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The Angiotensin Converting Enzyme Inhibitor Captopril Reduces Oviposition and Ecdysteroid Levels in Lepidoptera

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The role of angiotensin converting enzyme (ACE, peptidyl dipeptidase A) in metamorphic- and reproductive-related events in the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera, Noctuidae) was studied by using the selective ACE inhibitor captopril. Although oral administration of captopril had no effect on larval growth, topical administration to new pupae resulted in a large decrease of successful adult formation. Oviposition and overall appearance of adults emerging from treated larvae did not differ significantly from those emerging from non-treated larvae. In contrast, topical or oral administration of captopril to newly emerged adults caused a reduction in oviposition. By evaluating the effect of captopril on ecdysteroid titers and trypsin activity, we revealed an additional physiological role for ACE. Captopril exerted an inhibitory effect on ecdysteroid levels in female but not in male adults. Larvae fed a diet containing captopril exhibited increased trypsin activity. A similar captopril-induced increase in trypsin activity was observed in female adults. In male adults, however, captopril elicited reduced levels of trypsin activity. Our results suggest that captopril downregulates oviposition by two independent pathways, one through ecdysteroid biosynthesis regulation, and the other through regulation of trypsin activity. Apparently, fecundity is influenced by a complex interaction of ACE, trypsin activity, and ecdysteroid levels. Arch. Insect Biochem. Physiol. 57:123–132, 2004.

KEYWORDS: angiotensin converting enzyme; captopril; larval growth and development; metamorphosis; oviposition; egg viability; ecdysteroids; trypsin; *Spodoptera littoralis*

INTRODUCTION

Angiotensin converting enzyme (ACE, peptidyl dipeptidase A) is a Zn^{2+} metallopeptidase associated with the regulation of blood pressure in mammals. It increases blood pressure by removing a dipeptide from the C-terminus of angiotensin I, thus generating vasoconstricting angiotensin II. ACE also degrades and inactivates bradykinine, a vasodilatory peptide (Erdös and Skidgel, 1897; Johnston, 1992). In mammals, ACE exists as two isoforms, somatic ACE (sACE) with a molecular weight of 140–180 kDa and two highly homologous domains (N- and C-domains) that both are

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Abbreviations used: ACE = angiotensin converting enzyme; *Aea*-TMOF = *Aedes aegypti* trypsin modulating oostatic factor; *Neb*-TMOF = *Neobellieria bullata* trypsin modulating oostatic factor; sACE = somatic ACE; tACE = testicular ACE; 20E = 20-hydroxyecdysone

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catalytically active, and testicular ACE (tACE) with a single active site and a molecular weight of 90–110 kDa (Corvol et al., 1995). sACE is expressed in many different tissues, while tACE is unique to the testis. Whereas the role of sACE in the regulation of blood pressure and water and electrolyte balance is well understood, the exact function of tACE is unknown (Turner and Hooper, 2002).

Recently in several insects, a peptidyl dipeptidase that has very similar enzymatic properties to mammalian ACE has been found (Lamango and Isaac, 1994; Cornell et al., 1995; Wijffels et al., 1996; Schoofs et al., 1998). Two genes that code for ACE homologues, AnCE and Acer, were identified in *Drosophila melanogaster*. Since insects have an open circulatory system, the discovery of insect ACE homologues has led to speculations about new physiological roles for this enzyme. In the housefly *Musca domestica*, a soluble 67-kDa ACE has been purified, and its low molecular weight suggests that it only has one active site (single domain form). The physiological role for this enzyme is not known. At present, captopril (D-3-mercapto-2-methyl-propionyl-L-proline), a strong and specific inhibitor of ACE, is often used in the treatment of hypertension, and it has been reported that captopril displays the same potency for the inhibition of AnCE as for the inhibition of mammalian ACE (Williams et al., 1996).

Recent studies that were conducted with dipteran insects suggest a role for ACE in insect reproduction. Results from studies in which ACE inhibitors were fed to adult male mosquitoes (*Anopheles stephensi*) suggested that AnCE has an important influence on male fertility and that this effect could be mediated through the regulation of neuropeptide activity. Females that had been mated with these ACE-inhibited males showed a dramatic reduction in fecundity (Ekbote et al., 2003a). In addition, Vandingenen et al. (2001, 2002) treated female adults of the grey fleshfly *Neobellieria bullata* with captopril and studied the in vivo relationship between *Neb*-TMOF (trypsin modulating oostatic factor) and *Neb*-ACE. Since *Neb*-TMOF is an in vivo substrate for *Neb*-ACE, the captopril treatment had a direct effect on trypsin activity and vitellogenin concentrations. Vandingenen et al. (2001) also reported that captopril fed to female flies caused an increase in the liver meal-induced trypsin peak in the midgut and elevated levels of protein-induced yolk polypeptides in the hemolymph, but oocyte growth was not affected.

From other previous work, it is known that TMOF inhibits ecdysone biosynthesis in *N. bullata* and *Lymantria dispar* (De Loof et al., 1995; Gelman and Borovsky, 2000); however, the direct effect of captopril treatment on ecdysteroid biosynthesis has not been examined.

It appears that the effect of TMOF on trypsin biosynthesis occurs independently of its effect on ecdysteroid biosynthesis in the grey fleshfly. This follows from observations made by Bylemans et al. (1995), where injection of ecdysone together with Neb-TMOF did not significantly counteract the effect of TMOF on the inhibition of trypsin biosynthesis.

In addition to influencing egg production, in the silkmoth, *Bombyx mori*, ACE was found to be active at the time in metamorphosis when wing formation was observed (Quan et al., 2001). More evidence in support of a role for ACE in metamorphosis was provided by Siviter et al. (2002). During pupal development of *D. melanogaster*, ACE-like activity increased 3-fold at a mid-pupal stage, before declining to larval levels at the time of adult eclosion (Siviter et al., 2002).

In this report, we explore in a lepidopteran species, the Egyptian cotton leafworm, *Spodoptera littoralis*, the effects of the phenotypic knockout of ACE activity by its selective inhibitor captopril. *S. littoralis* is one of the major pest insects in the world and many populations of this insect have developed high levels of insecticide resistance (Oerke et al., 1994). In a first series of experiments, various developmental stages were tested by direct and residual treatment with captopril. For larval and pupal stages, we evaluated feeding, growth, and development with particular attention given to molting and metamorphosis. Oviposition and egg viability were also followed in treated male and female adults. Captopril was used at 10 µg/µl or dosed at 50 µg, as in vitro tests showed that

captopril completely inhibited ACE at 0.2 μM (Vermeirssen et al., 2002). In a second series of experiments, we determined for the first time the effect of captopril on ecdysteroid titers in the hemolymph of these different stages. Then, to address the mechanism responsible for the negative effects of captopril on oviposition, we measured its effects on trypsin activity in vivo and in vitro. Our objective was to test whether captopril downregulates oviposition by two independent pathways, one through ecdysteroid biosynthesis regulation, and the other through inhibition of trypsin activity.

MATERIALS AND METHODS

Chemicals

Captopril (D-3-mercapto-2-methyl-propionyl-L-proline) was purchased from Sigma Co. (Bornem, Belgium). All other chemicals were of analytical grade or were obtained as described in the text.

Insects

All stages of a continuous colony of *S. littoralis* were maintained under standard conditions of 23 ± 1°C, 70 ± 5% RH and a light:dark (16:8) photoperiodic regimen as described previously (Smagghe et al., 2002). Larvae were fed on an agar-based artificial diet that had been placed in multiwell culture plates, and adults were fed a 20% honey water solution.

Assay to Assess the Effects of Captopril on Larval Growth and Development

For larval bioassays, newly molted (0–1 d) larvae of different instars (hereinafter L_1– L_6) were selected and transferred to control diet or to artificial diet containing captopril. Captopril (75 μl; 10 μg/μl in methanol) was uniformly distributed on the diet surface of the experimental group, and after solvent evaporation, captopril was present as a film on the surface of the diet (Smagghe et al., 1999). Controls were treated only with methanol. Equal numbers of larvae were placed on the treated and control diet. There was a minimum of 2 replicate groups/treatment. The phenotypes of treated and control insects were evaluated to the larval-pupal molt.

For pupae, the effects of captopril on metamorphosis and adult formation were evaluated. New (0–6 h) pupae were topically treated with captopril (50 μg in 5 μl acetone), and two replicate groups of 20 pupae each were used. Controls were treated only with acetone. The phenotype of treated and control groups was followed to adult eclosion.

Effect of Captopril on Oviposition and Egg Viability Assay

The effect of captopril on egg production was measured by two different methods. In one protocol, L_1–L_6 larvae were fed on diet containing 10 μg/μl captopril. After adult emergence, oviposition was followed. In parallel, newly emerged (0–6 h) adults that had been fed on control diet during larval development were topically treated on the abdomen with 50 μg captopril (in 5 μl acetone). Captopril treatment was either administered once, at the time of adult eclosion, or, in a separate assay, every 2 days for 10 days. In addition, adults were continuously treated with captopril at 10 μg/μl by adding ACE inhibitor to the honey-water diet. To assess the effects of captopril on oviposition, groups of 10 newly emerged adults (sex ratio 1:1) were placed in a plastic box (10 × 10 × 15 cm) and the inside walls were covered with paper to provide oviposition sites (Smagghe and Degheele, 1994). After the first oviposition, the number of eggs laid per female was daily recorded for 8–10 days. Afterwards, egg viability was scored as a mean percentage ± SEM of first-instar larval emergence.

Trypsin Assay

Trypsin activity was measured by monitoring the digestion of casein, commonly used as a trypsin substrate (Bickerstaff and Zhou, 1993). Although casein is not a trypsin specific substrate, it is used to measure trypsin activity in *S. littoralis* as trypsin is the major digestive proteolytic enzyme in the cotton leafworm (De Leo et al., 1998). Briefly, casein was dissolved in sodium phosphate solu-

tion (50 mM, pH 8.5) and boiled gently for 10 min. The casein solution was diluted to 300 µg/ml with sodium phosphate buffer (50 mM, pH 7.5). To construct a standard curve, several tubes, each containing 400 µl of casein, were placed in a water bath at 30°C for 5 min. To each tube, 100 µl of the diluted trypsin solution was added and the mixture was incubated for 30 min. Protein content was measured using the Bradford assay (Bradford, 1976) with BSA standard and Coomassie blue. The effect of captopril on trypsin activity was followed in vitro by adding 100 µl of different concentrations of captopril to the incubation mixture containing a constant concentration of trypsin. To measure the effect of captopril on trypsin activity in vivo, larvae and *S. littoralis* adults of were fed captopril. Adults were fed honey water containing 1% captopril for 3 days and larvae were fed artificial diet containing 1% captopril for 4 days. Following feeding, both larvae and adults were homogenized and centrifuged in Tris/HCl buffer (50 mM, pH 7.4). After centrifugation, 100 µl of the diluted supernatant was added to 400 µl casein and trypsin activity was measured.

Ecdysteroid Titers

Larval and pupal hemolymph ecdysteroid levels and adult whole body ecdysteroid levels were determined 24 h after topically treating last-instar larvae, pupae, and adults with captopril (50 µg, in 5 µl acetone) and controls with acetone (Smagghe et al., 1995). Briefly, hemolymph from anaesthetized larvae and pupae was collected and transferred to 500 µl of ice cold 75% aqueous methanol. After the removal of antennae, wings, and legs, adults were homogenized in 1 ml of ice cold 70% aqueous methanol. All samples were centrifuged for 10 min at 21,460*g*, and the supernatant was transferred into ice cold tubes. The precipitate was washed with 500 µl of ice cold 75% aqueous methanol. After a third wash and centrifugation, combined supernatants were lyophilized and stored in the freezer until analysis.

Ecdysteroid content was determined using RIA, and tritium labeled ecdysone (63.5 Ci per mmol) (Gelman et al., 1997). The concentration of ecdysteroids was expressed as pg equivalents/µl hemolymph or /mg body weight.

RESULTS

Effect of Captopril-Containing Diets on Larval Growth and Development

Feeding of captopril at 10 µg/µl to first-sixth (last) instar larvae on a continuous basis did not inhibit food consumption, larval weight gain, or molting (data not shown). However, in last larval instars pupal molt was significantly delayed by a day ($P = 0.10$).

Although captopril had no effect on larval growth, the percentage of successful adult formation was significantly reduced from 77.5 ± 2.5% in controls (acetone-treated) to 30.0 ± 6.2 % after treatment with captopril (Fig. 1).

Effects of Captopril-Containing Diets on Oviposition and Egg Viability

Oviposition by adults that emerged from captopril-treated larvae (continuously treated with 10 µg/µl from the 1st through the 6th instar), was not significantly different from that of controls. After

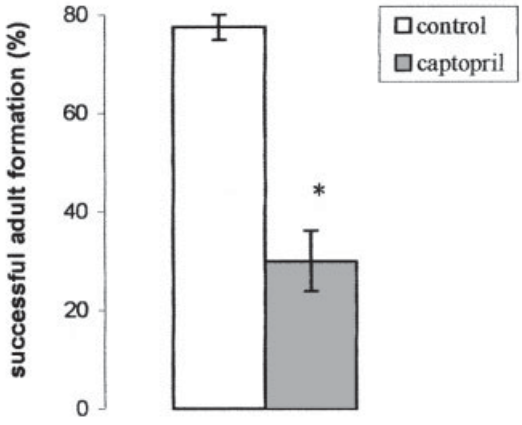

Fig. 1. Percentage of successful adult formation in *Spodoptera littoralis* after topical treatment with 50 µg captopril per new pupa. Data are expressed as means ± SEM based on 2–7 replicates, and (*) indicates a significant difference by a Student's *t*-test ($P < 0.01$) between the experimental and control groups.

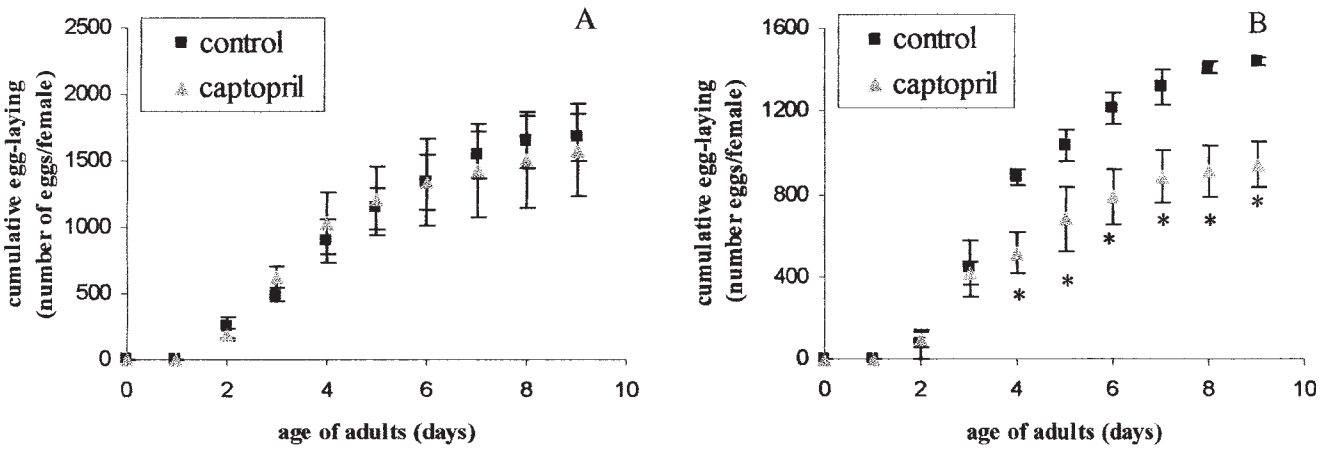

Fig. 2. Effect of ACE inhibition with captopril on oviposition of *Spodoptera littoralis* adults (**A**) after oral feeding captopril at 10 µg/µl continuously from the first to the last larval instar, (**B**) when 50 µg captopril was repeatedly administered topically at 2-day intervals in the adult stage. Data are expressed as means ± SEM based on 3 independent measurements, and (*) indicates a significant difference by a Student's *t*-test at $P < 0.15$ between experimental and control groups.

9 days, cumulative egg-laying per female was 1,576.3 ± 344.5 in the treated group and 1,682.7 ± 177.0 in the control group (Fig. 2A). As stated previously, captopril had no effect on larval development, nor on the overall appearance (e.g., size and condition of appendages), of adults that developed from captopril-fed larvae.

In contrast, when newly emerged adults were treated with captopril, a single topical application on the thorax caused a decrease in oviposition of 33 ± 10% as compared to control adults (data not shown). Repeated topical applications at 2-day intervals resulted in a dramatic decrease in egg laying as shown in Figure 2B. Similarly, when adults were treated with captopril dissolved in the honey-water diet, a significant reduction in oviposition was also observed (data not shown).

Treatment of either larvae or adults with captopril had no effect on egg hatch. In both control and experimental groups, percent hatch was greater than 90% (data not shown).

Effect of Captopril on Trypsin Activity In Vivo and In Vitro

In the in vitro assay, captopril at a concentration of 1 nM to 1mM had no effect on trypsin activity that varied between 33.11 and 159.29 ng/ml. In contrast, when captopril was fed to larvae and adults, a significant difference was observed between experimental and control groups, 2–4 days after feeding on artificial diet (Table 1). After 2, 3, and 4 days of treatment, trypsin activity increased by 1.62-, 1.67-, and 2.22-fold, respectively. Feeding female adults for 2 days with honey water containing captopril resulted in a 1.74-fold increase in trypsin activity (Table 2). In contrast, male adults fed honey water containing captopril for 2 days exhibited lower levels of trypsin activity.

Ecdysteroid Titer Reduction Using Captopril

As shown in Figure 3A, treatment of last instar (L_6) larvae and pupae with captopril did not significantly affect hemolymph ecdysteroid levels. In

TABLE 1. Trypsin Activity (ng/mg Protein) After Feeding Larvae of *Spodoptera littoralis* during the First Four Days of the Last Instar With Artificial Diet Containing 1% Captopril

Treatment	Day 1	Day 2	Day 3	Day 4
Control	2.83 ± 2.30^{aA}	9.52 ± 1.37^{aA}	8.86 ± 3.92^{aA}	9.93 ± 3.04^{aA}
Captopril	6.73 ± 7.06^{aA}	15.39 ± 0.64^{bB}	14.80 ± 3.90^{bB}	22.10 ± 2.46^{bC}

*Data are expressed as means ± SEM based on 2 independent measurements. Per treatment, significant differences by ANOVA at $P = 0.05$ between means in rows are indicated with lowercase letters (a and b) and in columns with capital letters (A–C).

TABLE 2. Trypsin Activity (ng/mg Protein) in Male and Female Adults of *Spodoptera littoralis* After 2 and 3 Consecutive Days of Oral Treatment With 1% Captopril in Honey Water Compared to Untreated Controls

		Day 2	Day 3
♀ Adult	Control	5.37 ± 1.04^{aA}	3.58 ± 6.54^{aA}
	Captopril	9.36 ± 1.37^{bB}	5.60 ± 1.88^{aB}
♂ Adult	Control	5.18 ± 2.48^{aA}	8.41 ± 5.30^{aA}
	Captopril	1.69 ± 1.37^{bB}	4.93 ± 2.31^{aB}

*Data are expressed as means ± SEM based on 3 replicates. For males as well as females, significant differences by ANOVA at $P = 0.05$ between means in rows are indicated with lowercase (a and b) and in columns with capital letters (A and B).

contrast, when female adults of *S. littoralis* adults were treated with captopril, there was a 5-fold significant ($P < 0.05$) decrease in whole body ecdysteroid levels. In captopril-treated females, the ecdysteroid titer was 45.43 ± 11.58 pg/mg body weight, whereas in controls it was 275.95 ± 99.96 pg/mg (Fig. 3B). Although inhibitory in female adults, captopril had no significant effect on ecdysteroid levels in male adults; ecdysteroid titers in treatment and control groups were 11.32 ± 2.55 pg/mg and 9.62 ± 3.85 pg/mg, respectively (Fig. 3B).

DISCUSSION

This is a first report on the effect of ACE-inhibition on ecdysteroid titers in the hemolymph of larvae and pupae and in whole body extracts of adults of a lepidopteran species, the cotton leafworm *S. littoralis*. In addition, we report the effect of captopril treatment on larval growth and development and on oviposition and egg viability. We also determined the effect of captopril on trypsin activity in vitro and in vivo in larvae and adults.

When administered orally to *S. littoralis* larvae, captopril did not affect larval development. Our results agree with those reported by Seinsche et al. (2000) who tested the effect of ACE-inhibitors on the development of *Heliothis virescens* larvae. They found that larvae injected with captopril, enalapril-maleate, and lisinopril, three inhibitors of ACE,

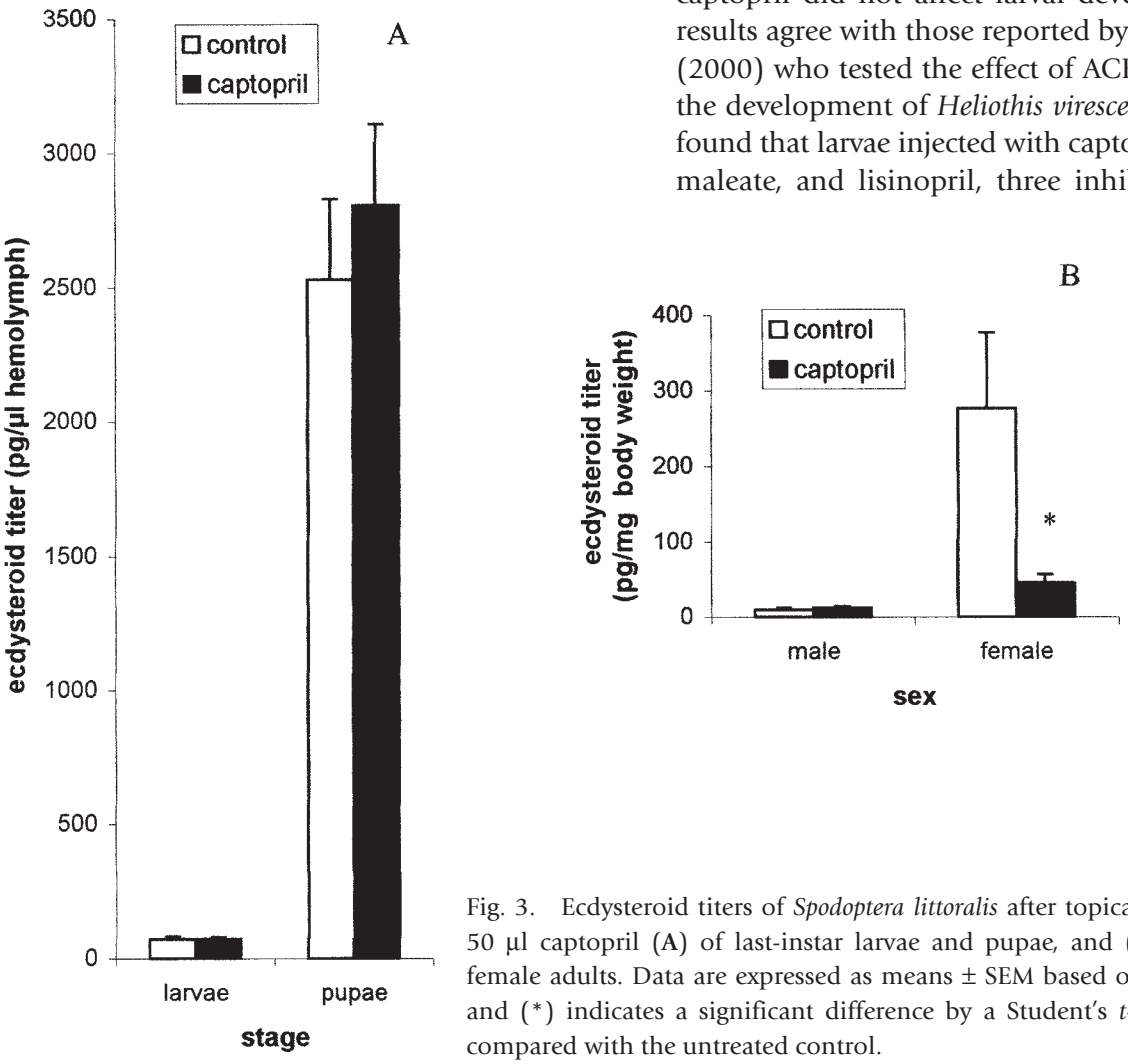

Fig. 3. Ecdysteroid titers of *Spodoptera littoralis* after topical treatment with 50 µl captopril (**A**) of last-instar larvae and pupae, and (**B**) of male and female adults. Data are expressed as means ± SEM based on 2–6 replicates, and (*) indicates a significant difference by a Student's *t*-test at $P < 0.05$ compared with the untreated control.

grew normally. On the other hand, combined application of ACE-inhibitors and helicokinins caused a reduction in weight gain and higher mortality rates in last instar *H. virescens* larvae. As a result of ACE inhibition, which, in turn, prevented the hydrolysis of helicokinins (by ACE), diuretic activity increased due to the elevated kinine titers. Our results show that application of captopril to *S. littoralis* also did not significantly affect larval development.

The large decrease in successful adult formation of *S. littoralis* after topical treatment of new (0–6 h) pupae with captopril shows that ACE has a role in metamorphosis of holometabolous insects. Siviter et al. (2002) previously suggested such a role for ACE based on their findings that larval-pupal transition of *D. melanogaster* was accompanied by a 3-fold increase in ACE-activity. This increase was attributed to the strong induction of Ance expression in the imaginal cells by 20E. Houard et al. (1998) described a 2-fold increase in ACE-activity during the early stages of *D. melanogaster* metamorphosis. Activity peaked between pupal stages P6 and P8, and 20E increased the expression of an ACE-like gene in imaginal wing disc cells of *B. mori* (Quan et al., 2001). Ekbote et al. (2003b) also reported that lepidopteran insects display an increase in ACE activity during metamorphosis. ACE activity increased approximately 4-fold during the last larval instar and early pupal stages of *Lacanobia oleracea*. It is possible that during metamorphosis, ACE contributes to the generation of biologically active peptides and/or signal termination of already active peptides.

ACE is not only thought to have a role during metamorphosis. Several studies suggest a physiological role for the enzyme in insect reproduction. In *D. melanogaster*, null alleles of Ance were larval lethal and a hypomorphic allele resulted in sterile male insects. The spermatocytes of these sterile males failed to develop beyond the primary spermatocyte stage (Tatei et al., 1995). When male *Anopheles stephensi* mosquitoes were treated with ACE-inhibitors and allowed to mate with blood-fed females, a dramatic reduction in fecundity was observed (Isaac et al., 1999). In another study in which *A. stephensi* females were fed a blood meal containing either captopril or lisinopril, the presence of the ACE-inhibitors did not affect feeding and mating behavior, but reduced fecundity in a dose-dependent manner (Ekbote et al., 2003a). Since treated insects displayed normal blood digestion and a normal development of oocytes, it is possible that ACE-inhibitors interfere with oocyte transfer along the oviducts. The report that ACE-like activity has been localized in the reproductive organs of both male and female insects provides additional evidence supporting a role for ACE in reproduction (Isaac et al., 1998; Loeb et al., 1998). In *Lacanobia oleracea*, the highest level of ACE activity was found in the reproductive tract. Almost all of the enzyme was found in the accessory glands of the male and in the spermatheca and bursa copulatrix of the female (Ekbote et al., 2003b). ACE activity was also localized in the testis of *N. bullata*, *Leptinotarsa decemlineata*, and *Locusta migratoria* (Schoofs et al., 1998).

The present study shows that there is no residual effect of captopril on oviposition. No significant difference in fecundity was observed between adults emerging from captopril-treated and non-treated larvae. But, when captopril was administered orally or topically to newly emerged adults, a decrease in oviposition was observed. Therefore, we may conclude that captopril can penetrate through the gut epithelium layer as through the skin. These results are in agreement with those reported for *A. stephensi* (Isaac et al., 1999; Ekbote et al., 2003a).

In contrast to these results are the reports of Vandingenen et al. (2001, 2002) and Hens et al. (2002) concerning the interaction between ACE, ACE-inhibitors, and trypsin modulating oostatic factor (TMOF). TMOF was first identified in the mosquito *Aedes aegypti* and named *Aea*-TMOF (Borovsky et al., 1990). A second TMOF-like hormone was purified from extracts of vitellogenic ovaries of the grey fleshfly *N. bullata* (*Neb*-TMOF) (Bylemans et al., 1994). *Aea*-TMOF as well as *Neb*-TMOF terminate protein meal-induced trypsin biosynthesis in the midgut, thereby impairing blood

digestion and causing a lack of amino acids necessary for vitellogenin synthesis by the fat body. *Neb*-TMOF also inhibits in vitro and in vivo ecdysone biosynthesis. It has been suggested that *Neb*-TMOF is activated by *Neb*-ACE (Vandingenen et al., 2001). When female grey fleshflies (2 days after adult eclosion) were fed on a diet containing captopril followed by a liver meal on day 4, an increase in trypsin levels of 19–36% and an increase in vitellogenin titer was observed. The captopril treatment might have reversed the effect of TMOF on trypsin and vitellogenin biosynthesis. In this scenario, ACE-inhibition should lead to an increase in fecundity; however, neither a stimulatory nor an inhibitory effect on egg-laying was observed by Vandingenen et al. (2001).

To have better insight into the negative effects of captopril on oviposition in *S. littoralis*, we measured the effect of captopril on trypsin activity in vitro and in vivo. The in vitro tests revealed that there is no direct effect of captopril on trypsin activity. However, captopril treatment of *S. littoralis* larvae and female adults resulted in an increase in trypsin activity, whereas treatment of male adults elicited a decrease in trypsin activity. Therefore, the results of the tests with female adults of *S. littoralis* are in compliance with the results reported by Vandingenen et al. (2001) using the grey fleshfly *N. bullata*, both in regard to captopril-induced trypsin activity and to the lack of stimulation of oviposition (*N. bullata*) or decreased levels of oviposition (*S. littoralis*) after treatment with captopril. And, although Isaac et al. (1999) reported that ACE reduced male fertility, this decrease in fertility actually resulted from a decrease in oviposition. In contrast to the significant effect of captopril on fecundity, captopril had no effect on *S. littoralis* egg hatch.

Several recent studies provide evidence for reciprocal interactions between ACE and ecdysteroid production (Loeb et al., 1998; Quan et al., 2001; Vandingenen et al., 2001; Siviter et al., 2002). Quan et al. (1998) reported that BmAcer expression is ecdysone-inducible. A 20E-induced synthesis of ACE-like activity was also observed in *D. melanogaster* (Siviter et al., 2002) and in *A. stephensi* (Ekbote et al., 1999). Moreover, Loeb et al. (1998) demonstrated that ACE-activity stimulates ecdysteroid synthesis, perhaps due to feedback effects. The experiments showed that both bovine ACE and bovine angiotensin II stimulate the synthesis of ecdysteroids by testis of *L. dispar* larvae and pupae, and yet inhibit the action of testis ecdysiotropin, a neuropeptide reported to be responsible for stimulating ecdysteroid production by testes. Vandingenen et al. (2001) suggested the reverse. In *N. bullata* ACE-inhibition would increase ecdysteroid titers by inhibiting the activation of *Neb*-TMOF; therefore, ACE activity suppressed ecdysteroid production. Our results showed no differences in ecdysteroid titers after captopril treatment of larvae and pupae. Nor was there a significant effect on treated male adults. Only when female *S. littoralis* adults were treated with captopril were ecdysteroid titers reduced.

Our results and those of other researchers indicate that there is an extremely complex relationship between fecundity (oviposition), vitellogenin production, trypsin synthesis, and 20E, ACE, and TMOF activity. Previous studies with *A. aegypti* demonstrated that an ecdysteroid peak is necessary to initiate vitellogenesis in the primary follicle and separation of the secondary follicle (Beckemeyer and Lea, 1980). In those insects in which ACE stimulates 20E biosynthesis, adding captopril, an ACE inhibitor, should correlate with a decrease in 20E production probably at the site of biosynthesis in the ovaria, leading in turn to a blockage of vitellogenesis. In addition, the effect of captopril is probably indirect via peptides such as TMOF, as Vandingenen et al. (2001) postulated that ACE activates TMOF. Thus, ACE inhibition can lead to an increase in trypsin activity, which, in turn, increases vitellogenin synthesis. Under these circumstances, an increase in oviposition could be expected. However, in our experiments, a decrease in oviposition was observed. This agrees with Vandingenen et al. (2001) who reported a lack of stimulation of oviposition in *N. bullata*. We hypothesize that ACE has multiple modes of action, and that the exact mechanism of captopril's activity is not clear. We suspect that in our experiments, the stimulatory effect of captopril on trypsin activity is counteracted by its

negative effect on 20E, so vitellogenesis is blocked and oviposition is decreased. The question as to whether there is a direct effect of 20E on trypsin or vice versa remains unanswered.

In conclusion, our results suggest that there is an important role for ACE in metamorphic- and reproductive-related events in the lepidopteran *S. littoralis*. There appears to be a relationship between ACE inhibition, trypsin activity, ecdysteroid titers, and oviposition levels, but further experiments are needed to clarify the mechanisms of action/interaction in these crucial life-cycle events.

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