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THE EFFECTS OF *MANDUCA SEXTA* DIURETIC HORMONE ON FLUID TRANSPORT BY THE MALPIGHIAN TUBULES AND CRYPTONEPHRIC COMPLEX OF *MANDUCA SEXTA*

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Summary

1. *Manduca sexta* diuretic hormone (Mas-DH) stimulates fluid secretion by adult Malpighian tubules of *M. sexta*, demonstrating its site of diuretic action in *M. sexta* for the first time. It was not possible to develop a suitable bioassay to measure fluid secretion in larval proximal tubules.

2. Mas-DH has an antidiuretic action on the cryptonephric complex of larval *M. sexta* because it increases fluid absorption from the rectum. It appears that in this complex Mas-DH is acting on a $Na^+/K^+/2Cl^-$ co-transporter, presumably on the basal membrane of the cryptonephric Malpighian tubules, because Mas-DH-stimulated fluid absorption by the cryptonephric complex is inhibited by bumetanide or the removal of Cl^-, Na^+ or K^+ from the haemolymph side of the tissue. This is the first demonstration of hormonal control of fluid absorption by the cryptonephric complex.

3. Concomitant with the stimulation of fluid transport, Mas-DH increases the amount of cyclic AMP secreted by adult Malpighian tubules and the cryptonephric complex. In addition, Mas-DH promotes cyclic AMP production by the larval proximal tubules.

Introduction

There is now a growing family of insect diuretic peptides (Kataoka *et al.* 1989; Blackburn *et al.* 1991; Kay *et al.* 1991*a*,*b*, 1992; Lehmberg *et al.* 1991), which are members of the same peptide superfamily as corticotropin releasing factor, sauvagine and urotensin I. Malpighian tubules are considered to be the major site of action of insect diuretic peptides where they cause an increase in the rate of fluid secretion. Diuretic peptides from *Acheta domesticus*, *Locusta migratoria* and *Periplaneta americana* have been shown to have such an effect, as well as increasing levels of cyclic AMP produced by the tissues (Kay *et al.* 1991*a*,*b*; Lehmberg *et al.* 1991; Kay *et al.* 1992).

The first diuretic peptide from this family to be isolated was from pharate adult whole-head extracts of *Manduca sexta* (Mas-DH; Kataoka *et al.* 1989). A second diuretic

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peptide from this same insect has recently been identified from adult corpora cardiaca–corpora allata complexes and separately from dissected medial neurosecretory cells (Mas-DPII; Blackburn *et al.* 1991). In both studies, an *in vivo* bioassay was used which measured diuresis in intact insects, but neither peptide has yet been shown to act on *M. sexta* Malpighian tubules. Kataoka *et al.* (1989) suggested that Mas-DH may stimulate the release of another peptide, which would act as a diuretic peptide on Malpighian tubules. More recently, Mas-DH has been shown to decrease fluid absorption from the rectum and increase intracellular levels of cyclic AMP in rectum and Malpighian tubules of larval *M. sexta in vivo* (Troetschler and Kramer, 1992). However, *in vitro* no significant increase in fluid secretion by adult Malpighian tubules or changes in cyclic AMP levels in larval, pharate adult or adult tubules was observed (Troetschler and Kramer, 1992). The exact site of action of this peptide in *M. sexta* is therefore unclear. This is surprising because Coast *et al.* (1992) have shown that Mas-DH stimulates fluid secretion and increases cyclic AMP levels in isolated tubules of adult *Acheta domesticus* and adult *Pieris rapae.*

In this study, the effects of Mas-DH on fluid transport and cyclic AMP production by Malpighian tubules of *M. sexta* adults and larvae, and the cryptonephric complex of larvae, are investigated to determine its site of action, as well as the possible role of cyclic AMP as a second messenger.

Materials and methods

Insects

Manduca sexta larvae were reared as described by Yamamoto (1969). Larval experimental animals were second-day, fifth instars, and adults were 12–48h post emergence.

Bioassays

Fluid absorption by the cryptonephric complex of M. sexta *larvae*

Methods for studying fluid transport across the larval cryptonephric complex using everted sacs were similar to those previously described for locust rectum (Hanrahan *et al.* 1984). However, in larval lepidopterans, the blind ends of the Malpighian tubules (which hang free in most other insects) are closely apposed to the rectum and the whole structure is enclosed within a perinephric membrane to form the cryptonephric complex. In this study, the whole cryptonephric complex was removed intact by carefully cutting the distal ends of the tubules where they emerge from the cryptonephric complex and ensuring that the perinephric membrane was not damaged. The cryptonephric complex was then everted so that the cut ends of the tubules were exposed on the inside of the sac and any fluid secreted by them would collect there. At hourly intervals, weight gain and tissue volume changes were determined by weighing the sacs (to within 0.25mg) before and after removal of fluid in the sac. The true rate of transepithelial fluid movement was determined by correcting for tissue volume changes. Fluid transport was measured hourly over 5h as described by Goh and Phillips (1978). With the tissue studied, rates are at near steady state after 3h. Effects of Mas-DH in physiological saline were determined by

adding small samples (10 μl) to the inside of the sacs (haemolymph side). In a typical experiment, physiological saline (10 μl) was added to the haemolymph side of the sacs for the first 3h, and the sacs were placed in 50ml of oxygenated physiological saline at 30˚C to obtain control rates. At the end of the third hour, Mas-DH or physiological saline (control) was added to the sacs. The rate of fluid transport was then measured for the next one or two hours and compared with control preparations over the same period and to rates during the previous control period for the same preparation. Results are expressed as $\mu l\,tissue^{-1}\,h^{-1}$.

To determine the effects of substituting Cl^-, K^+ or Na^+ in the experimental saline, the tissues were allowed to reach a steady state in the absence of a particular ion. Mas-DH or saline lacking one of these ions (control) was added at the start of the fourth hour to determine whether the substituted ion had any effect on stimulated or control rates of fluid absorption over the following hour. To determine the effects of restoring an ion, the substituted ion was added back at the start of the fourth hour and the tissue allowed to adjust to changes in ionic composition over the next hour. Mas-DH was then added at the start of the fifth hour and rates of fluid absorption were measured for 1h.

Bumetanide ($1mmol\,l^{-1}$ in saline) was added to the bathing saline (lumen side) or inside the sac (haemolymph) to determine its effects on fluid absorption across the cryptonephric complex under both control and Mas-DH-stimulated conditions. Bumetanide was made up as a $500mmol\,l^{-1}$ stock in dimethyl sulphoxide (DMSO) and diluted in saline for bioassay (final concentration 0.2%). DMSO was also assayed alone to determine whether it had any effect on fluid absorption. Bumetanide and DMSO were purchased from Sigma, St Louis, MO.

Fluid secretion by Malpighian tubules

The effects of Mas-DH on Malpighian tubule fluid secretion were determined using a modification of the bioassay described by Coast (1988). Briefly, short segments (6–12mm) of tubules were dissected free from male adult moths and transferred individually to small drops (5 μl) of saline beneath water-saturated liquid paraffin. One end of the tubule was occluded by clamping it into a strip of Sylgard, which ran alongside the drops of bathing fluid. The other end of the tubule was withdrawn from the saline into the liquid paraffin and anchored in place by clamping it into a second strip of Sylgard. The length of the tubule within the 5 μl drop of bathing fluid was 2.5–5mm. Secreted fluid escaped into the surrounding liquid paraffin from a small cut made just distal to the point at which the tubule was anchored. Tubules were allowed to equilibrate for 30min, after which the bathing fluid was replaced. Secreted fluid was collected over the following 60min in order to determine the basal (unstimulated) rate of fluid secretion. The bathing fluid was then substituted for fresh saline (controls) or saline containing Mas-DH, and the rate of secretion (experimental rate) was determined over a second period of 60min. To determine rates of secretion, droplets of tubule fluid secreted over 60min were displaced from the tubules with a fine glass rod, and their volume was calculated from measurements of diameter assuming them to be spherical when resting on the strips of Sylgard. Results are expressed either as absolute rates ($nl\,min^{-1}$) or as the difference in

the rate of secretion between the experimental and control periods ($\Delta nl min^{-1}$) so that each tubule segment served as its own control.

Cyclic AMP assay

To measure cyclic AMP production by the cryptonephric complex, whole tissues were dissected free, opened as flat sheets and incubated in 250 μl of oxygenated saline in Eppendorf tubes and at 30°C. Segments (1–1.5cm) of adult and larval proximal tubules were incubated in 100 μl of saline at 30°C in ELISA plates. The saline contained $0.5 mmol l^{-1}$ 3-isobutyl-1-methyl xanthine (IBMX, Sigma) to inhibit phosphodiesterase activity. The tissues were allowed to equilibrate as described for the fluid transport assays, and 50 μl samples were taken for cyclic AMP determination. These samples were replaced by fresh saline (control) or saline containing $10 nmol l^{-1}$ Mas-DH and allowed to incubate for a further 1h. 50 μl saline samples were again removed for cyclic AMP determination, and the amount of cyclic AMP produced was compared with that produced by control preparations over the same period and with rates during the previous control period for the same preparation. Saline samples were taken rather than liberating intracellular cyclic AMP from tissues, because Rafaeli *et al.* (1984) had previously determined that Malpighian tubules secrete cyclic AMP into the bathing medium. Cyclic AMP production was measured using a modification of the competitive binding assay of Gilman (1972) and as recently described by Coast *et al.* (1991). Results are represented as cyclic AMP produced (pmol) per tissue (cryptonephric complex or tubule segment).

IBMX was prepared as a $200 mmol l^{-1}$ stock solution in DMSO and diluted in physiological saline for tissue incubation (final concentration 0.25%).

Salines

The saline was based on that described by Chamberlin (1989) for *M. sexta* and contained in $mmol l^{-1}$: 3 Na_2HPO_4; 5 $MgCl_2$; 1 $CaCl_2$; 5.8 KOH; 7.7 potassium citrate; 2.8 sodium succinate; 10 glucose; 3.6 alanine; 9.4 glutamine; 12.8 glycine; 9.7 histidine; 5.6 malic acid; 7.4 proline; 8.9 serine; 4.6 threonine; 180 sucrose, 5 $NaHCO_3$. The saline was aerated with 95% O_2/5% CO_2, which adjusted the pH to 6.7. The osmolarity of the saline was $332 mosmol l^{-1}$, which is similar to that of *M. sexta* haemolymph (Chamberlin, 1989).

Ion substitutions were made by replacing Cl^- with gluconate, K^+ with Na^+, and Na^+ with K^+.

Manduca sexta *diuretic hormone*

Mas-DH used for cyclic AMP determination and actions on the cryptonephric complex was a gift from Dr J. Li (Sandoz Crop Protection). Mas-DH used to determine its effects on Malpighian tubule fluid secretion was synthesized by T. K. Hayes (Coast *et al.* 1992).

Osmolarity of salines

Changes in saline osmolarity due to the addition of Mas-DH or chemicals were monitored with a Westcor vapour pressure osmometer (model 5500; Logan, Utah) and

were found not to be significant. The osmolarities of the ion-substituted salines were also not significantly different from that of the physiological saline.

Statistical treatment

Differences between treatments were considered significantly different when Student's *t*-test indicated a *P* value of less than 0.01.

Results

Time course of fluid absorption across the cryptonephric complex

The rate of fluid absorption across the cryptonephric complex with time is shown in Fig. 1. Fluid absorption gradually falls from approximately 10 μl tissue^{-1} h^{-1} over the first 3h to a steady-state level of 3–4 μl tissue^{-1} h^{-1}, which persists over the next 2h. Fluid absorption can be increased two- to threefold after 3 and 4h by the addition of 5 nmol l^{-1} Mas-DH.

Dose–response relationship of Mas-DH on fluid absorption across the cryptonephric complex

Mas-DH increased fluid reabsorption by the cryptonephric complex in a dose-dependent manner, as shown in Fig. 2 (represented as increases over control values when only saline was present). A significant response was observed at 0.1nmol l^{-1}, and a maximum response was attained with a concentration between 1 and 5nmol l^{-1}.

Effect of cutting the perinephric membrane on fluid absorption across the cryptonephric complex

Cutting the perinephric membrane, which encloses the Malpighian tubules and rectum,

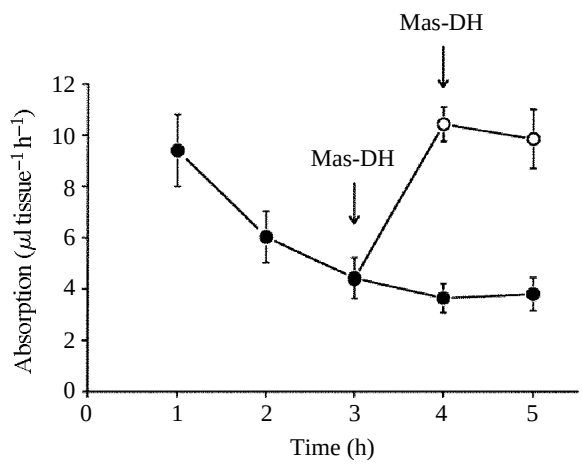

Fig. 1. Time course of fluid absorption across the larval cryptonephric complex. Filled circles represent control values when only saline was present, open circles show the effects of adding 5 nmol l^{-1} Mas-DH over the fourth and fifth hour (means ± S.E.M., *N*=9–16).

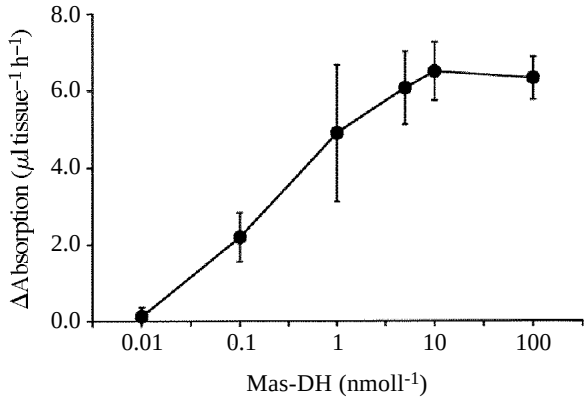

Fig. 2. Effect of increasing doses of Mas-DH on fluid absorption across the larval cryptonephric complex, shown as a semi-logarithmic plot (means ± S.E.M., *N*=6–12).

abolishes the stimulatory effects of Mas-DH on fluid absorption. The control rate of fluid absorption was 5.06±0.67 μl tissue^{-1} h^{-1}, which did not increase significantly (over the next hour) on the addition of 10nmol l^{-1} Mas-DH (5.78±0.72 μl tissue^{-1} h^{-1}; *N*=9).

Ion dependence of Mas-DH-stimulated fluid absorption across the cryptonephric complex

The effects of lumen or haemolymph ion substitution on fluid absorption by everted cryptonephric complex sacs of *M. sexta* are shown in Tables 1 and 2. Mean values were compared with controls when the tissue had attained a steady-state level of fluid absorption (3h after dissection) and when a full complement of ions was present. Substitution of ions throughout the experimental period, or restoring the missing ion after 3 h did not have a significant effect on the control rates of fluid absorption. If any one of the ions studied (Cl^-, K^+ or Na^+) was substituted on the lumen side of the tissue, Mas-DH caused increases in fluid absorption across the cryptonephric complex similar to those observed when a full complement of ions was present (Table 1). However, substitution of Cl^-, K^+ or Na^+ on the haemolymph side completely abolished the stimulatory effects of Mas-DH on fluid absorption (Table 2). The stimulatory effects of Mas-DH could be restored by adding back the missing ion to the haemolymph side of the sacs.

The effect of bumetanide on fluid absorption across the cryptonephric complex

The effects of bumetanide (which inhibits $Na^+/K^+/2Cl^-$ co-transport; Suki and Eknoyan, 1992) on fluid absorption across the cryptonephric complex of *M. sexta* under control and Mas-DH-stimulated conditions are compared in Table 3. Bumetanide had no significant effect on control rates of fluid absorption, but completely abolished the effects of Mas-DH when added to either the lumen or the haemolymph side of the sacs at a concentration of 1mmol l^{-1} (Table 3). When assayed alone, DMSO (0.2%) had no significant effect on rectal fluid absorption (results not shown).

Table 1. *Effect of lumen ion substitutions on fluid absorption across the larval cryptonephric complex*

Substituted ion	Control (μl tissue^{-1} h^{-1})	5 nmol l^{-1} Mas-DH (μl tissue^{-1} h^{-1})
None	3.65±0.57	10.44±1.15*
K^+	2.31±0.92	8.52±1.24*
Na^+	4.07±0.79	10.06±1.08*
Cl^-	3.79±0.69	9.73±1.27*

Means are significantly different from controls (normal saline, no Mas-DH), *P<0.01.
Values are mean ± S.E.M., N=6–12.

Table 2. *Effect of haemolymph ion substititions on fluid absorption across the cryptonephric complex*

	Ion substituted		Ion restored	
Ion	Control (μl tissue^{-1} h^{-1})	5 nmol l^{-1} Mas-DH (μl tissue^{-1} h^{-1})	Control (μl tissue^{-1} h^{-1})	5 nmol l^{-1} Mas-DH (μl tissue^{-1} h^{-1})
K^+	3.42±0.98	2.28±1.38	2.36±1.50	9.75±1.84*
Na^+	3.29±0.55	3.70±0.60	3.03±0.71	7.93±0.95*
Cl^-	4.20±0.57	3.73±0.66	4.00±0.73	9.20±1.21*

Means are significantly different from controls (normal saline, no Mas-DH), *P<0.01.
Values are mean ± S.E.M., N=8–12.

Table 3. *Effect of bumetanide on Mas-DH-stimulated fluid absorption across the larval cryptonephric complex*

	No bumetanide	1 mmol l^{-1} bumetanide	
Control (μl tissue^{-1} h^{-1})	3.06±0.34	3.42±0.75	
		Lumen	Haemolymph
5 nmol l^{-1} Mas-DH (μl tissue^{-1} h^{-1})	7.88±0.92*	3.00±0.58	2.81±0.42

Means are significantly different from controls, *P<0.01.
Values are mean ± S.E.M., N=6–9.

Effect of Mas-DH on adult Malpighian tubule fluid secretion

Basal (unstimulated) rates of fluid secretion vary from 0.2 to 0.8 nl min^{-1} in tubules taken from different insects 12–48h post emergence, but such variability is not seen in tubule segments taken from the same animal. The rate of secretion is virtually unchanged for up to 2h post equilibration, but when the bathing fluid is replaced with saline containing 10 nmol l^{-1} Mas-DH, there is a dramatic increase in fluid secretion as shown in Fig. 3. The rate of secretion immediately prior to adding Mas-DH was 0.25±0.36 nl min^{-1} but, after 3min (the shortest time interval over which rates could be measured), the rate had increased to 4.48±0.55 nl min^{-1}, and reached 6.26±0.09 nl min^{-1} after 6 min.

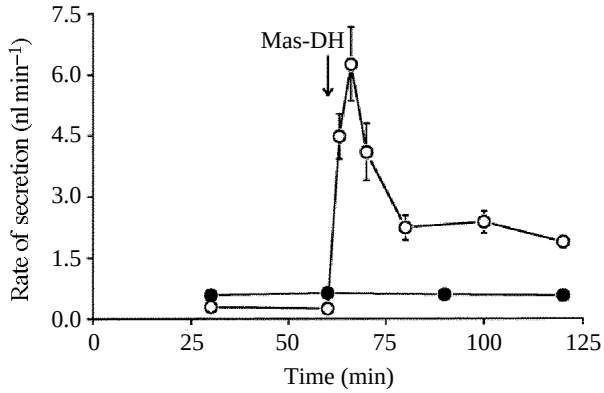

Fig. 3. Time course of fluid secretion by adult Malpighian tubules. Filled circles represent control values when only saline was present, open circles show the effects of adding 10nmol l^{-1} Mas-DH (means ± S.E.M., *N*=6–9).

Thereafter the rate fell, but 60min after addition of the peptide, tubule secretion was still about seven times the basal rate. Replacement of the bathing fluid with fresh saline at the start of the second hour (controls) had no significant effect on tubule secretion.

The effect of Mas-DH on tubule secretion is dose-dependent, as shown in Fig. 4. The response to the peptide is shown here as the difference between the basal and stimulated rates of secretion measured over periods of 60min. As little as 0.1nmol l^{-1} Mas-DH caused a significant increase in tubule secretion, and a maximal response was observed at a dose of 0.5nmol l^{-1}. The EC_{50} is 0.3±0.001nmol l^{-1}.

Effects of DH on cyclic AMP production by the Malpighian tubules and cryptonephric complex

The effects of Mas-DH on cyclic AMP production are presented in Table 4. Under control conditions (when no peptide was present) the amount of cyclic AMP produced by

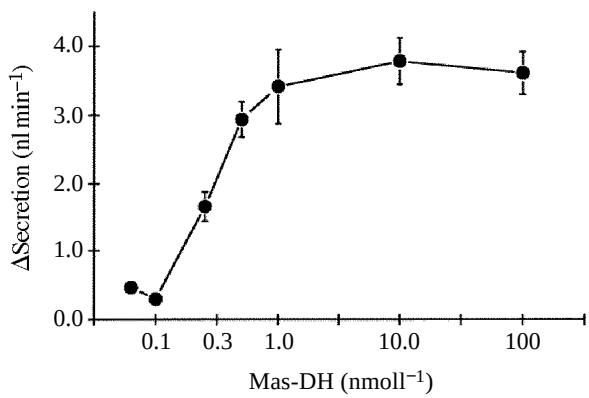

Fig. 4. Effect of increasing doses of Mas-DH on fluid secretion by isolated adult Malpighian tubules, shown as a semi-logarithmic plot (means ± S.E.M., *N*=7–9).

Table 4. *The effect of Mas-DH on cyclic AMP production by the Malpighian tubules and cryptonephric complex of* Manduca sexta

Tissue	Cyclic AMP (pmoltubule^{-1})	
	Control	10nmol l^{-1} Mas-DH
Adult tubules	1.44±0.52	17.70±5.65**
Larval proximal tubules	0.22±0.20	20.96±5.11**
Larval cryptonephric complex	9.13±0.87	18.55±1.16**

Means are significantly different from controls, $^{**}P<0.005$.
Values are mean ± S.E.M., N=5–12.

both adult and larval proximal tubules over a 1h period was less than 1.5pmoltubule^{-1}. This increased to approximately 20pmoltubule^{-1} over the next hour when Mas-DH was present.

Cyclic AMP levels secreted by the cryptonephric complex under control conditions are eight times greater than those observed for the adult and larval proximal tubules. However, there is a significant twofold increase in cyclic AMP production on the addition of 10nmol l^{-1} Mas-DH.

Discussion

In this study we demonstrate for the first time that Mas-DH acts on adult Malpighian tubules of *M. sexta* to increase their rate of fluid secretion. This peptide also causes several-fold increases in cyclic AMP production by the tubules of larvae and adults, demonstrating that this cyclic nucleotide probably acts as the second messenger for Mas-DH. Increases in both fluid secretion and levels of cyclic AMP production by the Malpighian tubules have also been reported for diuretic peptides isolated from *Acheta domesticus, Locusta migratoria* and *Periplaneta americana* acting on their source insects, and for Mas-DH acting on *A. domesticus* and *Pieris rapae* tubules (Kay *et al.* 1991*a*,*b*, 1992; Coast *et al.* 1992). In addition, Mas-DH acts on the larval cryptonephric complex of *M. sexta* to promote fluid absorption from the rectal lumen and cyclic AMP production. This is the first report of a peptide directly influencing fluid absorption across the cryptonephric complex of an insect.

Kataoka *et al.* (1989) and Troetschler and Kramer (1992) were unable to demonstrate any significant effect of Mas-DH on fluid secretion by isolated Malpighian tubules of *M. sexta*. The reason for this is unclear; however, measuring rates of fluid secretion by larval tubules also proved unreliable in this study (discussed later).

Troetschler and Kramer (1992) were also unable to show any significant increase in cyclic AMP levels in adult and pharate adult Malpighian tubules and larval recta *in vitro.* The cyclic AMP produced by these tissues (apparently whole-tissue homogenates) may have been degraded by phosphodiesterases before it could be measured, because no phosphodiesterase inhibitor (such as IBMX) was present in their incubation saline. When IBMX was absent from the saline in our study there was also no significant increase in cyclic AMP secreted by either adult or larval Malpighian tubules of *M. sexta* (N. Audsley,

unpublished observations). Additionally, Troetschler and Kramer (1992) used a locust saline for tissue incubation, which differs in ion content, osmolarity and pH from the saline used in this study. This locust saline may, therefore, have affected the physiological effects of Mas-DH on *M. sexta* tissues.

Mas-DH also acts to promote fluid absorption across the cryptonephric complex of larval *M. sexta*. The role of the cryptonephric complex in the reabsorption of water from the rectum has been studied in lepidopteran and coleopteran larvae. In *Tenebrio molitor* and *Onymacris plana*, the reabsorption of fluid from the rectal lumen is dependent on osmotic gradients created by the cryptonephric tubules, which actively accumulate KCl (and Na^+ and H^+; O'Donnell and Machin, 1991; Machin and O'Donnell, 1991). The source of these ions is the haemolymph *via* the perinephric fluid at the anterior end of the cryptonephric complex, where the low osmotic permeability of the perinephric membrane restricts the coupled movement of fluid. The rectum appears not to be an important source of ions (O'Donnell and Machin, 1991; Machin and O'Donnell, 1991). Until now there was no information available on the regulation of these processes, although from *in vivo* studies, Reynolds and Bellward (1989) showed that the rectum is the major site of fluid recycling in *M. sexta* larvae and proposed that this was subject to neural and/or hormonal control.

The present study provides the first evidence for hormonal control of the function of the cryptonephric complex. The results suggest that Mas-DH stimulates the cryptonephric tubules actively to accumulate Cl^-, Na^+ and K^+ from the haemolymph side of the tissue, not from the rectal lumen. This presumably creates an osmotic gradient between the tubules and the rectal lumen, so that fluid is withdrawn osmotically across the rectal epithelium. In support of this hypothesis, removal of any one of these ions on the haemolymph side of the tissue only, completely abolishes the absorption of fluid stimulated by Mas-DH. Likewise, cutting the perinephric membrane also abolishes the effect of Mas-DH, presumably by destroying the structural integrity of the perinephric space.

The precise transport mechanisms involved and their location in the cryptonephric complex remain to be determined by measuring electrochemical gradients using ion-sensitive microelectrodes, as described for the cryptonephric complex of *T. molitor* and *O. plana* by O'Donnell and Machin (1991) and Machin and O'Donnell (1991). However, the inhibition of Mas-DH-stimulated fluid absorption in the cryptonephric complex by the removal of Cl^-, Na^+ or K^+, and by bumetanide, would suggest that Mas-DH is acting on a $Na^+/K^+/2Cl^-$ co-transporter, presumably on the basal membrane of the Malpighian tubules. Hegarty *et al.* (1992) demonstrated a similar mechanism in the Malpighian tubules of *Aedes aegypti* where bumetanide inhibits cyclic-AMP-stimulated fluid, Na^+, K^+ and Cl^- secretion. Likewise, in *Rhodnius prolixus*, 5-hydroxytryptamine (5-HT) stimulates a similar $Cl^-/Na^+/K^+$ step on the basal membrane of the Malpighian tubules and functions *via* cyclic AMP (O'Donnell and Maddrell, 1984).

It would appear that Mas-DH has two distinct sites of action in larval *M. sexta*, producing two very different effects. It stimulates cyclic AMP production in the proximal tubule segments, which lie free in the haemolymph and are not associated with the rectum. It is assumed that this is a diuretic response, although this has not been shown

directly, because it has not been possible to develop a reliable fluid secretion assay with larval tubules. The assumption is based on the well-established role of cyclic AMP as a second messenger in the response of Malpighian tubules to diuretic peptides. For example, in Malpighian tubules from *R. prolixus*, *Aedes aegypti* and *Acheta domesticus* (Maddrell, 1980; Petzel *et al.* 1987; Kay *et al.* 1991*a*), crude extracts of neurohaemal tissue containing diuretic peptides cause an increase in intracellular cyclic AMP production which precedes or parallels the stimulation of fluid secretion. In *Aedes aegypti* and *Acheta domesticus*, a clear correlation exists between intracellular cyclic AMP levels and the rate of tubule fluid secretion. Moreover, Coast *et al.* (1992) showed that Mas-DH stimulates cyclic AMP production and fluid secretion by the Malpighian tubules of *Pieris rapae* and *Acheta domesticus*. In addition, using an *Acheta domesticus* saline as the bathing fluid, it has been possible to show that Mas-DH can cause a twofold stimulation of fluid secretion by the larval proximal tubules of *M. sexta* (results not shown). However, this proved to be an unreliable assay. Mas-DH also increases cyclic AMP production by the cryptonephric complex and stimulates fluid absorption from the rectal lumen. Even though Mas-DH appears to have a diuretic effect on the cryptonephric tubules rather than on the rectal epithelium, the overall response is antidiuretic (reabsorption of fluid from the rectal lumen). Such a combined action for a diuretic factor was suggested previously by Nicolson (1991). In the desert beetle *Onymacris rugatipennis*, a diuretic factor from the corpus cardiacum caused a pronounced stimulation of fluid secretion by the Malpighian tubules, but much of this fluid was recycled to the haemolymph *via* the midgut and there was no observable increase in fluid excretion from the insect. Nicolson proposed that in xeric insects the term 'clearance hormone' might more accurately describe the role of the so-called diuretic hormone, the function being to increase the rate of clearance of toxic wastes (such as nicotine) present in the diet from the haemolymph without incurring an increase in net loss of water. Nicotine is transported at high rates by larval tubules (Maddrell and Gardiner, 1976) and its clearance from the haemolymph will be greatly increased at high rates of fluid secretion by reducing passive diffusion back into the haemolymph (Maddrell, 1980). The term clearance hormone would seem to be an appropriate description of the conflicting effects of Mas-DH in larval *M. sexta*. Stimulation of fluid secretion by the proximal tubule segments would increase the rate of clearance of toxic wastes from the haemolymph, whilst an increase in the active accumulation of ions by the distal segments associated with the cryptonephric complex would ensure that water is recycled to the haemolymph from the rectum, *via* the perinephric space. This combined action of a diuretic peptide may be restricted to those insects in which a cryptonephric complex is present because, in locusts, a diuretic peptide that stimulates tubule secretion has no effect on ion or water transport in the hindgut (G. M. Coast, J. Meredith and J. E. Phillips, in preparation). In these insects, hindgut function is regulated by other factors; for example, ion transport peptide in the ileum (Audsley *et al.* 1992) and chloride transport stimulating hormone in the rectum (Phillips *et al.* 1982). Clearance of waste products from the locust was proposed to be due to the combined action of diuretic factors acting on the Malpighian tubules and antidiuretic factors/chloride transport stimulating hormone acting on the rectum (Proux *et al.* 1984). The presence of antidiuretic factors acting on the hindgut of either adult or larval

lepidoptera, rather than the cryptonephric tubules as proposed for Mas-DH in this study, remains to be demonstrated.

Mas-DH has a diuretic effect on Malpighian tubules from adult *M. sexta*. At a supramaximal dose ($10 nmol\,l^{-1}$), the peptide produces a 25-fold stimulation of fluid secretion, and the absolute rate may be as high as $2.5 nl\,mm^{-1}\,min^{-1}$. The Malpighian tubules of the adult are extremely long, so the rate of secretion from a single tubule could be in excess of $500 nl\,min^{-1}$. By way of comparison, the maximal rate of secretion in *Acheta domesticus* tubules is about $0.5 nl\,mm^{-1}\,min^{-1}$, equivalent to a single tubule rate of $4–5 nl\,min^{-1}$, and in *R. prolixus* the maximal rate of secretion by a single tubule is $200 nl\,min^{-1}$ (Maddrell and Gardiner, 1980). The high rate of secretion by Malpighian tubules of the adult insect may be associated with the need to secrete excess larval water rapidly shortly after emergence, although post-eclosion diuresis in *M. sexta* is not as marked as in some other lepidopterans.

In conclusion, the effects of Mas-DH on fluid movement by Malpighian tubules and cryptonephric complex are consistent with a stimulation of ion transport *via* a cyclic-AMP-dependent mechanism. For adult tubules, and probably the free (proximal) portions of the larval tubules, this translates into an increase in fluid secretion, i.e. a diuretic effect. However, because of the anatomical arrangement of the cryptonephric tubules, the effect of stimulated ion transport is to promote the reabsorption of fluid from the rectum, i.e. an antidiuretic effect.

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