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Cypermethrin inhibits egg development in the ixodid tick, *Amblyomma hebraeum*

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Abstract

The pyrethroid insecticide, cypermethrin (CyM), stimulates vitellogenesis in *Ornithodoros moubata* (an argasid tick) by stimulating the release of the normal vitellogenesis-inducing factor (a neuropeptide) and subsequent release of the vitellogenic hormone [Inverteb. Reprod. Dev. 15 (1989) 19]. Here we examine the effects of CyM on egg development in the ixodid tick, *Amblyomma hebraeum*. Ovary weight, oocyte size, and vitellin content of the ovary were measured after CyM treatment; in partially fed ticks, none of these parameters were affected significantly. However, CyM treatment caused an inhibition of ovary development, as well as reduction of both hemolymph 20-hydroxyecdysone (20E; the vitellogenic hormone in this species) and vitellogenin (Vg)-concentrations in engorged ticks. In addition, the degree of salivary gland degeneration (which is triggered by 20E) was slightly reduced in CyM-treated engorged ticks. These results indicate that CyM acts differently in *Amblyomma* compared to *Ornithodoros*. Instead of stimulating vitellogenesis, CyM inhibits egg development perhaps in part as a result of inhibiting release of 20E.

Keywords: Cypermethrin; Vitellogenesis; 20-Hydroxyecdysone; *Amblyomma hebraeum*; Ticks; Pyrethroid

1. Introduction

The roles of juvenile hormone (JH) and 20-hydroxyecdysone (20E) during vitellogenesis are well understood in insects, and provide useful models for the study of vitellogenesis in other arthropods. Our detailed understanding of some insect systems serves to emphasize our rudimentary understanding of the hormonal control of vitellogenesis in most chelicerates, including ticks [1]. For example, there is only circumstantial evidence for JH being the vitellogenic hormone in mites [2] and for 20E being the vitellogenic hormone in spiders [3].

Vitellogenesis in the argasid tick, *Ornithodoros moubata*, is initiated by a peptide ('vitellogenesis inducing factor'; VIF) from the synganglion [4]. This neuropeptide triggers the release of 'fat body stimulating factor' (FSF), which in turn stimulates yolk synthesis in fat body tissue [5]. The identity of this second factor is unknown, although circumstantial evidence from several studies suggest that it may be JH [6,7] or an ecdysteroid [8]. So far,

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however, attempts to identify JH in ticks by gas chromatography/mass spectrometry have failed [9,10].

Evidence for an ecdysteroid stimulating vitellogenesis in ixodid ticks is mounting. In engorged *Amblyomma hebraeum*, hemolymph ecdysteroid-concentration increases in parallel with yolk accumulation by ovaries [11] and with hemolymph vitellogenin (Vg)-concentration [12]. Likewise, an increase of Vg-synthesis by fat body corresponds to an increase of hemolymph ecdysteroid-concentration in female *Ixodes scapularis* [13]. Sankhon et al. [14] stimulated in vitro Vg-synthesis and release from fat body of *Dermacentor variabilis* with 20-hydroxyecdysone (20E), but not with the JH-mimic, methoprene. More recently, Friesen and Kaufman [12] demonstrated that injection of partially fed *A. hebraeum* females with 20E triggered a marked increase of hemolymph Vg-concentration.

Pyrethroid insecticides stimulate the continuous firing of action potentials in neurons of both the central and peripheral nervous systems of insects [15]. Neurosecretory cells of both *Rhodnius prolixus* [16] and the stick insect, *Carausius morosus* [17], show increased spontaneous activity after application of pyrethroid, a response which may result in increased secretion of neurohormones from these cells. Indeed, sublethal doses of CyM induced ovarian development in the ladybird beetle, *Henosepulachna vigintioctopunctata*, which the authors attribute to the release of neurosecretion [18]. Similarly, CyM-treated, unfed, virgin female argasid ticks undergo vitellogenesis to the same degree as normal, fed, mated females [19], which the authors attribute to an ultimate release of VIF. The latter study indicates that even without a blood meal and mating, a female argasid tick can synthesize Vg and produce eggs if given an effective stimulus. These findings for argasid ticks led us to consider that, if CyM can stimulate egg development in ixodid ticks as well, it would provide evidence for neurosecretory control over vitellogenesis, and may prove to be a valuable pharmacological tool for identifying an ixodid tick VIF.

In this study, the effects of CyM on vitellogenesis were tested in the ixodid tick, *A. hebraeum*. In order to directly measure Vg in hemolymph or tissues in response to CyM treatments, an enzyme-linked immunosorbent assay (ELISA) was used [12]. Results obtained using this ELISA, along with other assays for vitellogenesis, indicate that the effects of CyM are not the same in *A. hebraeum* as in argasid ticks [20,19]. In addition, this study provides further evidence that an ecdysteroid may act as the vitellogenic hormone in *A. hebraeum*.

2. Materials and methods

2.1. Ticks

Our colony of *A. hebraeum* was kept in darkness at 27 °C and >95% relative humidity. Tick feeding occurred on rabbits as described by Kaufman and Philips [21]. Ticks were allowed to engorge and detach spontaneously, or were forcibly removed from the host below the 'critical weight' necessary to begin vitellogenesis [22,23]. In this study weights of partially fed females (below critical weight) ranged from 180 to 275 mg, and engorged females ranged from 900 to 3500 mg. After removal from the rabbit, ticks were cleaned with water, weighed, and stored individually in gauze-covered glass vials until needed for dissection and hemolymph collection.

2.2. Application of CyM

CyM was purchased from Chem Service (West Chester, PA) and Zeneca Ag Products (Calgary, AB), and was designated by the manufacturers as 92–93% pure. CyM was diluted in acetone to working concentration (0.05–30 mg/ml), and stored in darkness at room temperature in glass vials. Ticks were divided into groups of defined weight range. In all groups CyM was applied topically to the dorsal integument, just behind the scutum, via a 1 µl glass capillary tube or micropipettor. CyM doses ranged from 0.05 to 30 µg/tick in 1 µl acetone for partially fed ticks. Engorged ticks received 0.05–30 µg CyM/100 mg body weight. Controls received acetone alone, or nothing. After treatment, all ticks were held in individual glass vials in darkness at 27 °C and >95% relative humidity. Each treatment group was stored in a separate, sealed, plastic container to

avoid inadvertent exposure to unintended doses of CyM. The containers were opened at least once every other day during a five or ten day incubation period to renew the air. At the end of the incubation period the ticks were re-weighed and the salivary glands, the ovary, and a sample of hemolymph collected.

2.3. Collection of hemolymph and tissue

Tick survival was determined at the time of dissection. If, during hemolymph collection or dissection, the gut was noticed to be ruptured, that tick was discarded.

Ticks were stuck to disposable petri dishes with cyanoacrylate glue and cooled in a refrigerator for 20 min. Cooling inhibits gut contraction, thereby reducing the risk of piercing the delicate gut wall and contaminating the hemolymph [11]. A small incision was made in the cuticle and hemolymph collected in a calibrated glass micropipette; exact sample volume was calculated from the length of the fluid column. Samples were diluted at least 1:10 in 100% methanol for radioimmunoassay (RIA), or 1:4 in phosphate-buffered saline (PBS; 35 mM NaH_2PO_4, 60 mM Na_2HPO_4, and 150 mM NaCl, pH 7.0) for ELISA. Samples for RIA were stored at −17 °C and all other samples were stored at −70 °C until analyzed.

After collection of hemolymph, ticks were flooded with 1.2% NaCl (approximately isosmotic with tick hemolymph), the dorsal cuticle removed using a microscalpel, and the salivary glands and ovaries dissected out for further analysis (see below).

2.4. Measurement of fluid uptake by salivary glands

After the removal of a salivary gland, the salivary duct was tied off with a fine strand of silk thread peeled from 8-0 Dermalon (a gift from Davis and Geek, Pearl River, New York). Glands were gently blotted with filter paper to remove the adhering extraglandular fluid, weighed to the nearest 10 μg, and suspended in a solution of 10 μM dopamine (Sigma Chemicals) in Tissue Culture Medium 199 (TC199; Gibco) for 10 min. Glands were then blotted and re-weighed. The net weight increase of a gland was used as a measure of its fluid secretory competence (see [24] for further details).

2.5. Assay for ovarian development

Ovarian development was assessed by three measures: ovary weight, oocyte size, and vitellin (Vt) content in ovary homogenates. After removal of an ovary, the length of the eight largest oocytes was measured on the stage of a light microscope. The ovary was then weighed and homogenized in 3 ml distilled water. Homogenates were frozen at −17 °C. At the time of analysis, frozen samples were thawed and centrifuged at 13,000*g* for 5 min to remove insoluble material, and the supernatant used to quantify Vt content. Because of the heme-moiety attached to tick Vt, absorbance of ovary homogenates can be measured at a wavelength specific for heme (400 nm) from which is subtracted an absorbance non-specific for heme (500 nm). The difference between the two values provides a quantitative assay for Vt content [25].

2.6. RIA for 20E

20E-concentration in tick hemolymph was determined by the RIA procedure described by Kaufman [26]. Briefly, a methanol extract of each hemolymph sample was dried in a vacuum centrifuge and diluted in 0.2 M borate-BSA (5 mg/ml) buffer (pH 8.5). Samples were then incubated overnight at 4 °C with ~8000 cpm ^{3}H-ecdysone (NEN) per tube and an ecdysone antiserum (E-22-succinyl-thyroglobulin; supplied by Dr. L.I. Gilbert, University of North Carolina, or Dr. E. Chang, University of California, Davis). 20E standards (0–500 pg) were treated similarly. Antiserum-bound ligand was precipitated with a crude suspension of protein A, prepared according to the procedures of Kessler [27]. The pellet was resuspended in 50 μl distilled water and 4 ml scintillation fluid (Scintiverse E; Fisher Scientific), and radioactivity monitored by liquid scintillation spectroscopy. The limit of detection using this protocol was about 10 pg 20E. Further details are presented in Kaufman [26]. Since the ecdysone antiserum cross-reacts with several ecdysteroid

analogues, all RIA data are expressed as '20E-equivalents.'

2.7. ELISA for Vg

Vg and Vt were quantified using an indirect competitive ELISA as described by Friesen and Kaufman [12]. Partially purified Vt from day 10 ovaries was used as the standard for determining the concentration of unknown samples. Briefly, wells of a 96-well microtitre plate were coated with 1 μg partially purified Vt in 100 μl PBS (containing 0.02% NaN_3) and allowed to sit in a sealed container overnight at 4 °C. Simultaneously, anti-Vg polyclonal antibodies were mixed with known concentrations of partially purified Vt (standard) or with hemolymph samples (diluted to 0.1 and 0.01 mg/ml total protein); equilibration occurred overnight at 4 °C. The next day, the standard and sample mixtures were added to the wells, allowing the plate-bound Vt to compete for antibody binding with the Vt or Vg in solution. Two hours later, the wells were incubated with secondary antibody (alkaline phosphatase (AP)-goat anti-rabbit IgG; Bio-Rad) for a further 2 h and the color reaction performed using an AP substrate kit (*p*-nitrophenylphosphate and diethanolamine kit; Bio-Rad). Bound Vt-antibodies were quantified by measuring the absorbance of each well at 405 nm using a microtitre plate reader (Bio-Tek).

Absorbance values of known Vt-concentration were plotted on a logarithmic scale to create a standard curve from which concentration of Vg or Vt in unknown samples were calculated and reported as 'Vt-equivalents.' The sensitivity of this ELISA to *A. hebraeum* Vg is approximately 5 ng of Vt-equivalents

2.8. Statistical analysis

Statistical analyses were performed using Statview 4.02 software (Abacus Concepts). Data are reported as means ± SEM (*n*). Significant differences were identified using one-way ANOVA at a 5% significance level. Statistical significance in figures is indicated as follows: (*) $0.01 < P < 0.05$; (**) $0.001 < P < 0.01$; (***) $P < 0.001$.

3. Results

3.1. Effects of CyM on partially fed ticks

Weight loss [as % initial body weight (bw)] acetone-treated partially fed control ticks increased significantly ($P < 0.004$) from day 5 (12 ± 1.6% bw) to day 10 (19 ± 1.5% bw) post-treatment. Five days after treatment, partially fed ticks which received greater than 0.2 μg CyM had lost significantly more weight (up to 24 ± 1% loss of bw) than acetone-treated controls (Fig. 1A). Tick sur-

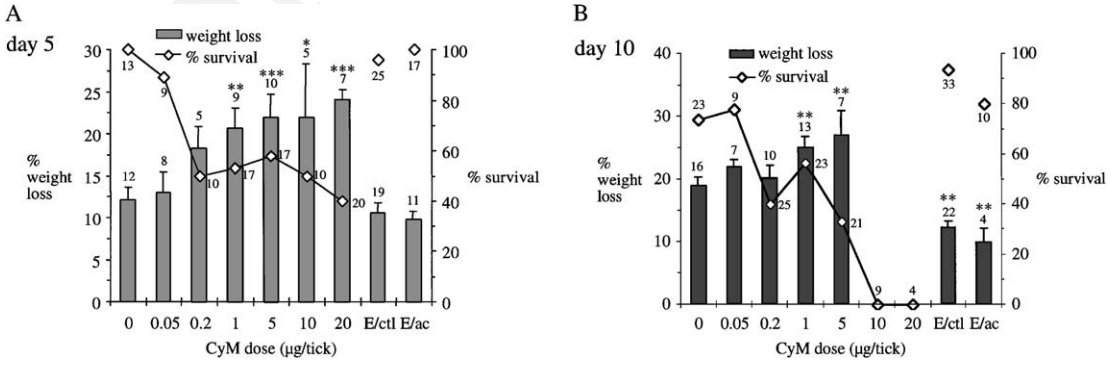

Fig. 1. Weight loss and mortality of CyM-treated partially fed ticks on (A) day 5 and (B) day 10 post-treatment. (E/ctl): untreated engorged ticks; (E/ac): acetone-treated engorged ticks. Mortality is reported as percent of ticks surviving treatment (*n*). Weight loss here, and data in subsequent figures, are reported as means ± SEM (*n*). Statistically significant differences from partially fed acetone-treated control (0 μg) are indicated as follows: (*) $0.01 < P < 0.05$, (**) $0.001 < P < 0.01$, (***) $P < 0.001$.

vival 5 days post-treatment dropped from 100% in control ticks to 40% in ticks treated with 20 μg CyM per tick (Fig. 1A). By day 10, ticks treated with 1 and 5 μg CyM/tick lost significantly more weight (25 ± 1.7 and $27 \pm 4\%$ bw, respectively), relative to acetone-treated control ticks. Beginning at 74% for controls, the percent of ticks surviving to day 10 generally decreased with dose, with no ticks surviving at doses of 10 or 20 μg per tick.

CyM had no dose-dependent effect on the ovary from partially fed ticks on day 5 or 10. There were no significant differences in ovary weight (Fig. 2A), oocyte size (Fig. 2B), or ovary Vt content (Fig. 2C) across partially fed treatment groups on day 5 or 10; a single exception was that oocyte length of 20 μg CyM-treated ticks on day 5 was significantly smaller than acetone-treated controls (95 ± 5 μm for CyM treated and 135 ± 18 for controls; Fig. 2B).

There were no differences in ovary weight (as % bw) between engorged and partially-fed ticks on day 5. However, by day 10, the ovary weights of engorged ticks were much higher than for partially fed ticks ($3.4 \pm 1.0\%$ for acetone-treated engorged, and $1.0 \pm 0.1\%$ for partially fed acetone-treated controls; Fig. 2A). Oocytes were also significantly larger in acetone-treated engorged (256 ± 12 μm on day 5; 425 ± 23 μm on day 10) compared to partially fed acetone-treated control ticks (135 ± 18 μm on day 5; 162 ± 21 μm on day 10; Fig. 2B). Ovaries contained more Vt in acetone-treated engorged (11.7 ± 1.5 abs/g ovary on day 5; 19.5 ± 3.2 abs/g ovary on day 10) than partially fed acetone-treated controls (4.7 ± 1.1 abs/g ovary on day 5; 7.0 ± 2.6 abs/g ovary on day 10; Fig. 2C). Acetone treatment had no significant effect on ovary weight, oocyte length, or ovary Vt content in engorged ticks on day 5 or day 10.

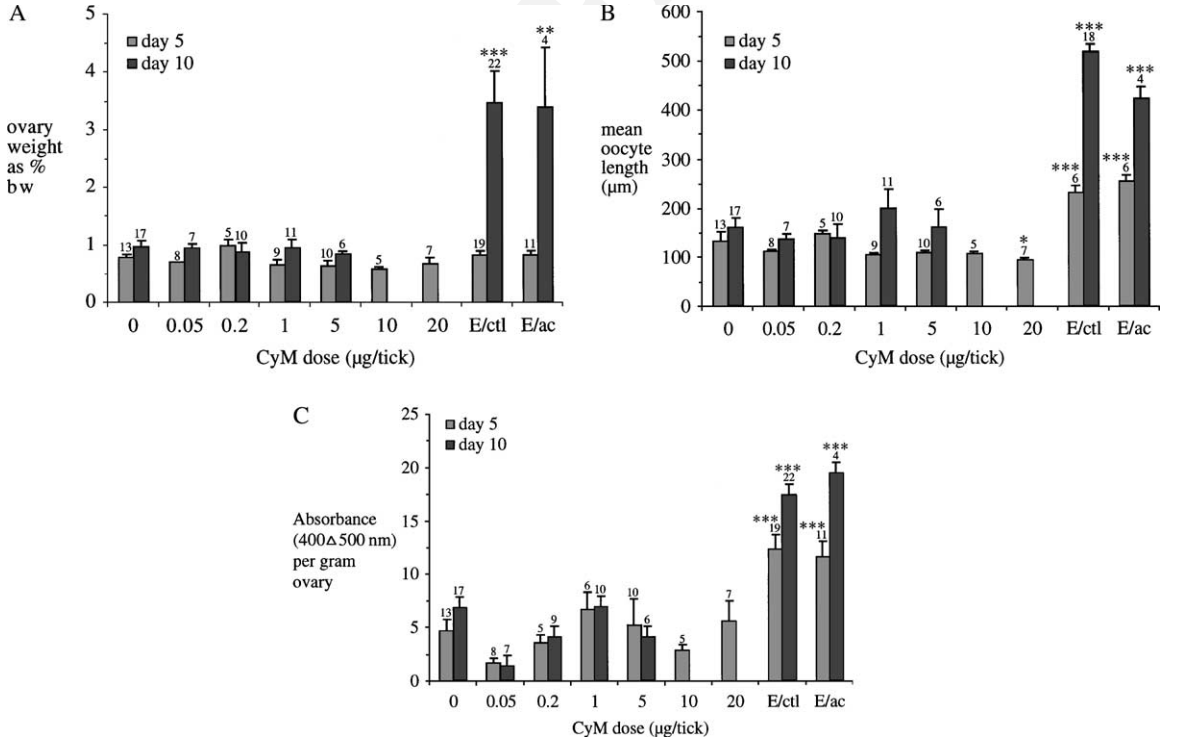

Fig. 2. Effect of CyM treatment on ovary development in partially fed ticks on day 5 (light grey bars) and 10 (dark-grey bars) post-treatment. (A) ovary weight; (B) mean length of 8 of the largest oocytes; (C) ovary Vt content, measured as absorbance per gram ovary at heme-specific wavelength (400–500 nm; see Section 2). Abbreviations and statistically significant differences from partially fed control (0 μg) are indicated as in Fig. 1.

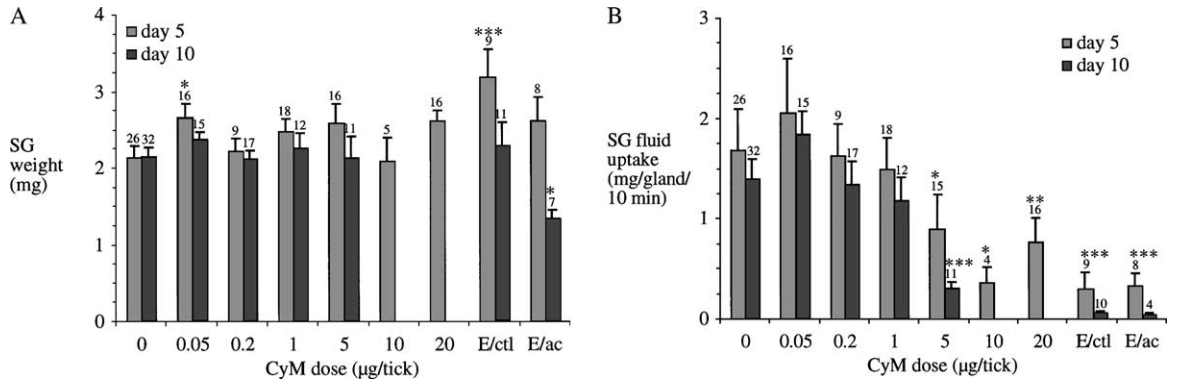

Fig. 3. Effect of CyM treatment on (A) salivary gland (SG) weight and (B) salivary fluid secretory competence in partially fed ticks on day 5 (light grey bars) and day 10 (dark grey bars) post-treatment. Abbreviations and statistically significant differences from partially fed control (0 µg) are indicated as in Fig. 1.

CyM (up to 20 µg/tick) had no significant effect on wet weight of salivary glands from partially fed ticks (Fig. 3A). However, salivary gland fluid uptake was significantly reduced compared to controls both on day 5 (from 1.69 ± 0.41 mg/gland/10 min for controls to 0.36 ± 0.16 mg/gland/10 min (10 µg CyM)) and day 10 (from 1.4 ± 0.2 for controls to 0.31 ± 0.07 mg/gland/10 min (5 µg CyM); Fig. 3B).

3.2. Effects of CyM on engorged ticks

Weight loss (compared to initial bw) increased significantly ($P < 0.001$) from day 5 ($8.7 \pm 1.0\%$) to day 10 ($12.5 \pm 0.7\%$) in untreated engorged ticks (Fig. 4A); however, there was no significant difference in weight loss in acetone-treated engorged ticks between day 5 and day 10.

There was no significant difference in weight loss across treatment groups by day 5 post-engorgement (Fig. 4A). Tick survival on day 5 dropped from 97% (acetone controls) to 20% (30 µg CyM/g bw), and then recovered somewhat at the 100 and 300 µg CyM/g bw doses (Fig. 4A). A CyM dose of 30 µg/g caused a significant increase in weight loss in engorged ticks on day 10 [up to $15.1 \pm 2.6\%$ (30 µg CyM/g bw) from 10.8 ± 0.7 (acetone controls); Fig. 4A], but at 300 µg CyM/g bw, the weight loss ($4.9 \pm 0.96\%$) was significantly less than controls. The percent of

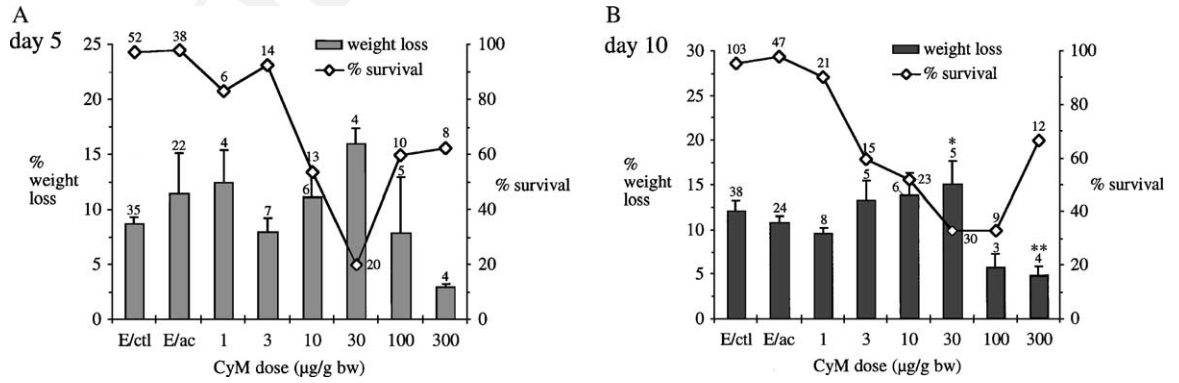

Fig. 4. Weight loss and survival of CyM-treated engorged ticks on (A) day 5 and (B) day 10 post-treatment. Mortality is reported as percent of ticks surviving treatment (*n*). Abbreviations and statistically significant differences from engorged acetone-treated control are indicated as in Fig. 1.

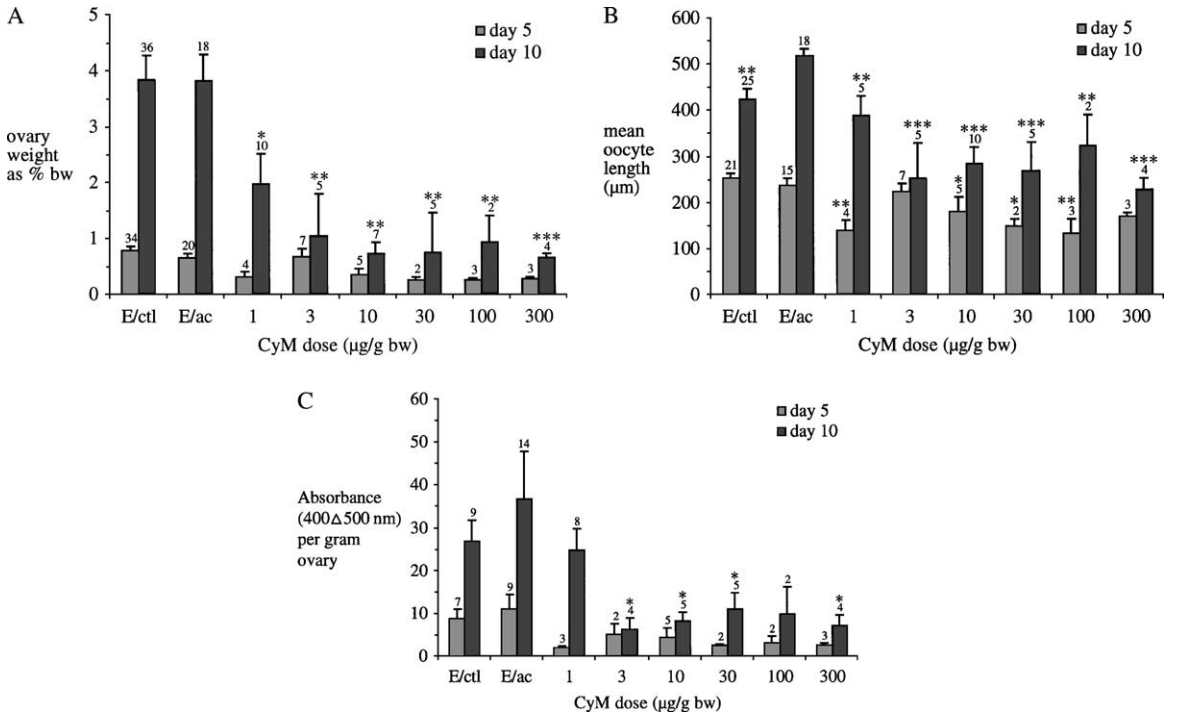

Fig. 5. Effect of CyM treatment on ovary development in engorged ticks on day 5 (light grey bars) and 10 (dark-grey bars) post-treatment. (A) ovary weight; (B) mean length of eight of the largest oocytes; (C) Vt content of ovary, measured as absorbance at heme-specific wavelength (400–500 nm; see Section 2). Abbreviations and statistically significant differences from engorged acetone-treated control are indicated as in Fig. 1.

ticks which survived to day 10 decreased with CyM dose; at doses of 30 and 100 μg/g only 33% of ticks survived compared with 96% for acetone-treated controls (Fig. 4B). At 300 μg/g doses, day 10 tick survival on day 10 had recovered somewhat to 67%.

Although ovary weight in day 5 ticks was not significantly affected by CyM, CyM caused a significant reduction in oocyte length (from 238 ± 15 μm in acetone controls to 134 ± 31 μm in 100 μg CyM/g bw-treated ticks; Fig. 5B). There was no significant change in yolk content, however, at any dose of CyM (Fig. 5C). On day 10, however, doses of 1–3 μg CyM/g bw inhibited all observed indices of ovary development (Fig. 5). Ovary weight was reduced to 0.67 ± 0.07% bw (300 μg CyM/g bw) compared with 3.84 ± 0.42% bw in acetone-treated controls (Fig. 5A). Oocyte length was reduced from 519 ± 15 μm (acetone controls) to 230 ± 24 μm (300 μg CyM/g bw; Fig. 5B); untreated control ticks (425 ± 23 μm) also had significantly smaller oocytes than acetone controls. Ovary Vt content was also reduced on day 10, by up to 80% in ticks treated with 300 μg CyM/g bw (from 37 ± 11 abs/g ovary for acetone controls to 7 ± 2 abs/g ovary for CyM-treated ticks).

Salivary glands of day 5 engorged ticks treated with over 10 μg CyM/g bw were significantly heavier (up to 5.3 ± 0.3 mg) than those of acetone controls (2.7 ± 0.2 mg; Fig. 6A). By 10 days post-engorgement, salivary gland weight in acetone-treated controls had fallen to 1.6 ± 0.1 mg, but there was a significant recovery of weight (4.83 ± 0.80 mg) at 30 μg CyM/g bw (Fig. 6A). Salivary gland fluid secretory competence on day 5 increased by up to 2.5-fold in ticks treated with 10 μg CyM/g bw, compared to acetone-treated controls (1.38 ± 0.14 mg/gland/10 min for CyM treated and 0.56 ± 0.10 mg/gland/10 min for controls; Fig. 6B). Higher doses of CyM did not have this effect, however (Fig.

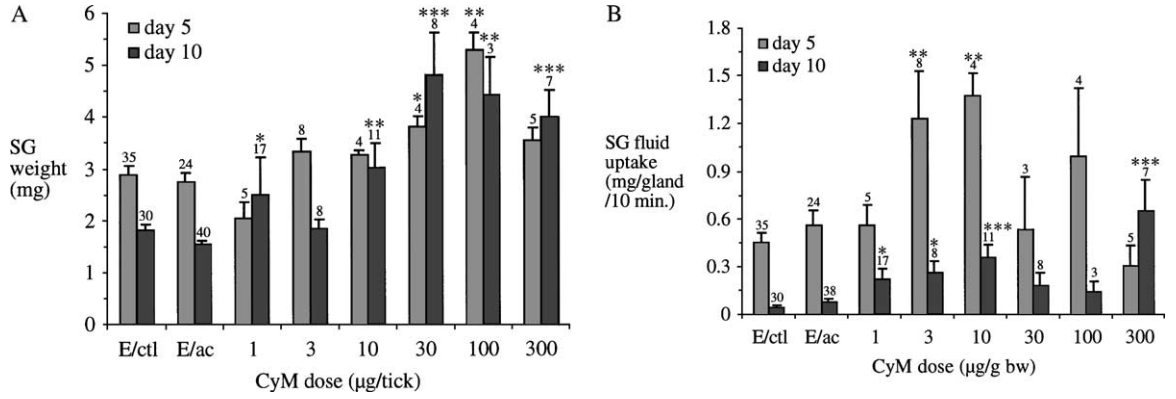

Fig. 6. Effect of CyM treatment on (A) salivary gland (SG) weight and (B) salivary fluid secretory competence in engorged ticks on day 5 (light grey bars) and day 10 (dark grey bars) post-treatment. Abbreviations and statistically significant differences from engorged acetone-treated control are indicated as in Fig. 1.

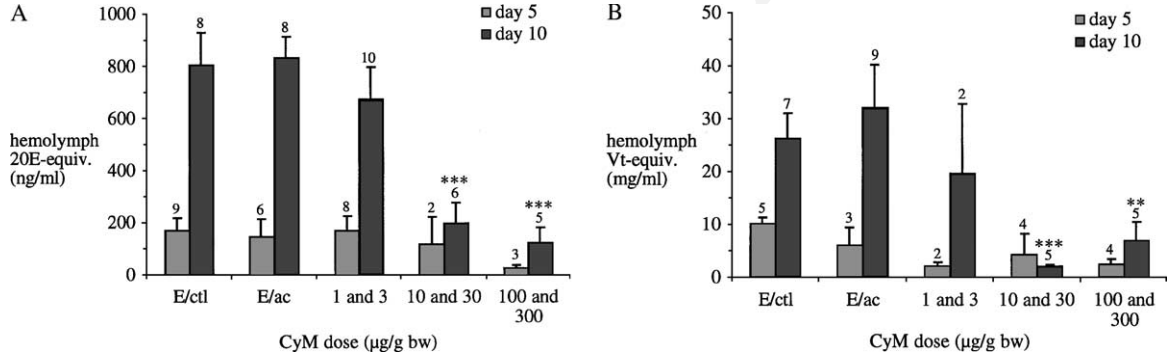

Fig. 7. Effects of CyM on hemolymph (A) 20E-concentration and (B) Vg-concentration in engorged ticks on day 5 (light grey bars) and day 10 (dark grey bars) post-treatment. Abbreviations and statistically significant differences from engorged acetone-treated control are indicated as in Fig. 1.

6B). Likewise, on day 10, CyM at most doses caused significant increase in fluid secretory competence compared to controls (Fig. 6B).

Due to difficulty in obtaining hemolymph samples on days 5–10 post-engorgement, CyM treatment groups were pooled as follows: 'low dose' (1 and 3 µg CyM/g bw), 'medium dose' (10 and 30 µg CyM/g bw), and 'high dose' (100 and 300 µg/g bw). There was no significant effect of CyM treatment on hemolymph 20E- or Vg-concentrations by day 5 (Fig. 7). However, by day 10, hemolymph 20E-concentration was reduced from 837 ± 80 pg/µl in acetone-treated controls to 123 ± 59 pg/µl in the high dose group (Fig. 7A). Likewise, on day 10, CyM-treated ticks had hemolymph Vg-concentration as low as 2.3 ± 0.3 µg/µl (medium dose) relative to acetone controls (32.1 ± 8.2 µg/g bw; Fig. 7B).

4. Discussion

As described in Section 1, sublethal doses of CyM can be used to stimulate the release of neurohormones and to trigger associated physiological events. Thus, Taylor et al. [19] applied CyM to unfed, virgin female *O. moubata*, and stimulated Vg-synthesis and ovarian development to levels characteristic for normal engorged ticks. CyM did not have similar effects in this study, however: in

partially fed ticks, CyM did not stimulate ovary development (Fig. 2). In engorged ticks, CyM inhibited ovary growth and egg development (Fig. 5), as well as 20E and Vg-synthesis (Fig. 7). Thus, at least part of CyM's inhibitory effect on Vg-synthesis is likely to be the result of inhibiting 20E-secretion (Fig. 7A). In *A. hebraeum*, ecdysteroids are synthesized in the epidermis in response to a neuropeptide from the synganglion [28]. We do not know whether CyM inhibits 20E-synthesis by blocking the release of the ecdysteroidogenic neuropeptide, or by a direct action on the epidermis.

The effects of CyM on oocyte development were more pronounced on day 10 than day 5, likely because in *A. hebraeum* vitellogenesis has only begun by day 5, whereas it is almost complete by day 10 [12]. Although by day 5 there were no significant differences in ovary weight or ovary Vt content, a decreasing trend is apparent in engorged ticks treated with higher doses of CyM (Figs. 5A and C). Day 5 oocytes from engorged ticks were significantly smaller after CyM treatments (Fig. 5B), being approximately the same size as day 5 partially fed oocytes (Fig. 2B). This suggests that oocyte growth had been delayed prior to or during the cytoplasmic growth phase. In addition, by day 10, all of the parameters of egg development were reduced by CyM treatment to the same levels as day 5 acetone-treated controls. These results strongly suggest that CyM inhibits Vg uptake by oocytes.

Salivary gland degeneration in female ixodid ticks following engorgement is triggered by 20E [11]. By day 10, high doses of CyM inhibited hemolymph 20E-concentration in engorged ticks by 85% by day 10. There was a similar trend by day 5, thought the difference was not significant, perhaps because of the very small number of ticks available for this experiment (Fig. 7A). In partially fed ticks removed from the host below the critical weight, hemolymph ecdysteroid-concentration does not rise [29], and the salivary glands do not degenerate [24,30]. CyM, perhaps due to a toxic effect, reduced salivary fluid transport (Fig. 3B). In engorged ticks, on the other hand, CyM significantly increased salivary gland weight and fluid transport (Fig. 6). At first sight, perhaps it was through its effect on reducing hemolymph ecdysteroid-concentration (Fig. 7A). But there are some problems with this explanation. The reduction of hemolymph ecdysteroid-concentration by CyM occurred only at 10 μg CyM/g and above, whereas an increase in salivary gland weight and fluid transport began as low as 3 μg CyM/g on day 5, and 1 μg CyM/g on day 10 (Fig. 6B). Moreover, even at 10–100 μg/g, CyM reduced hemolymph ecdysteroid-concentration only to 100–200 ng/ml. At this level, ecdysteroid-concentration should still have been high enough to cause salivary gland degeneration [30,29]. Thus, the mechanism whereby CyM increases salivary gland weight and fluid transport remains and enigma. However, similar effects on salivary gland fluid secretion were observed in *A. hebraeum* using the avermectin, MK-243 (Friesen et al., submitted manuscript), which also causes a marked reduction of haemolymph ecdysteroid-concentration in this tick [31].

In insects, pyrethroids cause paralysis and death by stimulating repetitive action potential firing in neurons [15,32]. While CyM was the most effective pyrethroid tested at stimulating ovarian development in the ladybird beetle, it was also the most toxic; 36–56% of animals treated with 0.05 μg CyM died [18]. However, in ticks, Taylor et al. [19] found that >80% of mated, unfed *O. moubata* survived a dose of 20 μg CyM/tick until day 10 post-treatment, and CyM was the least toxic of the six pyrethroids tested. As reported here, CyM seemed to be more toxic in *A. hebraeum* than in *O. moubata* (see Figs. 1 and 4). CyM also appeared to be more toxic to partially fed ticks than to engorged ticks; by day 10, a dose of 10 μg CyM/tick (~36–55μg/g, corrected for body weight), caused 100% death in partially fed ticks (Fig. 1B), compared to 66% death in engorged ticks at 100 μg CyM/g (Fig. 4B). The reason for this difference is obscure.

The effect of CyM on vitellogenesis in *A. hebraeum* is significantly different from that observed for *O. moubata* and *Ornithodoros parkeri* [20,19, 33,4]. In the latter, a compelling argument was made that CyM releases VIF, a neuropeptide from the synganglion, which then triggers apparently normal vitellogenesis and egg development. In *A. hebraeum,* CyM exerts only inhibitory effects on

the reproductive system, but the sites and mechanisms of action remain largely unknown.

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