Pestycydy, 2007, (3-4), 33-38.
ISSN 0208-8703

# The effect of *Aea*-TMOF on egg maturation in *Heliothis virescens*: a preliminary study

Maciej A. PSZCZOLKOWSKI[1,2], Crystal RHINE[3],
Sonny B. RAMASWAMY[2,4], and Dov BOROVSKY[5]

*[1] Department of Agriculture, Missouri State University, Mountain Grove, MO 65711, USA, e-mail: MPszczolkowski@missouristate.edu*
*[2] Department of Entomology, Kansas State University, Manhattan, KS 66506, USA*
*[3] Department of Anatomy & Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA*
*[4] Agricultural Research Programs, Purdue University, West Lafayette, IN 47907, USA*
*[5] Florida Medical Entomology Laboratory, University of Florida – IFAS, Vero Beach, FL 32962, USA, e-mail: dobo@ifas.ufl.edu*

**Abstract:** Trypsin Modulating Oostatic Factor from the mosquito, *Aedes aegypti,* (*Aea*-TMOF) inhibits juvenile hormone (JH) - stimulated egg chorionation and patency in the follicular epithelium cells of *Heliothis virescens*. *Aea*-TMOF exhibits highest inhibitory effect on oocytes or follicular epithelium cells when it is administered together with JH I rather than with JH III. These results indicate that *Aea*-TMOF specifically inhibits JH I-dependent events during egg maturation in *Heliothis virescens*. Preliminary pharmacological analysis of the *Aea*-TMOF effect on patency suggests that the decapeptide hormone acts upstream of the protein kinase-dependent step during the JH activated cellular signaling pathway.

**Keywords:** patency, egg chorionation, juvenile hormone, Lepidoptera, Diptera

## INTRODUCTION

Insect reproduction (including oocyte growth and maturation) is hormonally controlled by juvenile hormone (JH), ecdysteroids and neuropeptides. In many insects oocyte growth results from a combination of three processes: (1) synthesis of female specific yolk proteins, vitellogenins, in the fat body, (2) their release into the hemolymph and (3) their uptake by oocytes. The latter process includes an important step, termed patency, which results in an increase in intercellular spaces in oocyte follicular epithelium allowing the hemolymph-borne vitellogenins to pass through the oocyte's cell membrane and be sequestered [1]. In the noctuid, *Heliothis virescens,* oocyte maturation and growth are mediated solely by JH, which stimulates patency [2], vitellogenin synthesis, uptake, and egg chorionation [3, 4]. No information is available about possible hormonal factors that inhibit or terminate oocyte maturation, caused by JH stimulation, in *Heliothis virescens.*

In several dipterans, follicular epithelial cells synthesize oostatic peptides. The yellow fever mosquito *Aedes aegypti* produces Trypsin Modulating Oostatic Factor (*Aea*-TMOF) and the fleshfly *Neobellieria bullata* synthesizes two peptide hormones *Neb*-TMOF and *Neb*-colloostatin [5, 6]. *Aea*-TMOF and *Neb*-TMOF indirectly affect the oocytes by inhibiting the *de novo* biosynthesis of trypsin in mosquito gut. These peptides stop the digestion on the protein meal in the gut into free amino acids necessary for vitellogenin biosynthesis [5, 6]. Fleshfly-produced *Neb*-colloostatin, however, may act directly on the follicles preventing vitellogenin uptake [7], exhibiting *in vivo* a bifunctional activity of preventing vitellogenin uptake and ovarial development in the beetle, *Tenebrio molitor* [8]. A question arises whether or not *Aea*-TMOF also may affect ovarial development directly, and in a non-dipteran species.

This study tested a hypothesis that mosquito *Aea*-TMOF affects ovarial maturation in the moth *Heliothis virescens.* We posed following questions: (1) Does *Aea*-TMOF inhibit ovarial maturation *in vivo*? (2) Does *Aea*-TMOF inhibit ovarial patency *in vitro*? (3) What is the signal transduction pathway that is activated by *Aea*-TMOF during patency?

## MATERIALS AND METHODS

### Insects and chemicals

Details of source and conditions for raising the test animals are given elsewhere [2]. *Aea*-TMOF was synthesized as described earlier [9]. JH I was purchased from SciTech Chemicals (Prague, Czech Republic, EU). Remaining

reagents including JH III, protein kinase A (PKA) activator, 8-Br-cAMP, and protein kinase C (PKC) activator, phorbol 12,13-dibutyrate, PDBu, were purchased from Sigma (St. Louis, MO, USA).

**Experimental design**

The first experiment was designed to determine the effects of *Aea*-TMOF on oocyte maturation stimulated by standardized dose of juvenile hormones. Egg chorionation assay was used as described earlier [3]. Virgin *H. virescens* females were allatectomized by decapitation immediately after adult emergence and before the start of synthesis of endogenous JH. Six hours later, female abdomens were injected either with 5 $\mu$l of corn oil (negative control), 5 $\mu$g of either JH I or JH III alone in the same volume of corn oil (positive control) or 5 $\mu$g of either JH I or JH III in combination with various doses of *Aea*-TMOF (0.1, 1, and 10 $\mu$g). Forty-eight hours later, oocytes were dissected and chorionated eggs counted. In a second experiment the effect of *Aea*-TMOF on patency was studied. Ovaries were removed from 24h-old virgin females and incubated in medium containing either $10^{-9}$ M JH I or JH III alone or in combination with various concentrations of *Aea*-TMOF. After 2 h incubation, patency index was assessed by morphometric analysis of folliclular epithelial cells [2]. A third experiment was designed to delineate proximate mechanism(s) by which *Aea*-TMOF affects the follicular epithelium during patency period. Because JH I causes patency by activating PKA and JH III activates PKC [2], oocytes were incubated with standard concentrations of either $10^{-9}$ M 8-Br-cAMP or $10^{-9}$ M PDBu alone, or in combination with $10^{-10}$ M *Aea*-TMOF in experimental system that was described in details in earlier study [2].

**Statistics**

All data sets passed tests for normality with $P<0.05$ (GraphPad Software Inc., San Diego, CA). Sample means ± SEM were compared among control and experimental groups with analysis of variance (ANOVA), followed by multiple Bonferroni comparisons, or with Student's t-test (for pair-wise comparison) and considered significantly different at $P<0.05$.

## RESULTS

**Effects of Aea-TMOF in decapitated females**

Decapitated females do not synthesize JH and their ovaries are not exposed to JH. Injection of oil did not stimulate oocyte chorionation. Injections of JH I or

JH III alone resulted in chorionation of about 200 and 110 oocytes, respectively (Figure 1A). Concurrent injection of 0.1-10 $\mu$g *Aea*-TMOF inhibited chorionation of oocytes stimulated by JH I, but not those stimulated by JH III (Figure 1A).

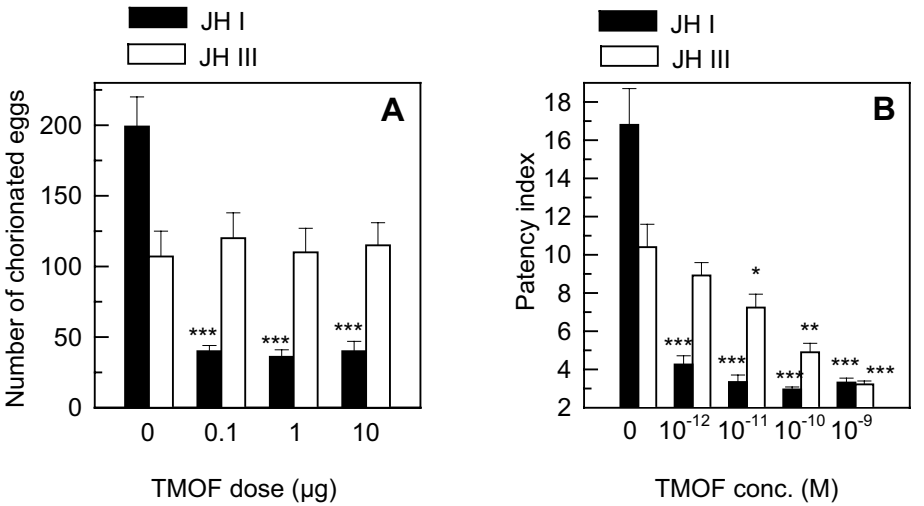


**Figure 1.** Inhibitory effects of *Aea*-TMOF on egg chorionation (A) and patency (B) stimulated by either JH I or JH III in *H.virescens*. Each datum point refers to mean ± SEM obtained from 20 insects (panel A) or 45-53 pairs of follicular epithelium cells (panel B).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ANOVA, followed by Bonferroni comparison of means among control and TMOF-treated groups.

**Effects of Aea-TMOF on patency caused by JH homologues**

Follicular epithelial cells exposed to JH and *Aea*-TMOF concurrently exhibited lower degree of patency than those exposed only to JH. However, *Aea*-TMOF was more effective in inhibiting patency stimulated by JH I (concentrations as low as $10^{-12}$ M were inhibitory), whereas patency stimulated by JH III slightly decreased at $10^{-11}$M *Aea*-TMOF. Higher concentration of *Aea*-TMOF ($10^{-9}$ M) equally suppressed the levels of both JH III- and JH I evoked patency (Figure 1B).

**Effects of Aea-TMOF on patency caused by protein kinase activators**

*Aea*-TMOF, at a concentration of $10^{-9}$ M, had no effect on patency caused by either PKA or PKC activators. Patency index caused by $10^{-9}$ M 8-Br-cAMP equaled 14.43 ±1.34, and that caused by the same concentration of 8-Br-cAMP in presence of $10^{-9}$ M *Aea*-TMOF equaled 14.23 ±1.48 ($P > 0.05$, N=20, Student's

t-test). Similarly, no difference in patency evoked by $10^{-9}$ M PDBu alone (11.61 ±1.23) and $10^{-9}$ M PDBu in combination with $10^{-9}$ M *Aea*-TMOF (10.94 ±1.16) (P >0.05, N=20, Student's t-test) was observed.

## DISCUSSION

*H.virescens* females decapitated prior to the onset of JH biosynthesis do not produce vitellogenin and oocyte maturation is inhibited. This process can be restored by the application of JH I or JH III (Figure 1A). Oocyte maturation restored by JH I is inhibited by *Aea*-TMOF (Figure 1A). At present, we do not know whether *Aea*-TMOF directly acts on the oocytes developing in isolated abdomens. Indirect action, including suppression of vitellogenin biosynthesis in the fat body, could also produce similar effects. However, in our *in vitro* experiments *Aea*-TMOF inhibited patency in absence of any tissue other than ovaries (Figure 1B). We conclude that our results demonstrate for the first time a direct effect exerted by insect oostatic peptide on developing oocytes during the reproductive cycle in a moth. We can only speculate how *Aea*-TMOF inhibits patency. Because *Aea*-TMOF had no effect on patency caused by either PKA activator or PKC activator, it is likely that *Aea*-TMOF inhibits signal transduction pathways upstream of protein kinase-dependent events, perhaps by interference with functioning of postulated [2] membrane JH receptors, or by binding to an oocyte receptor, resembling gut TMOF receptor in *Aedes aegypti,* and evoking a signal transduction pathway that stops a JH mediated pathway in *H.virescens* follicular epithelial cells.

Our data suggest that *Aea*-TMOF inhibits mostly JH I-dependent events during oocyte maturation. This peptide had better pronounced inhibitory effects on patency caused by JH I than that caused by JH III, and did not prevent egg chorionation caused by JH III. How *Aea*-TMOF inhibits chorionation in oocytes exposed to JH I in abdomens of decapitated females, is currently not known. One possible explanation is that patency is such an important event in *H.virescens* reproduction that oocyte maturation cannot proceed if patency is inhibited. This hypothesis, however, needs further testing. One possible strategy here would be constantly inhibiting patency in *H.virescens* with oostatic peptides administered *per os* and measuring egg output.

**Acknowledgements**

A part of this study was funded by Merck-Merial Research Grant Program. This is paper no. 08-57-A, Kansas State University Agricultural Experiment Station.

## REFERENCES

[1] Davey K.G., Am. Zool., 1981, 21, 701-705.
[2] Pszczolkowski M.A., Peterson A., Srinivasan A., Ramaswamy S.B., J. Insect. Physiol., 2005, 51, 445-453.
[3] Ramaswamy S.B., Shu S., Park Y.I., Zeng F., Arch. Insect Biochem. Physiol., 1997, 35, 539-558.
[4] Shu S., Park Y.I., Ramaswamy S.B., Srinivasan A., J. Insect Physiol., 1998, 44, 1111-1117.
[5] Borovsky D., J. Exp. Biol., 2003, 206, 3869-3875.
[6] De Loof A., Bylemans D., Schoofs L., Janssen I., Spittaels K., Vanden Broeck J., Huybrechts R., Borovsky D., Hua Y.J., Koolman J., Sower S., Insect. Biochem. Mol. Biol., 1995, 25, 661-7.
[7] Bylemans D., Proost P., Samijn B., Borovsky D., Grauwels L., Huybrechts R., Van Damme J., Van Beeumen J., De Loof A., Eur. J. Biochem., 1995, 228, 45-49.
[8] Wasielewski O., Rosiński G., Arch. Insect. Biochem. Physiol., 2007, 131-141.
[9] Borovsky D., Mahmood F., Regul. Pept., 1995, 57, 273-281.