Diuretic and antifeedant actions by *Manduca sexta* diuretic hormone in lepidopteran larvae

L. L. Keeley, J. S. Chung* and T. K. Hayes

Laboratories for Invertebrate Neuroendocrine Research, Department of Entomology, Texas A & M University, College Station (Texas 77843, USA)
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Abstract. Neuroendocrine disruption of water balance in insect larvae was evaluated as the basis for a new approach to pest insect control. Effects on water balance and food consumption were measured for larvae of *Heliothis virescens* (cotton budworm) treated with synthetic *Manduca sexta* diuretic neurohormone (MasDH). Synthetic MasDH (50–250 nM) caused dose-dependent increases in fluid secretion by larval Malpighian tubules in vitro; higher concentrations resulted in lower fluid secretion. Last instar larvae injected with 20–100 pmol MasDH exhibited increasing weight loss. Larvae injected with 500 pmol MasDH dramatically reduced both water excretion and food consumption by 70%. These latter results indicate that high titers of diuretic hormone can suppress feeding damage by larval insects by depressing water excretion and food consumption.
Key words. Water balance; diuresis; neurohormone; *Manduca*; *Heliothis*; insect control; peptide biotechnology.

Endocrine imbalances in higher organisms produce physiological dysfunctions that weaken or destroy affected individuals. On this basis, hormone mimetics have proven useful for the control of weeds and some pest insects. The concept of producing hormone imbalance in insects for pest control purposes was suggested first by Williams[1,2] with reference to the juvenile hormones and ecdysteroids that regulate insect molting, metamorphosis and reproduction. During the past decade, advances in insect neuroendocrinology, peptide chemistry and genetic bioengineering make it reasonable to consider manipulating neuroendocrine-dependent processes as a new basis for insect pest control[3–9].

Insects employ neurohormones as master regulators for the synthesis and secretion of the ecdysteroids and juvenile hormones and for regulating homeostasis of critical physiological processes such as intermediary metabolism, hemolymph metabolite biosynthesis and salt and water balance[8]. Salt and water balance are critical insect processes especially susceptible to neuroendocrine disruption. Prevention of dehydration is important in small animals such as insects, and excess diuretic hormone titers may produce diarrhea and death, especially in soft-bodied larvae. Identification and synthesis of a diuretic neurohormone from the tobacco hornworm, *Manduca sexta*[10], provides an opportunity to test whether exogenous administration of large amounts of a diuretic neuropeptide can cause a significant water imbalance in lepidopteran larvae. The results from these studies suggest that high titers of diuretic hormones can alter water balance and feeding behavior in lepidopteran larvae and have potential for application in insect control strategies.

Materials and methods

Fluid secretion by isolated Malpighian tubules was measured in response to synthetic *Manduca sexta* diuretic hormone (MasDH) prepared in our laboratories[11]. The MasDH was tested on Malpighian tubules isolated from 24-h-starved, feeding-stage, fifth instar larvae of *Heliothis virescens* (Noctuidae: Lepidoptera) using a modified, Ramsay bioassay[12].

In vivo effects by MasDH were measured in feeding-stage fifth instar *H. virescens* larvae. Larvae were placed on ice for temporary immobilization and injected manually behind the head capsule with 4 µl of MasDH in modified (50 mM NaCl, 20 mM KCl) Reddy-Wyatt medium[13]. Control larvae were injected with 4 µl of medium. Larvae were weighed and placed into a petri dish either with or without food. Larvae were reweighed at the end of each experimental period, and fecal pellets were collected and weighed for each larva. The amount of water excreted in the feces was estimated from the difference in fecal wet weight and the dry weight after 24 h at 110 °C.

For studies on food consumption, diet portions were weighed and presented to individual larvae at the start of each experimental feeding period. The percent water in the initial diet was determined by comparing the fresh and dry weights of a portion of the same diet dried 24 h at 110 °C. From the percent water, the initial quantity of dry diet presented to the larva was estimated. At the end of the experimental feeding period, the uneaten diet was dried and the amount of food consumed calculated as the difference between the estimated starting dry weight and the final dry weight. For the 24-h studies, fresh diet was provided at 0, 4, 8 and 12 h.

Results and discussion

Basal fluid secretion rates were nearly identical for all tubules in the absence of MasDH (fig. 1). Fluid secretion increased in a dose-dependent manner when the tubules were exposed to concentrations of 50–250 nM MasDH. However, exposure of tubules to concentrations of MasDH $\geq$ 500 nM reduced fluid secretion to the minimal response observed at 50 nM MasDH. Decreased tubule fluid secretion in the presence of $\geq$ 500 nM MasDH suggests that tubules had reduced sensitivity when exposed

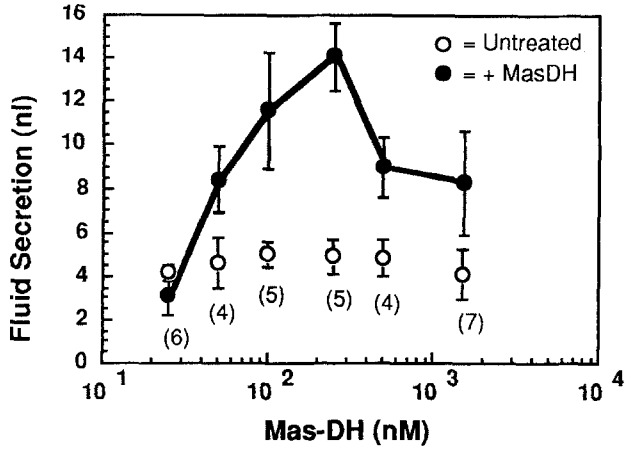

Figure 1. MasDH dose-response for fluid secretion by isolated Malpighian tubules from 24-h-starved, feeding-stage, fifth instar *H. virescens* larvae. Values are means ± SEM for the number of replicate larval tubules shown in parenthesis. Open circles represent basal fluid secretion by untreated tubules in 40 µl modified Reddy-Wyatt medium; closed circles represent the response by the same tubules after the addition of MasDH to the bathing medium to provide the final concentration indicated. The rate of fluid transfer was estimated based on the rate of transfer of [^{14}C]urea [12].

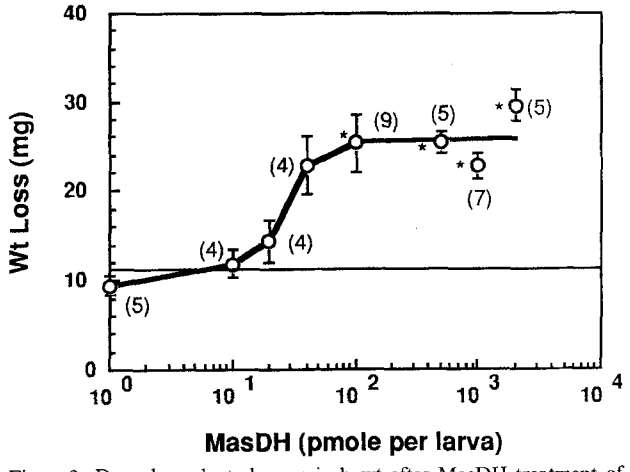

Figure 2. Dose-dependent changes in b. wt after MasDH treatment of feeding stage, fifth instar *H. virescens* larvae. Larvae were fed until treatment but unfed during the 4-h study period following medium- or MasDH-injection. The horizontal line indicates the mean weight loss for medium-injected control larvae (n = 14). Values are means ± SEM for the number of replicate larvae shown in parenthesis. Asterisks indicate treated means that differ significantly ($p < 0.05$) from the control mean.

to high levels of the hormone. These results demonstrated that MasDH affected fluid secretion by Malpighian tubules from *H. virescens* larvae and, therefore, it was reasonable to expect that MasDH could influence the water balance of intact larvae.

MasDH administration to *H. virescens* larvae produced a dose-dependent weight loss. All treated and control-injected larvae were held without food to eliminate possible effects by differences in feeding. All larvae lost weight during a 4-h observation period (fig. 2). Medium-injected control larvae lost 10 mg of body weight. Larvae treated with < 20 pmol MasDH lost the same amount of weight as the controls. However, weight loss was dose-dependent between 20–100 pmol MasDH (= 95–473 ng MasDH) per larva and reached a maximum of 26 mg for MasDH doses of ≥ 100 pmol. This latter weight loss was significant when compared to the weight loss by control larvae, and suggested that MasDH produced in vivo water loss.

Experiments were conducted to determine whether MasDH caused weight loss in feeding animals, and whether MasDH effects could be sustained beyond 4 h by treatment with higher doses of the hormone. Normal, feeding *H. virescens* larvae were injected with 500 pmol of MasDH. This dose exceeded by 5-fold the dose necessary for maximum in vivo weight loss during a 4-h period. Unlike the previous experiment, larvae were provided food after treatment. Body weight, food consumption and fecal water content were measured at intervals for 24 h following MasDH treatment. Medium-injected control larvae had a starting live weight of 253 ± 13 mg and gained 18 ± 2 mg during the first 4 h after treatment compared to MasDH-treated larvae which had an initial live weight of 237 ± 20 mg and lost 9 ± 2 mg ($p \leq 0.05$). This loss constituted an overall 27 mg decrease in weight between the treated and control larvae which agreed with the results of the previous experiment. However, in this experiment, food was consumed by both groups as evidenced by weight gain in the controls and lesser weight loss by MasDH-treated larvae. In fact, MasDH-treated larvae ate 68% less food than control larvae during the first 4 h after treatment (fig. 3). During subsequent observation intervals, weight gains by both MasDH-treated and control larvae were equal, but MasDH-treated larvae still consumed 50% less food than control larvae

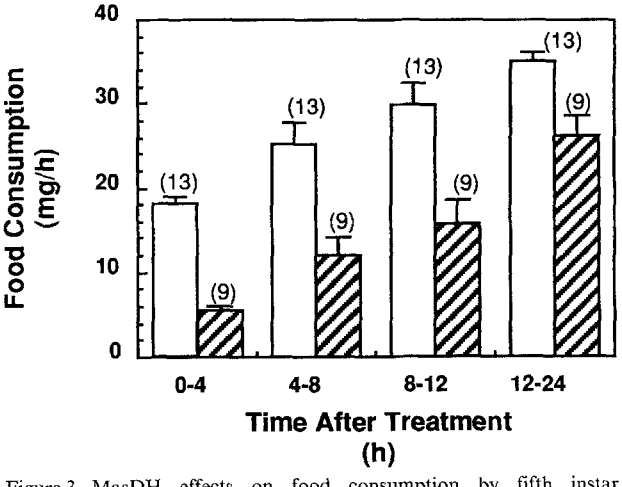

Figure 3. MasDH effects on food consumption by fifth instar *H. virescens* larvae. Fifth instar *H. virescens* larvae were injected with 500 pmol of MasDH in 4 µl modified Reddy-Wyatt medium. Larvae were permitted to feed before and after treatment. Open bars represent medium-injected control larvae; striped bars represent MasDH-injected test larvae. Values are means ± SEM for the number of replicate larvae shown in parenthesis. Mean values of control and MasDH-treated larvae differ significantly ($p \leq 0.002$) at each time interval.

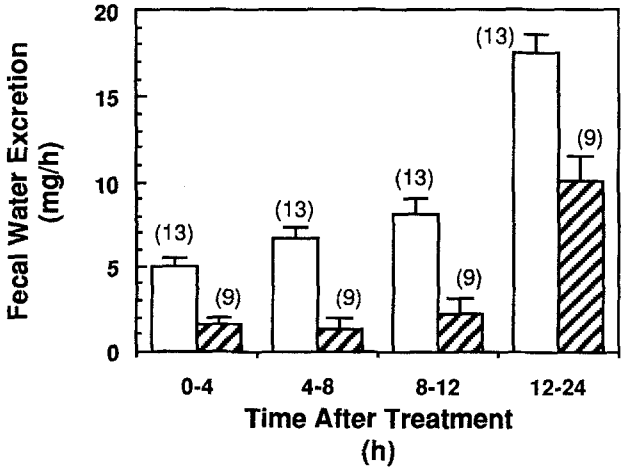

Figure 4. MasDH effects on the production of fecal water by fifth instar *H. virescens* larvae. Treatments and conditions are identical to figure 3. Mean values of control and MasDH-treated larvae differ significantly ($p \leq 0.001$) at each time interval.

between 4 and 12 h after treatment, and 26% less food between 12 and 24 h after treatment.

MasDH-treated larvae produced significantly less feces than control larvae at all times during the 24 h following treatment. Treated larvae produced only 10 ± 1 mg of feces during the first 4 h; whereas, control larvae produced 26 ± 2 mg of feces ($p \leq 0.05$). Total fecal production by treated larvae was still 40% below that of control larvae after 24 h ($p \leq 0.05$). Furthermore, feces from MasDH-treated larvae were drier than feces from control larvae. Fecal moisture ranged between 60 and 67% for treated larvae during the 24-h observation period compared to 72–77% for control larvae. Therefore, control larvae excreted a total of three times more water than MasDH-treated larvae during the first 4 h (fig. 4). Control larvae showed proportionate increases in fecal water with growth, but MasDH-treated larvae lost a constant 1–2 mg of fecal water per h during the first 12 h. Although water excretion by MasDH-treated larvae increased by more than 5-fold between 12 and 24 h after treatment, control larvae continued to excrete a total of 70% more.

The in vitro studies confirmed that MasDH affected fluid secretion by Malpighian tubules from *H. virescens* larvae and, therefore, could potentially affect larval water balance. MasDH treatment of larvae appeared to cause dose-dependent water loss between 20 and 100 pmol (fig. 2), but 500 pmol MasDH decreased water excretion (fig. 4), possibly by lowering the responsiveness of the tubules as suggested in figure 1 for doses $\geq$ 500 nM. Furthermore, the 500 pmol dose profoundly suppressed larval feeding activity (fig. 3). Although the regulation of water balance is a complex process that involves both excretion by the Malpighian tubules and water resorption by the hindgut/rectum, it is possible to speculate on the cause for decreased larval feeding based on the results with Malpighian tubules in vitro. Malpighian tubules exposed to $\geq$ 500 nM MasDH showed decreased hormone sensitivity and low fluid secretion (fig. 1). We speculate that this decreased sensitivity may result from a down-regulation of the tubules' diuretic hormone receptors. Such a response could be a mechanism to prevent diarrhea and dehydration if diuretic hormones should attain high titers in vivo. We estimate that injection of 500 pmol of MasDH per larva (live wt = 250–300 mg; hemolymph vol. 50–60 µl) should produce final hemolymph concentrations of approximately 10 µM MasDH. This estimated hemolymph concentration of MasDH exceeded by 20-fold the in vitro MasDH concentration that decreased tubule fluid secretion. Therefore, it can be suggested that injection of larvae with a 500 pmol MasDH dose might lower tubule responsiveness to hormonal stimulation in vivo and suppress water excretion.

Previous studies on *H. virescens* larvae during the feeding-stage indicated that water is excreted continuously in response to released diuretic factors[12]. Presumably, these hormones prevent edema from the high rate of water intake that occurs during this period of intense feeding on moisture-rich food. Hence, reductions in tubule sensitivity and fluid secretion in response to excessive titers of diuretic hormone would require that larval feeding be correspondingly reduced to prevent edema from further water intake.

These results with MasDH suggested that administration of agents with diuretic/antidiuretic activity may have a significant potential for future application in pest insect control, especially for lepidopteran larvae. Although the original prediction of rapid water loss and dehydration in response to high doses of MasDH was not supported, high doses of MasDH appeared to lower Malpighian tubule sensitivity and result in larval water retention and suppressed feeding. Hormonal suppression of feeding would also be useful as a pest-control strategy. Treated larvae would either starve to death or linger in the environment until destroyed by natural mortality factors. Either of the latter situations would reduce crop damage. Because of the peptide nature of neurohormones, they cannot be administered directly to insects for control purposes; however, alternatives are feasible. Development of oral delivery systems for peptide therapeutics is an area of intense research by pharmaceutical firms, and advances in this area for human therapy may be useful in insects as well. It is feasible that nonpeptide neurohormone analogs can be developed or discovered that will not require per os administration[6, 14]. Isolation of diuretic hormone receptors will permit screening for natural or synthetic chemicals that interact with the receptors and can serve as agonists or antagonists for diuretic hormones. This approach may identify existing natural products that can be used for insect control and illustrate the structural properties of molecules that can be used as models for synthesizing diuretic hormone analogs. Alternatively, genes for diuretic/antidiuretic neurohormones may be engineered into insect baculovirus cloning-ex-

pression vectors (BEV)[15], and the hormone super-expressed during infection of lepidopteran larvae to synergize the viral pathology[6]. In a preliminary experiment, *Bombyx mori* larvae infected with *B. mori* BEV transformed with a synthetic MasDH coding sequence expressed under control of the viral polyhedrin promoter resulted in a 30% decrease in hemolymph volume and larval death one day earlier than controls[16]. Although these latter effects are modest from the standpoint of insect control, they suggest that the concept of manipulating insect neurohormone levels is possible.

In conclusion, these studies provide evidence that insect water balance can be disturbed in vivo by exogenously administered diuretic peptides. The means to alter insect neurohormone titers are not presently available, but water balance and its neuroendocrine regulation are a likely focus for future research on insect pest control using advances in the peptide and genetic bioengineering technologies.

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Modulation of C2 and C3 gene expression of human peripheral blood monocytes by interleukin 1β, interferon γ, tumor necrosis factor α and lipopolysaccharide

H. Shimizu[a], T. Sakano[b]*, A. Fujie[a], S. Nishimura[a] and K. Ueda[a]

[a] *Department of Pediatrics, Hiroshima University School of Medicine, and* [b] *Hiroshima Prefectural Hospital, Hiroshima 734 (Japan)*
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Abstract. The effect of interleukin 1β (IL-1β), interferon γ (IFN-γ), tumor necrosis factor α (TNFα) and lipopolysaccharide (LPS) on the expression of the C2 and C3 genes in human adherent monocytes was studied. Stimulation of monocytes with IFN-γ increased both C2 and C3 mRNA. IL-1β also increased C2 mRNA level, whereas C3 gene expression was not enhanced. TNFα failed to increase either C2 or C3 mRNA. LPS increased C2 mRNA, but suppressed C3 gene expression. These results suggest that C2 and C3 production by monocytes is regulated by IL-1β and IFN-γ in the local tissues.
Key words. IL-1β; IFN-γ; LPS; C2; C3.

Monocytes have many activities including phagocytosis, superoxide release, and production of IL-1β and TNFα, and play a central role in the inflammatory disorders[1]. Complement components are also produced by monocytes and are thought to be important in local inflammatory reactions in such diseases as rheumatoid arthritis (RA)[2, 3] and systemic lupus erythematosus (SLE)[4]. It is likely that synthesis of complement components in local tissues by monocytes/macrophages is regulated by several cytokines. Therefore, we studied the effect of IL-1β, TFNα, IFN-γ and LPS on C2 and C3 gene expression in human peripheral blood monocytes.

Materials and methods

Materials. RPMI 1640, L-glutamine and penicillin-streptomycin (Gibco, Grand Island, NY), Hanks' balanced salt solution (HBSS, Nissui, Tokyo, Japan), phosphate buffered saline (PBS, Nissui), cesium chloride (Boehringer Mannheim, Indianapolis, IN), guanidine-isothiocyanate (Fluka, Buchs, Switzerland), Hybond-N+, multiprime DNA labeling systems (Amersham, Arlington Heights, IL), [^{32}P]dCTP (ICN, Irvine, CA), ITS+ (Collaborative Research, Bedford, MA), Falcon 3803 Primaria culture dishes (Becton Dickinson Labware, Lincoln Park, NJ), and LPS (*E. coli* 026 : B6, Dif-