Immunocytochemistry studies have showed that AT-immunoreactivity has a widespread and sexually dimorphic distribution in the nervous system of adult locusts and cockroaches [61].

All three types of allatostatins in insects have been isolated on the basis of their inhibition of JH secretion by CA *in vitro* [55,62,63]. Allatostatins have also been shown to inhibit muscle contractions in a variety of visceral organs from different insect groups. In *L. migratoria*, for example, B-type allatostatin suppresses spontaneous contractions of the oviduct *in vitro* [55,56].

In addition, ASTs have been shown to have effects on female reproduction. A-type allatostatin inhibited vitellogenin release *in vitro* by the fat body of cockroach females, *B. germanica* [64]. *In vitro* ovarian ecdysteroid biosynthesis in the cricket, *Gryllus bimaculatus* is inhibited by a B-type allatostatin [65]. In *Bombyx mori*, a peptide of this family is known to inhibit the synthesis of ecdysteroids by conspecific prothoracic glands [56]. There are only a few reports on the *in vitro* effects of allatostatins in female insects. Effects such as reduced growth of oocytes or reduced titres of JH in the haemolymph in certain cockroaches, are achieved only by injecting known allatostatins *in vivo* at high and sometimes in repeated doses [62,63]. Similar injections of cricket AST-A and AST-B into female crickets, *G. bimaculatus*, resulted in a decrease of body and ovary weight, decrease of ovary ecdysteroid biosynthesis, and an increased vitellogenin titer in the haemolymph [65].

SUMMARY

The results regarding the insect gonadotropic peptides presented here may evoke mixed feelings in the reader. The factors isolated from the insect gonads and brains that stimulate or inhibit the oocyte growth belong to the gonadotropic peptides group. This group also comprises the peptides isolated from the insect gonads, which do not directly influence the reproduction process (or their role in it has not been recognized so far). Moreover, the content of the present article is not uniform owing to some peptides being described in variable detail. This happens because many laboratories dealing with insect physiology and biochemistry isolate a whole cocktail of peptides, but characterize mostly a single biological effect of peptide, and neglect the rest. The research is mostly descriptive, not analytical.

The aim of this review is arrangement of the data and stimulation of a broader group of scientists to undertake fundamental, coordinated research on the individual peptide factors. There is a long way to go in the understanding of the aspect of insect reproduction. But we can predict that it will eventually be possible to use the peptides described here as safe agents for harmful insect population control.

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