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TARGET ORGAN SPECIFICITY OF MAJOR NEUROPEPTIDE STIMULANTS IN LOCUST EXCRETORY SYSTEMS

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Summary

The major stimulant of ileal fluid reabsorption in *Locusta migratoria* and *Schistocerca gregaria* corpora cardiaca, ion-transport peptide (ITP), had no stimulatory action on fluid secretion by isolated Malpighian tubules of *S. gregaria*, nor did it have a synergistic or antagonistic effect in combination with locustakinin (Lom-K) or *Locusta*-diuretic hormone (*Locusta*-DH). Stimulants of locust Malpighian tubules (Lom-K and *Locusta*-DH) had no action on either active transport of Cl^- (measured as short-circuit current, I_{sc}) or the rate of fluid reabsorption across *S. gregaria* ilea and recta *in vitro*. Thus, hormonal control of these major organs of the excretory system appears to be clearly separated.

Lom-K and *Locusta*-DH acted synergistically to stimulate secretion by *S. gregaria* Malpighian tubules, and the diuretic response was more rapid than the response of the ileum and rectum to hindgut stimulants. Taken together, these data suggest that, in the initial phase of post-prandial diuresis, urine flow will exceed fluid uptake in the hindgut, thereby allowing excess water to be eliminated.

Key words: *Locusta*-DH, locustakinin, ion-transport peptide, locust, *Schistocerca gregaria*, excretion, Malpighian tubule, ileum, rectum, bioassay, diuretic hormone.

Introduction

The excretory process in insects such as locusts involves secretion by the Malpighian tubules of a KCl-rich primary urine, containing most small solutes found in the haemolymph, followed by selective reabsorption of essential substances in the hindgut as required for homeostasis. Secretion by Malpighian tubules is driven by a proton gradient (maintained by an apical V-type ATPase secreting protons into the lumen), which in turn drives a luminal K^+/H^+ antiporter (Zhang et al., 1994). Cl^- follows passively, moving *via* a paracellular or transcellular shunt and a basal $Na^+/K^+/Cl^-$ cotransporter (Hegarty et al., 1991; Wang et al., 1996; O'Donnell et al., 1998). Fluid reabsorption in the anterior (ileum) and posterior (rectum) hindgut is largely driven by an apical electrogenic Cl^- pump and is associated with passive movement of K^+ as the major cation. Most of the primary urine is recovered in the hindgut, particularly under dehydrating conditions, and this recycling of fluid concentrates substances destined for excretion.

Rapid progress has been made in isolating and sequencing the neuropeptides that control secretion by Malpighian tubules in a variety of insects (for a review, see Coast, 1996). These include corticotropin-releasing factor (CRF)-related diuretic peptides and the insect kinin family of neuropeptides (Coast, 1995). CRF-related peptides are C-terminally amidated peptides 30–47 amino acid residues in length that act through cyclic AMP to stimulate the $Na^+/K^+/Cl^-$ cotransporter (Audsley et al., 1993) and to open basal Na^+ channels (Clark et al., 1998). In contrast, kinins are small (6–15 amino acid residues) amidated peptides that are believed to stimulate Cl^- secretion by opening a cellular or paracellular shunt (Pannabecker et al., 1993; O'Donnell et al., 1998) to produce an increase in the rate of Na^+/KCl transport. These two families of diuretic peptides act synergistically in controlling primary urine production, permitting maximal stimulation at lower concentrations of hormone than would otherwise be required (Coast, 1995).

The situation is less clear for neuropeptide stimulants of hindgut reabsorption (for a review, see Phillips et al., 1998). However, the major stimulant of ileal reabsorption from the brain and nervous corpus cardiacum (NCC), ion-transport peptide (ITP; a peptide 72 amino acid residues in length with an amidated C terminus) has been isolated, sequenced, synthesised and its biological actions on hindgut transport processes elucidated (Audsley et al., 1992a; Meredith et al., 1996; King et al., 1999). The ileum is functionally analogous to the proximal convoluted tubule of vertebrate kidneys: ITP stimulates iso-osmotic fluid reabsorption by fourfold in this locust segment by stimulating electrogenic Cl^- transport and increasing K^+ conductance (Audsley et al., 1992a). ITP also causes a submaximal stimulation (40 %) of rectal Cl^- transport.

In the present paper, we show that *Locusta migratoria* diuretic factors are equally effective on *Schistocerca gregaria* isolated Malpighian tubules. The deduced amino acid sequences of ITP from *Schistocerca gregaria* and *Locusta migratoria* cDNAs are identical, as are reciprocal bioassay results using isolated preparations of ilea and recta, and corpora cardiaca (CC) homogenates of these two species (Macins et al., 1999). Moreover, ileal short-circuit current (I_{sc}) bioassays and western blots probed with ITP antibodies on five genera of locusts and grasshoppers also suggest little, if any, differences between ITP structure or ileal transport physiology among members of this group (Macins et al., 1999).

To determine how the control of the secretory and reabsorptive functions of the insect excretory system might be integrated, we first considered whether the major stimulant of ileal reabsorption in locusts, ITP, has any effect on isolated Malpighian tubules either alone or in combination with diuretic factors. Conversely, do the stimulants of locust Malpighian tubules, i.e. *Locusta*-DH and Lom-K, have any action on ion and water reabsorption in the hindgut bioassays? We have also examined the time course of the biological responses to these peptides as this might also be of importance in the overall control of the insect excretory system. In this paper, we show that, at least *in vitro*, these stimulants of secretion and reabsorption in the locust excretory system are organ-specific and do not interact. Stimulation of tubule secretion appears to precede stimulation of fluid uptake in the hindgut, which could be important for the elimination of excess water after feeding.

Materials and methods

Animals

Adult 5- to 8-day-old male locusts (*Schistocerca gregaria*) used in Malpighian tubule experiments were purchased from Blades Biological (Edenbridge, Kent, UK). They were fed freshly germinated wheat supplemented with bran, and water was provided *ad libitum*. Locusts used in the hindgut assays were raised as previously described (Audsley et al., 1992a,b).

Diuretic assay

Synthetic *S. gregaria* ITP (Scg-ITP), locustakinin (Lom-K) and *Locusta*-diuretic hormone (*Locusta*-DH) were tested for diuretic activity using Malpighian tubules from the desert locust. This bioassay has been described in detail for the migratory locust (*Locusta migratoria*; Coast et al., 1993), and the same procedures were used for *S. gregaria*. Briefly, anteriorly directed tubules were cut at their junction with the midgut and at the level of the midgut caeca. Tubule segments (equivalent to approximately 80 % of the entire length) were transferred individually to small (5 µl) drops of saline beneath water-saturated liquid paraffin, and both their proximal and distal ends were clamped into strips of Sylgard. Unless stated otherwise, the bathing fluid used was based upon locust hindgut saline (Hanrahan et al., 1984) and had the following composition (in mmol l^{-1}): NaCl, 100.0; $NaHCO_3$, 4.0; K_2SO_4, 5.0; $MgSO_4$, 10.0; $CaCl_2$, 5.0; alanine, 2.9; arginine, 1.0; asparagine, 1.3; glutamine, 5.0; glycine, 11.4; histidine, 1.4; lysine, 1.4; proline, 13.1; serine, 1.5; tyrosine, 1.0; valine, 1.8; glucose, 10.0; Hepes, 25.0; pH adjusted to 7.0 with NaOH. Urine escaped from a small cut made close to the proximal end of the tubule, and samples were collected at 20–30 min intervals following a 20 min equilibration period. Diuretic activity was calculated as the difference (Δ; nl min^{-1}) between the rates of secretion measured before and after peptide addition, with each tubule serving as its own control. To reduce the variability between assays, peptides were normally tested alongside tubules stimulated maximally with 100 nmol l^{-1} *Locusta*-DH, and results are expressed as a percentage of the maximal rate of secretion. Experiments were performed at room temperature (22±3 °C).

Hindgut short-circuit current bioassay

Locust ilea and recta were mounted as flat sheets in modified Ussing chambers containing 2 ml of physiological saline bubbled with 95 %O_2/5 %CO_2 at 23±2 °C, as previously described (Hanrahan et al., 1984; Irvine et al., 1988). The electrical current (short-circuit current, I_{sc}) required to keep the transepithelial electropotential difference (V_t) clamped at 0 mV was continuously monitored. Changes in short-circuit current (ΔI_{sc}) caused by stimulants have previously been shown to be a direct measure of active electrogenic transport of Cl^- from the lumen side, the major mechanism driving passive K^+ absorption and secondary fluid transport. The I_{sc} was briefly stopped periodically to measure open-circuit V_t. Transepithelial resistance (R_t; i.e. ion permeability, mostly due to K^+) was calculated using Ohm's law from I_{sc} and V_t as previously described (Hanrahan et al., 1984).

Hindgut fluid transport rate bioassay

Cannulated everted rectal and ileal sacs bathed bilaterally in locust physiological saline and bubbled with 95 % O_2/5 % CO_2 at 23±2 °C were used to measure the hourly rate of fluid absorption (to the haemolymph side) in the absence of an initial osmotic concentration difference, as described by Lechleitner and Phillips (1989). The second hour was considered a control period, and potential stimulants were then tested during the third hour by addition to the haemolymph side (inside the sac). To confirm the viability of preparations at the end of the experiments, corpus cardiacum homogenate (1 gland-equivalent) containing hindgut stimulants (e.g. ITP, ion transport peptide; CTSH, chloride transport stimulating hormone) was added during the fourth or fifth hour. Changes in tissue volume were also followed, as previously described (Lechleitner and Phillips, 1989).

Chemicals

Synthetic ITP, synthesised as previously described (King et al., 1999), was dissolved in phosphate buffer (117 µmol l^{-1}) and shipped on dry-ice from Vancouver to London and stored at −20 °C. This stock solution was diluted ⩾1000-fold for

Malpighian tubule bioassays. Preliminary experiments showed that a 1:1000 dilution of phosphate buffer in locust tubule saline had no effect on tubule secretion or on the diuretic activities of *Locusta*-DH and Lom-K (results not shown). The diuretic peptides were prepared as described previously (Coast, 1998a), and stock solutions were made up in 60 % acetonitrile. Working concentrations were prepared by direct dilution (⩾100-fold) into locust tubule saline, thereby avoiding 'dilution artefacts' that may arise from the adsorption of peptides onto the walls of microcentrifuge tubes. The final concentration of acetonitrile was no more than 0.6 %, which had no effect on tubule secretion (results not shown).

Statistical analyses

Results are presented as the means ±1 S.E.M., with the number of determinations (*N*) shown in parentheses. Tests for significance were made using the computer program Instat 3.01 (GraphPad Software Inc., San Diego, CA, USA), with $P<0.05$ being accepted as significant. Dose–response curves were fitted to a generalised four-parameter logistic equation using the computer program FigP (Biosoft, Cambridge, UK). Significance of differences for hindgut studies was determined by Student's *t*-test, with $P<0.05$ being considered significant.

Results

Locustakinin and Locusta*-DH are potent stimulants of primary urine production by* S. gregaria *tubules*

Lom-K and *Locusta*-DH are potent stimulants of fluid secretion by *L. migratoria* tubules, the maximum response to Lom-K being approximately 75 % of that obtained with *Locusta*-DH. Both peptides were tested on *S. gregaria* tubules at concentrations ranging from 1 pmol l^{-1} to 100 nmol l^{-1}. The results are shown in Fig. 1A,B, in which diuretic activities are expressed as a percentage of the response to 100 nmol l^{-1} *Locusta*-DH. At 100 nmol l^{-1}, *Locusta*-DH increased tubule secretion more than threefold. The concentration required for half-maximal stimulation (EC_{50}) was 2.1 nmol l^{-1} (95 % confidence limits 1.1–4.2 nmol l^{-1}), which does not differ significantly (unpaired *t*-test: $P=0.7059$; d.f.=8) from that obtained previously with *L. migratoria* tubules (1.9 nmol l^{-1}; Coast, 1995). Lom-K was also active on *S. gregaria* tubules: at 100 nmol l^{-1}, the rate of fluid secretion was increased 2.5-fold. The EC_{50} (0.22 nmol l^{-1}; 95 % confidence limits 0.15–0.31 nmol l^{-1}) does not differ significantly (unpaired *t*-test: $P=0.9239$; d.f.=8) from that obtained with *L. migratoria* tubules (0.31 nmol l^{-1}; Coast, 1995).

In the migratory locust, locustakinin and *Locusta*-DH act synergistically in stimulating primary urine production (Coast, 1995). This was tested in *S. gregaria* Malpighian tubules using threshold concentrations of the two diuretics added separately or together. As shown in Fig. 2, the sum of the separate activities of 0.1 nmol l^{-1} Lom-K and 1 nmol l^{-1} *Locusta*-DH is less than 30 % of the response obtained when the peptides are used in combination.

Scg-ITP has no effect on primary urine production

Scg-ITP was tested for diuretic activity at 11.7 and 117 nmol l^{-1} alongside tubules stimulated with 10 or 100 nmol l^{-1} *Locusta*-DH and Lom-K. The results are presented in Table 1. At neither concentration did ITP have any significant effect on fluid secretion compared with the controls (saline addition).

The bathing fluid used in the diuretic assay is hypo-osmotic to locust hindgut saline (Hanrahan et al., 1984), which contains 100 mmol l^{-1} sucrose and is buffered with bicarbonate/CO_2

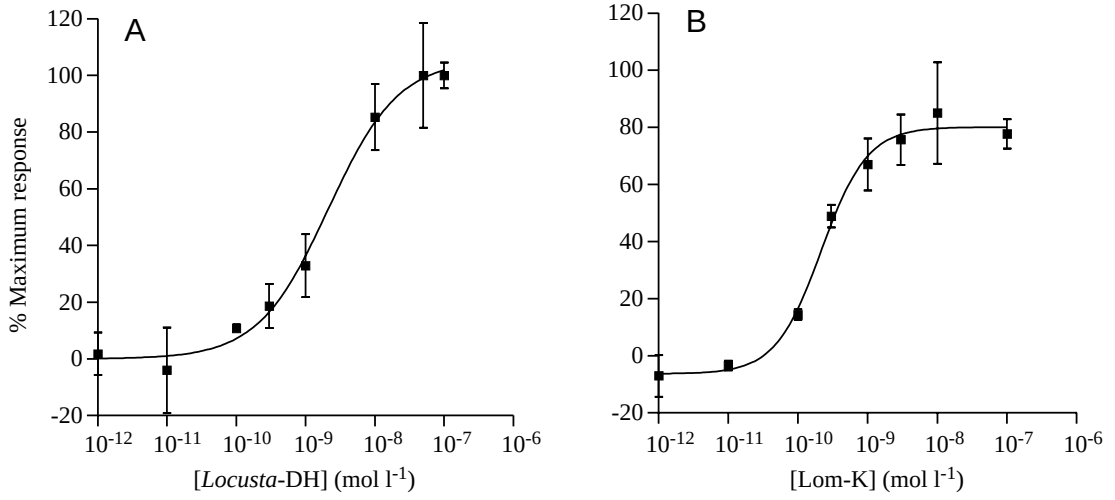


Fig. 1. Locustakinin and *Locusta*-DH are potent and efficacious stimulants of *Schistocerca gregaria* Malpighian tubules. Dose–response curves for the stimulation of fluid secretion by (A) *Locusta migratoria* diuretic hormone (*Locusta*-DH) and (B) locustakinin (Lom-K). Results are expressed as a percentage of the response of tubules stimulated maximally with 100 nmol l^{-1} *Locusta*-DH. Data points are the means and vertical lines ±1 S.E.M. for 5–7 replicates. Activities and potencies are very similar to those obtained in conspecific assays with *Locusta migratoria* tubules.

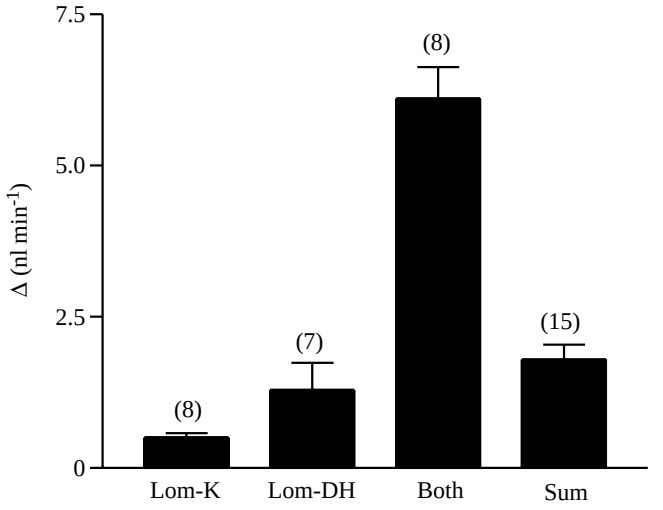


Fig. 2. Locustakinin (Lom-K) and *Locusta migratoria* diuretic hormone (Lom-DH) act synergistically to stimulate fluid secretion in *Schistocerca gregaria* tubules. Following the combined addition (Both) of 0.1 nmol l^{-1} Lom-K and 1 nmol l^{-1} Lom-DH, the increase in the rate of fluid secretion (Δ; nl min^{-1}) is more than three times greater than the sum (Sum) of their individual activities. Bars show the means and vertical lines +1 S.E.M. for the number of determinations shown in parentheses.

rather than Hepes. Scg-ITP (117 nmol l^{-1}) was therefore tested for diuretic activity in locust hindgut saline aerated for 30 min prior to the experiment with 95 % O_2/5 % CO_2. Not surprisingly in view of its higher osmolarity, tubules secrete more slowly in hindgut saline (0.43±0.04 nl min^{-1}, N=29) compared with the saline used in the diuretic assay (1.86±0.06 nl min^{-1}, N=272). Nevertheless, both *Locusta*-DH (100 nmol l^{-1}) and Lom-K (100 nmol l^{-1}) significantly increased the rate of fluid secretion compared with controls (saline addition), whereas Scg-ITP had no effect (Fig. 3).

There remained the possibility that Scg-ITP might have an antidiuretic effect on Malpighian tubules, reducing the activity of Lom-K and *Locusta*-DH. To examine this possibility, tubules were stimulated with submaximal concentrations of Lom-K (0.25 nmol l^{-1}) or *Locusta*-DH (2.5 nmol l^{-1}) with or without the addition of 117 nmol l^{-1} Scg-ITP. Lom-K alone increased the rate of fluid secretion by 0.80±0.21 nl min^{-1} (N=5), which is not significantly different (two-tailed t-test; P=0.785) from the response obtained when the peptide was added together with Scg-ITP (change 0.87±0.15 nl min^{-1}; N=7). Likewise, the response to *Locusta*-DH alone (change 0.97±0.09 nl min^{-1}; N=7) does not differ significantly (two-tailed t-test; P=0.952) from that obtained in combination with Scg-ITP (change 0.96±0.14 nl min^{-1}; N=6).

*Malpighian tubule stimulants (*Locusta*-DH and Lom-K) have no effect on either ileal or rectal absorption*

Doses of *Locusta*-DH and Lom-K (100 nmol l^{-1}) sufficient to stimulate Malpighian tubules fully had no significant effect on I_{sc} (i.e. Cl^- transport rate), R_t (an indicator of K^+ permeability) or V_t in either *S. gregaria* ileum or rectum (Figs 4, 5). Test preparations all responded at the end of the experiment to addition of 5 gland-equivalents of CC homogenate containing ITP/CTSH or to 5 mmol l^{-1} cyclic AMP with large increases in I_{sc} and V_t and with reductions in R_t (ilea only shown), which were quantitatively similar to those previously observed by several authors (for reviews, see Phillips et al., 1986, 1998).

Using a second bioassay, *Locusta*-DH and Lom-K at 100-fold higher dosages (10 μmol l^{-1}) had no effect on the rate of fluid transport across *S. gregaria* ileal sacs (Fig. 6A). All preparations subsequently responded to the addition of 1 gland-equivalent of CC homogenate with a four- to fivefold increase in the rate of fluid transport. No significant changes in tissue volume were observed under any treatment (data not shown). The results were essentially similar for *S. gregaria* rectal sacs

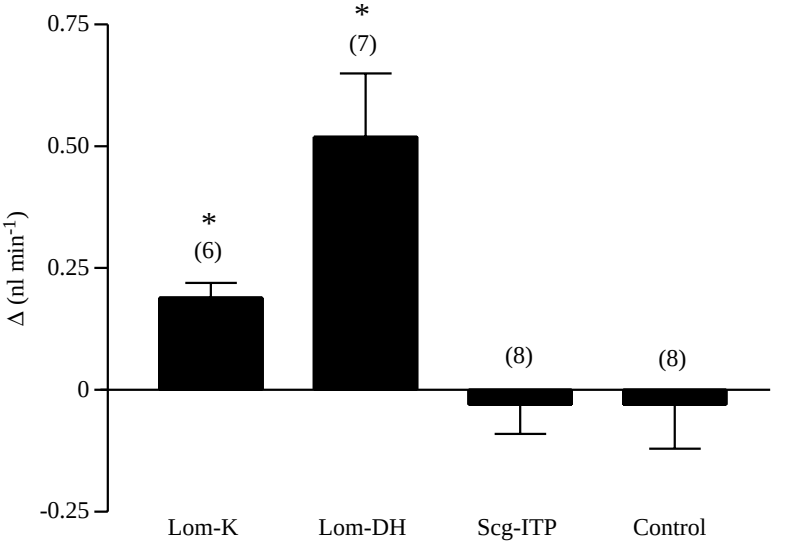


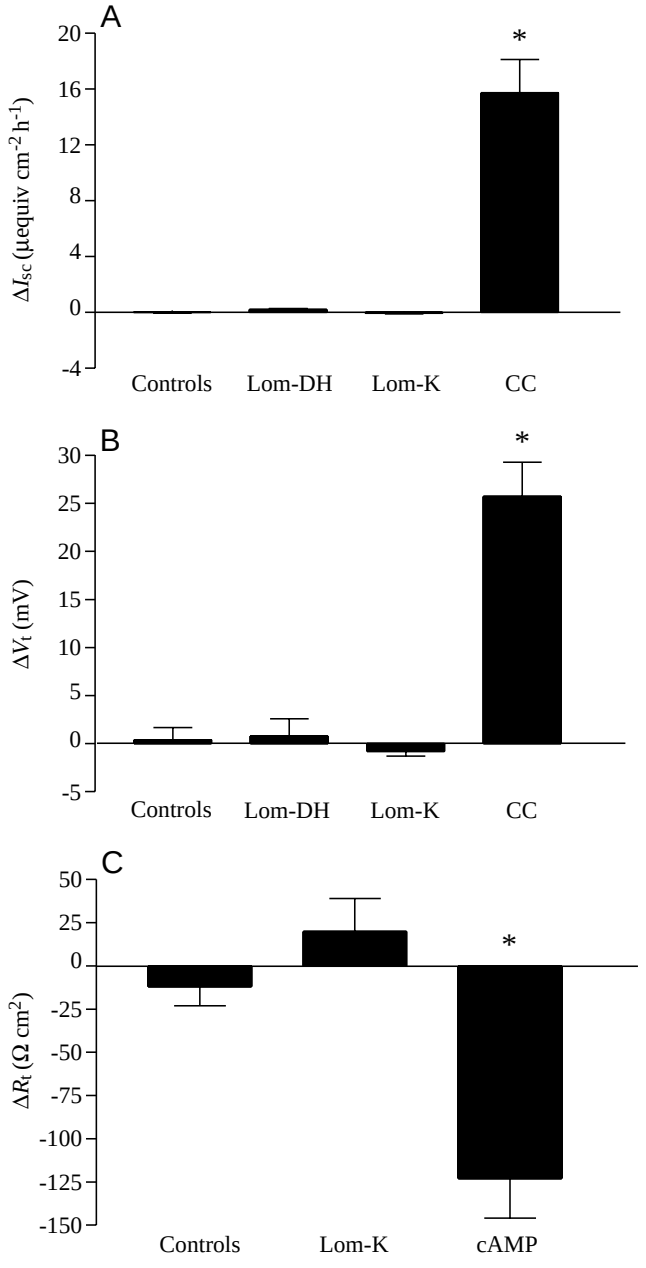
Fig. 3. Diuretic activity of *Locusta migratoria* diuretic hormone (Lom-DH; 100 nmol l^{-1}), locustakinin (Lom-K) (100 nmol l^{-1}) and *Schistocerca gregaria* ion-transport peptide (Scg-ITP) (117 nmol l^{-1}) measured in hindgut saline aerated with 95 % O_2/5 % CO_2 for 30 min. Results are shown as the change in rate of secretion (Δ; nl min^{-1}) following peptide addition. Bars show the means and vertical lines +1 S.E.M. for the number of determinations shown in parentheses; asterisks indicate significant difference (P<0.05) from controls (saline addition).

Table 1. *The effect of* Locusta-*DH, Lom-K and Scg-ITP on the rate of fluid secretion by* Schistocerca gregaria *tubules*

Peptide	Peptide concentration (nmol l^{-1})	Rate of fluid secretion (nl min^{-1})		
		Basal	Stimulated	Change
Locusta-DH	10	2.25±0.31 (5)	4.15±0.65 (5)	1.90±0.40*
Locusta-DH	100	1.34±0.19 (7)	4.41±0.34 (7)	3.07±0.46*
Lom-K	10	1.42±0.08 (5)	3.33±0.31 (5)	1.91±0.26*
Lom-K	100	1.50±0.16 (7)	3.80±0.17 (7)	2.29±0.24*
Scg-ITP	11.7	2.38±0.22 (6)	1.79±0.21 (6)	−0.59±0.08
Scg-ITP	117	1.72±0.14 (8)	1.18±0.16 (8)	−0.54±0.12
Control		2.11±0.12 (9)	1.53±0.10 (9)	−0.58±0.14

Results are given as the mean ±1 S.E.M. for the number of determinations shown in parentheses; asterisks indicate a significant difference (P<0.05) from the control value (saline addition).

Locusta-DH, *Locusta migratoria* diuretic hormone; Lom-K, locustakinin; Scg-ITP, synthetic *Schistocerca gregaria* ion-transport peptide.



(Fig. 6B,C), but the response of the sacs to CC homogenate at the end of the experiments was small. Previous reports are for an increase in the rate of fluid transport of 50–100 % in response to CC.

Time course of the response to Malpighian tubule and hindgut stimulants

Faecal water loss is determined by the difference between rates of primary urine production and fluid reabsorption in the hindgut and could depend in part on the time course of the biological response of these target structures to diuretic and antidiuretic peptides, respectively. The responses of Malpighian tubules to the addition of Lom-K (10 nmol l^{-1}) or *Locusta*-DH (50 nmol l^{-1}) are shown in Fig. 7A. Diuresis begins within 2–3 min (the shortest interval over which urine production can be measured) and is maximal within 5 min. The response is equally as rapid when the two diuretics are added together, but at much lower concentrations (0.1 nmol l^{-1} Lom-K and 1 nmol l^{-1} *Locusta*-DH), with maximal urine production occurring within 5 min of peptide addition (Fig. 7B).

Figs 8 and 9 show the time course of stimulation in isolated ilea and recta respectively. In both tissues, the electrical parameters (I_{sc}, Figs 8A, 9A; V_t, Figs 8B, 9B; R_t, Figs 8C, 9C) change within 6 min of the addition of corpora cardiaca homogenate (maximal dose) and the changes reach maximal values within 12 min. These changes are sustained for over 2 h despite the removal of stimulant after 1 h. The time courses of increases in fluid transport rates were similar to

Fig. 4. Effects of *Locusta migratoria* diuretic hormone (Lom-DH; 100 nmol l^{-1}) and locustakinin (Lom-K) (100 nmol l^{-1}) on electrical parameters (short-circuit current, I_{sc}; transepithelial electropotential difference, V_t; transepithelial resistance, R_t) reflecting ion absorption across *Schistocerca gregaria* isolated ilea. The responsiveness of hindgut preparations was tested at the end of all experiments by adding corpora cardiaca homogenate (CC; 5 gland-equivalents or 5 mmol l^{-1} cAMP). Data points show the means and vertical lines ±1 S.E.M. (N=3–8); an asterisk indicates a significant difference (P<0.05) from other treatments.

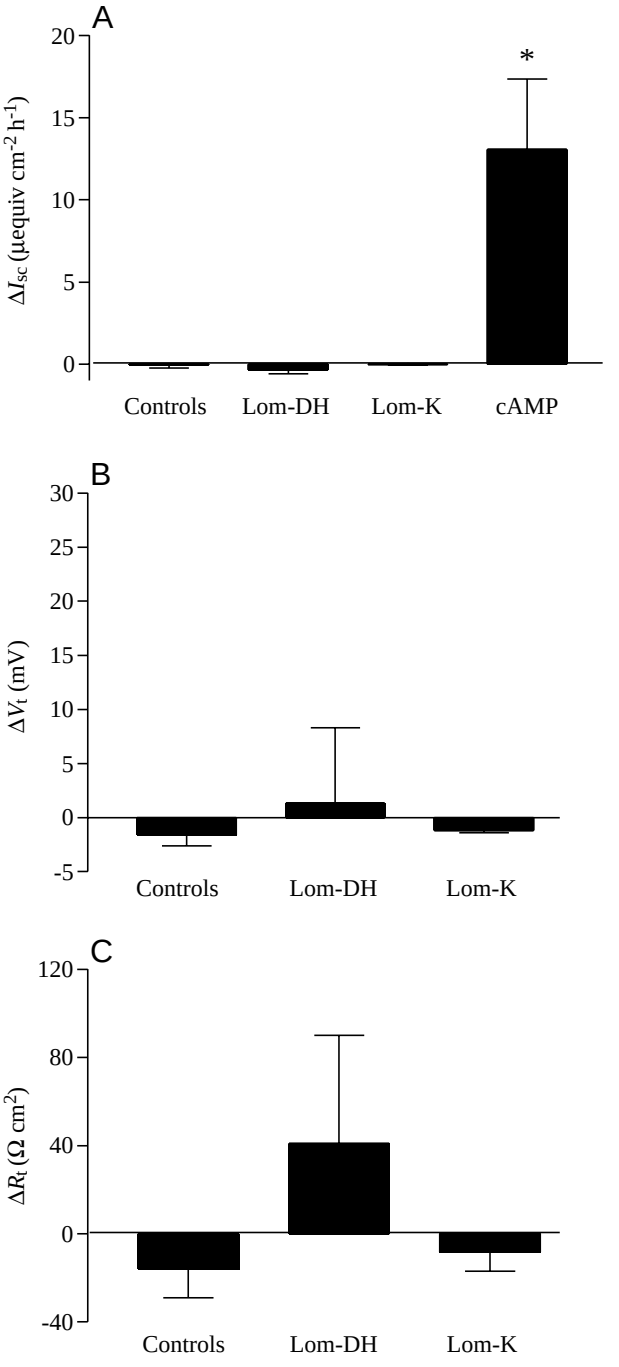


Fig. 5. Effects of *Locusta migratoria* diuretic hormone (Lom-DH; 100 nmol l^{-1}) and locustakinin (Lom-K) (100 nmol l^{-1}) on electrical parameters (short-circuit current, I_{sc}; transepithelial electropotential difference, V_t; transepithelial resistance, R_t) reflecting ion absorption across *Schistocerca gregaria* isolated recta. The responsiveness of hindgut preparations was tested at the end by adding 5 mmol l^{-1} cyclic AMP. Data points show the means and vertical lines ±1 S.E.M. (N=3–6); an asterisk indicates a significant difference (P<0.05) from other treatments.

those of changes in electrical variables, although these changes could only be measured at 30 min (recta) or 60 min (ilea) intervals.

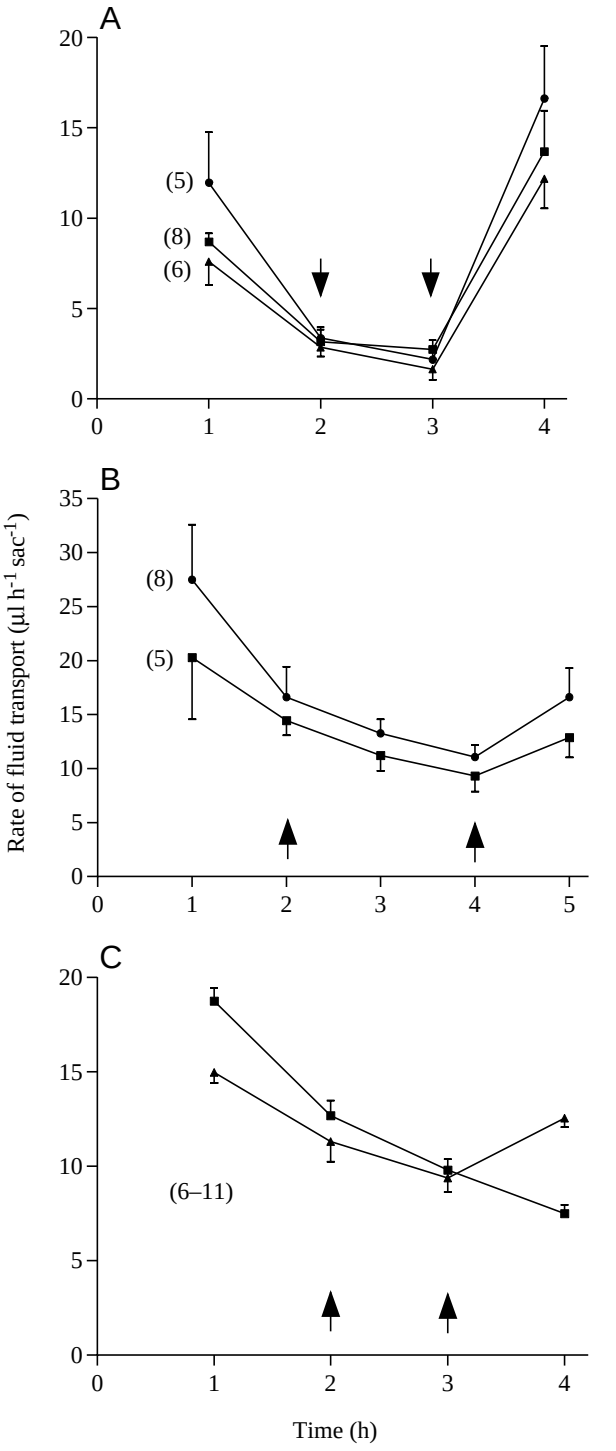


Fig. 6. Effect of *Locusta migratoria* diuretic hormone (*Locusta*-DH) (filled squares; 10 μmol l^{-1}) and locustakinin (Lom-K) (filled triangles; 10 μmol l^{-1}) on the net rate of fluid transport measured hourly across everted ileal (A) and rectal (B,C) sacs of *Schistocerca gregaria*. After a control period (no treatment), neuropeptides were added (first arrow) for 1 or 2 h. Control preparations (filled circles) were not exposed to neuropeptides during this time. Finally, corpus cardiacum homogenate (1 gland-equivalent) was added in the last hour (second arrow) to test the responsiveness of all hindgut preparations. Bars show the means and vertical lines ±1 S.E.M. for the number of determinations shown in parentheses.

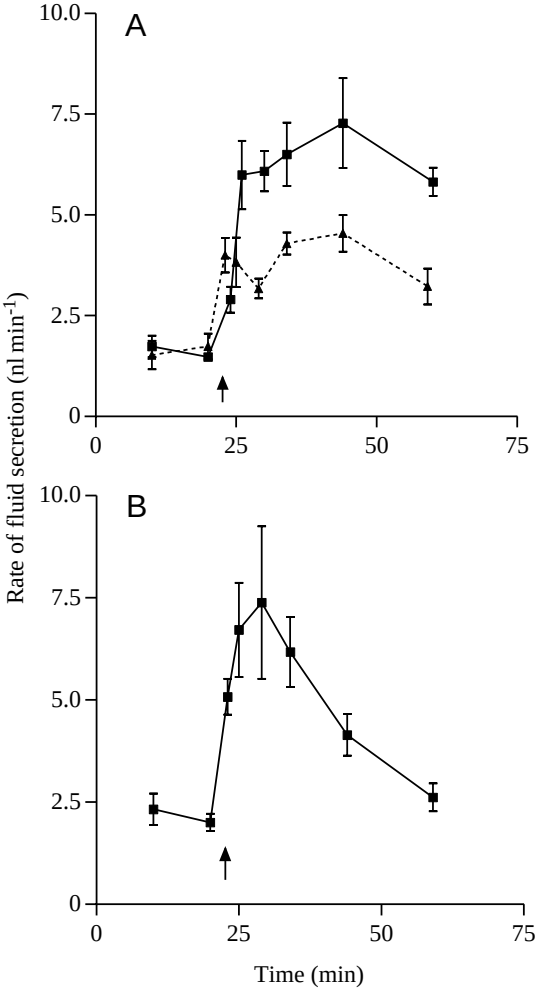


Fig. 7. Time course for the stimulation of tubule secretion by (A) 10 nmol l^{-1} locustakinin (Lom-K) (triangles; dashed line) and 50 nmol l^{-1} *Locusta migratoria* diuretic hormone (*Locusta*-DH) (squares; solid line), and (B) 0.1 nmol l^{-1} Lom-K and 1 nmol l^{-1} *Locusta*-DH added together. Data points are the means and vertical lines ±1 S.E.M. (*N*=5–6). Vertical arrows indicate the time of addition of diuretic peptides. The rate of fluid secretion increases rapidly following peptide addition and is maximal within 5 min.

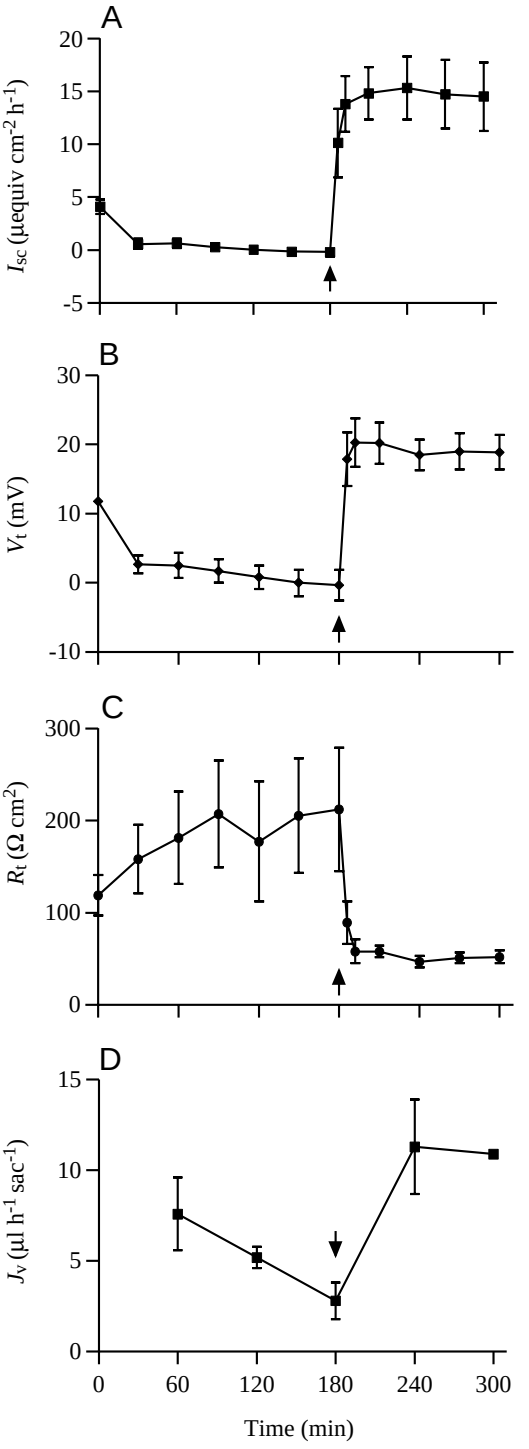


Fig. 8. A comparison of the time courses of changes in short-circuit current I_{sc} (A), transepithelial electropotential difference V_t (B), transepithelial resistance R_t (C) and fluid secretion rates (J_v; D) in isolated ilea stimulated (arrows) with a maximal dose of corpora cardiaca homogenate (5 gland-equivalents) between 3 h and 4 h. Data points show the means and vertical lines ±1 S.E.M. (*N*=3–5).

Discussion

Diuretic factors have been purified from Lepidoptera, Dictyoptera, Diptera, Orthoptera and Coleoptera (for a review, see Coast, 1998b). To date, there is evidence of ITP-like antidiuretic peptides in Orthoptera, Dictyoptera (Macins et al., 1999) and Coleoptera (J. Meredith and J. E. Phillips, unpublished observations). In locusts, the availability of pure neuropeptides that stimulate fluid secretion by Malpighian tubules (*Locusta*-DH and Lom-K) and ileal fluid reabsorption (Scg-ITP) and of well-characterised *in vitro* bioassays have permitted us to test organ specificity in the control of the overall insect excretory process. While different locust species were originally used in the purification of these neuropeptides, *Locusta migratoria* diuretic peptides are equally effective on isolated Malpighian tubules from *Schistocerca gregaria*. Likewise, the hindgut stimulant ITP has the same deduced amino acid sequence in *L. migratoria* and *S. gregaria*, and reciprocal bioassays of hindgut I_{sc} (a

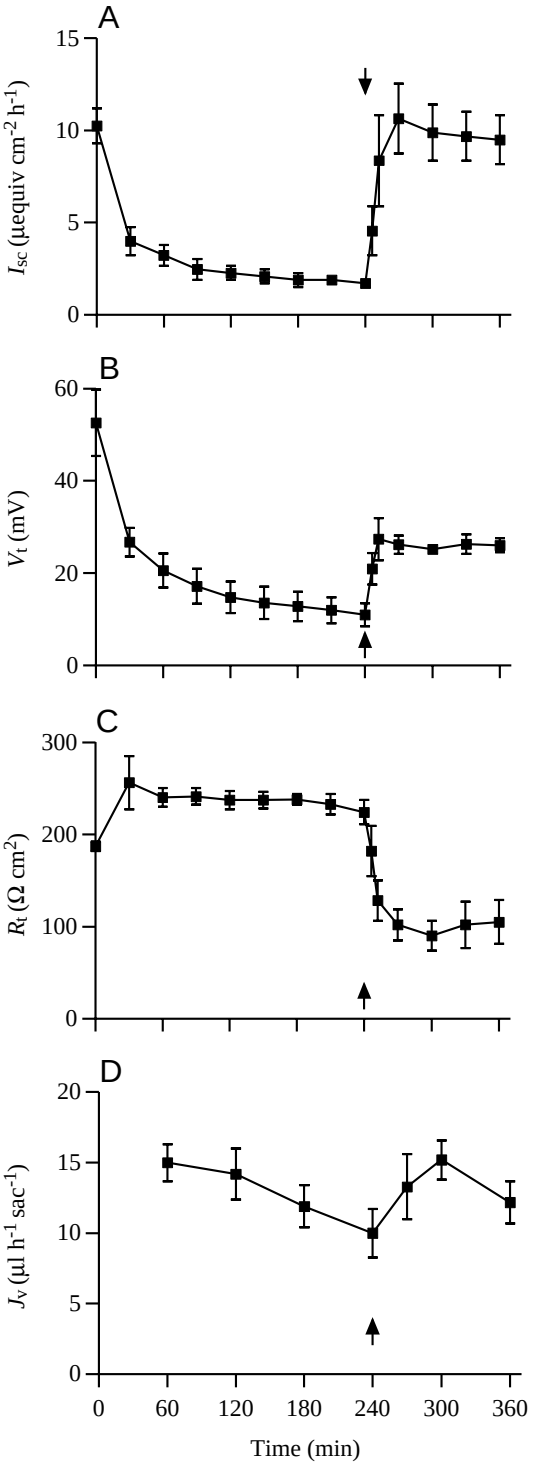


Fig. 9. A comparison of the time courses of changes in short-circuit current I_{sc} (A), transepithelial electropotential difference V_t (B), transepithelial resistance R_t (C) and fluid secretion rates (J_v; D) in isolated recta stimulated (arrows) with a maximal dose of corpora cardiaca homogenate (5 gland-equivalents) between 4 h and 5 h. Data points show the means and vertical lines ±1 S.E.M. (N=3–4).

measure of the rate of Cl^- transport) are quantitatively indistinguishable (Macins et al., 1999).

Both diuretic (*Locusta*-DH and Lom-K) and antidiuretic (ITP) neuropeptides give half-maximal stimulation at approximately 2 nmol l^{-1} or less in their respective bioassays. Even at much higher doses, ITP has no stimulatory or inhibitory action on fluid secretion by isolated Malpighian tubules of *Schistocerca gregaria*. Neither ileal nor rectal Cl^- (I_{sc}) or fluid transport rate was stimulated by *Locusta*-DH or Lom-K. Because of a limited supply of synthetic ITP, we did not test whether *Locusta*-DH or Lom-K might inhibit ITP stimulation of hindgut fluid transport.

It has been proposed that fluid recycling through the insect excretory system increases several-fold after feeding (Phillips and Audsley, 1995), thus increasing toxic waste elimination. In the present study, we provide evidence indicating that the secretory and reabsorptive phases of this recycling are controlled separately. To evaluate further the cooperative role of *Locusta*-DH, Lom-K and Scg-ITP in the control of fluid excretion, the time course of haemolymph titres of these neuropeptides must be determined. However, we can speculate on the likely sequence of events on the basis of the time course for the biological responses to Malpighian tubule and hindgut stimulants. In the migratory locust, circulating levels of *Locusta*-DH increase within minutes of feeding (Audsley et al., 1997). If diuretic and antidiuretic peptides were released more or less simultaneously, the rapid response of Malpighian tubules to diuretic peptides (maximum urine flow within 5 min) would mean that the rate of urine production would exceed the rate of fluid reabsorption from the hindgut, which takes approximately 12 min to reach maximum values. Synergism between Lom-K and *Locusta*-DH will further ensure an early onset to diuresis, because it requires only small amounts of these peptides to be released into the circulation for urine production to be stimulated maximally. In this initial phase of post-prandial diuresis, urine flow is therefore expected to exceed fluid uptake from the hindgut, allowing excess water to be eliminated. This period of diuresis will be prolonged if the release of antidiuretic peptides (Scg-ITP and CTSH) occurs some time after the release of diuretic peptides. Later, under the influence of antidiuretic factors, a sustained increase in Cl^- transport (and hence fluid uptake) in the ileum and rectum will mean that much of the fluid entering the hindgut is returned to the haemolymph. During this phase, accelerated rates of tubule secretion and hindgut reabsorption allow for the rapid clearance of toxic wastes without an unacceptable loss of water. Finally, with removal of the stimulus for hormone release, diuretic and antidiuretic peptides will be degraded or otherwise removed from the circulation, and urine flow will fall below the rate of fluid uptake in the hindgut, thereby reducing faecal water loss to a minimum.

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