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ACTIONS OF ION-TRANSPORT PEPTIDE FROM LOCUST CORPUS CARDIACUM ON SEVERAL HINDGUT TRANSPORT PROCESSES

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Summary

1. *Schistocerca gregaria* ion-transport peptide (Scg-ITP), a neuropeptide isolated from locust corpora cardiaca, stimulates ileal Cl^- transport (I_{sc}) in a dose-dependent manner and causes increases in Na^+, K^+ (I_K) and fluid reabsorption (J_v) as previously observed with crude extracts of corpus cardiacum and with cyclic AMP. Unlike cyclic AMP, Scg-ITP does not stimulate ileal NH_4^+ secretion.

2. H^+ secretion (J_H) in the ileum, which is not affected by cyclic AMP, is almost completely abolished by Scg-ITP. Although ITP may act *via* cyclic AMP as second messenger to stimulate NaCl, KCl and fluid reabsorption, it apparently acts through a different intracellular pathway to influence J_H.

3. Scg-ITP is unlikely to be the chloride transport stimulating hormone previously reported to act on the rectum, because it did not produce a maximum rectal I_{sc} response and had no effect on either rectal J_v (which is Cl^--dependent) or I_K.

Introduction

In a companion paper, the purification of ion-transport peptide (ITP) from the locust corpus cardiacum (CC) is reported. ITP is the major stimulant in the CC that acts on the dominant transport process in locust ileum, namely electrogenic Cl^- absorption measured using short-circuit current (I_{sc}). ITP was partially sequenced (31 of an estimated 65 residues identified) and was found to belong to a family of crustacean neuropeptides of which hyperglycaemic hormone was the first to be characterized (Kegel *et al.* 1989). In this paper the various actions of ITP on locust hindgut epithelia are reported; therefore, a review of their transport processes is in order.

Using *in vitro*, voltage-clamped preparations of locust hindgut described by Hanrahan *et al.* (1984), the ion-transport processes across the locust rectum have been characterized and localized, leading to a detailed epithelial model (reviewed by Phillips *et al.* 1986,

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1988). Those in the ileum, while less extensively studied, have been found to be similar in properties to ion-transport processes in the rectum (Irvine *et al.* 1988; Phillips *et al.* 1988; Thomson *et al.* 1991). Studies indicated an electrogenic Cl^- pump in the apical membrane, a Na^+/K^+-ATPase pump in the basolateral membrane, with both apical and basal K^+ and Cl^- conductances. More recently, Thomson *et al.* (1988*a*,*b*) described apical secretory mechanisms for both H^+ and NH_4^+ in the rectum and similar processes have since been observed in the ileum (Audsley, 1991; Thomson *et al.* 1991).

Cyclic AMP was found to stimulate KCl absorption in both the ileum and rectum, and Na^+ transport in the ileum only. This second messenger also inhibits active acid secretion in the rectum, whereas it stimulates total ammonia secretion in the ileum but not the rectum (Lechleitner, 1988; Thomson *et al.* 1988*a*). Stimulation of Cl^- transport in both segments by forskolin and theophylline also supports the view that cyclic AMP acts as the second messenger in both tissues for unknown natural stimulants (Chamberlin and Phillips, 1988; Audsley and Phillips, 1990). In the case of the rectum, all conditions that are associated with stimulation of rectal I_{sc} are accompanied by elevated cyclic AMP levels in this tissue (Chamberlin and Phillips, 1988). Hanrahan and Phillips (1984*a*,*b*) were able to show that cyclic AMP acted specifically on the rectum to stimulate the apical Cl^- pump and both apical K^+ and basolateral Cl^- conductances. The observed stimulation of secondary fluid transport (J_v) by cyclic AMP in both locust rectum (Proux *et al.* 1984) and ileum (Lechleitner *et al.* 1989*a*,*b*) is undoubtedly a consequence of this primary action on ion-transport processes. In support of this view, stimulation of J_v is abolished in Cl^--free saline.

To identify natural stimulants that activate the cellular control systems by studies with cyclic AMP, short-circuited recta and ilea have been used as bioassays to survey the whole locust endocrine system. Stimulants of I_{sc} across both hindgut segments were found in both lobes of the CC and also in the ventral ganglia (Audsley and Phillips, 1990; Audsley, 1991; Spring and Phillips, 1980*a*,*b*). These studies showed that crude CC extracts affect all the ion and fluid transport processes in both segments in the same way as does exogenous cyclic AMP, with two exceptions. Crude CC extracts, but not cyclic AMP, inhibit ileal acid secretion (Thomson *et al.* 1991; Audsley, 1991), whereas cyclic AMP but not crude CC stimulates ileal ammonia secretion (Lechleitner, 1988; Audsley, 1991).

Phillips *et al.* (1980) partially purified a chloride transport stimulating hormone (CTSH) from *Schistocerca gregaria* CC (predominantly in the storage lobe; NCC) using rectal I_{sc} as an assay. Girardie *et al.* (1989, 1990) report that neuroparsins (Nps), which were purified from the NCC of *Locusta migratoria*, doubled the rate of fluid absorption across everted rectal sacs. However, the actions of Nps on specific ion-transport processes have not been reported. It is not clear whether Nps act to change the osmotic permeability or ion-transport rates, or whether CTSH is related to Nps.

The partial sequence of ITP reported in the companion paper is completely different from that of Nps, but it remains to be established whether ITP is different from CTSH.

In this paper we address two questions arising from previous studies. (1) Does purified ITP stimulate or inhibit all the transport processes in locust ileum that are influenced by crude CC extracts? That is, do different neuropeptides control individual transport

processes or does one peptide act on all of them? Given the multiple actions of exogenous cyclic AMP, we hypothesized that ITP should have similar multiple actions. This prediction is confirmed by the results of this paper. (2) Can ITP also account for all the stimulatory and inhibitory actions of crude CC extracts on locust rectum or do separate neuropeptides control the major transport events in locust ileum and rectum? Is ITP identical to the partially purified stimulant (CTSH) previously reported to act on rectal I_{sc} and, presumably as a consequence, on passive K^+ and secondary fluid absorption? We report that ITP has no (or much reduced) effects on some rectal transport processes compared to the effects of crude CC.

There has been rapid progress recently in characterizing insect diuretic peptides acting on Malpighian tubule secretion. These peptides have been identified using a variety of assays including *in vivo* preparations to measure fluid excretion (Kataoka *et al.* 1989; Blackburn *et al.* 1991), immunological cross-reactivity with an antibody to arginine vasopressin (Proux *et al.* 1986; Schooley *et al.* 1987) and *Manduca sexta* diuretic hormone (Lehmberg *et al.* 1991) and increases in fluid secretion and intracellular cyclic AMP levels in isolated Malpighian tubules (Coast *et al.* 1990; Kay *et al.* 1991*a,b*). These assays do not demonstrate the primary physiological actions of these diuretic peptides, i.e. on the specific ion-transport mechanisms causing enhanced fluid secretion. The emphasis of these studies has been on neuropeptide structure rather than specific cellular functions. The same is true for the action of Nps on rectal fluid absorption. In the present paper, in contrast, we emphasize the specific action of ITP on ileal and rectal ion transport.

Materials and methods

The experimental animals were adult *Schistocerca gregaria* Forskål, 2–3 weeks past their final moult. They were reared at 28 °C and 55 % relative humidity under a 12 h:12 h light:dark cycle, and fed a diet of lettuce and a mixture of dried grass, bran and milk powder. Ilea from females were used because of their larger size.

Salines

The physiological saline was based on the composition of locust haemolymph (Hanrahan *et al.* 1984) and contained (mmol l^{-1}): 100 NaCl, 5 K_2SO_4, 10 $MgSO_4$, 10 $NaHCO_3$, 5 $CaCl_2$, 10 glucose, 100 sucrose, 2.9 alanine, 1.3 asparagine, 1.0 arginine, 5 glutamine, 11.4 glycine, 1.4 histidine, 1.4 lysine, 13.1 proline, 6.5 serine, 1.0 tyrosine and 1.8 valine. It was bubbled with 95 % O_2/5 % CO_2 and adjusted to pH 7.2. This saline was used in all experiments unless otherwise stated.

Electrogenic chloride transport

To test the effects of purified ion-transport peptide (ITP), recta and ilea were mounted as flat sheets between two modified Ussing chambers and voltage-clamped at 0 mV, as described by Hanrahan *et al.* (1984) for locust rectum. Each chamber contained 2 ml of saline which was stirred by vigorously bubbling with a mixture of 95 % O_2/5 % CO_2 at 22±2 °C. Short-circuit current (I_{sc}), a direct continuous measurement of electrogenic Cl^-

transport in both the ileum and rectum (Williams *et al.* 1978; Hanrahan *et al.* 1984; Irvine *et al.* 1988), was recorded continuously on a strip chart recorder (Soltec 1242, Soltec Corp., Sun Valley, CA). As this assay was sensitive to acetonitrile, samples of HPLC fractions to be assayed were dried in polypropylene microcentrifuge tubes [Robbins Scientific, CA; rinsed with a 0.5 % bovine serum albumin (BSA) solution] by centrifugal evaporation (Speed-vac, Emerston Insruments Inc., Ontario). BSA was necessary to prevent loss of neuropeptide activity due to non-specific binding to the surface of the tubes: if active peptide was dried alone it became insoluble. Fractions were resuspended in small volumes (10–100 μl) of physiological saline and samples were added to the haemocoel side of ilea or recta once a steady-state level had been reached (1–2 h after dissection) to give the desired concentration. BSA, which does not affect hindgut I_{sc}, was also added to the bathing saline to reduce non-specific binding of active peptide to the walls of the Ussing chamber. Concentrations of pure ITP are given in CC equivalents ml^{-1}, 1.0 CC equivalent is estimated to equal 0.487 pmol of this peptide (Audsley *et al.* 1992).

Fluid absorption

Methods for studying ileal and rectal fluid transport using everted hindgut sacs were similar to those previously described for locust rectum (Hanrahan *et al.* 1984) and ileum (Lechleitner *et al.* 1989*a*). At hourly intervals, weight gain and tissue volume changes were determined by weighing ilea or recta (to within 0.1 mg) 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 (J_v) was measured hourly over 5 h as described by Goh and Phillips (1978). Usually rates are at near steady state after the first hour. Effects of ITP in saline were determined by adding small amounts (1–3 μl) to the inside of the sacs (haemocoel side). Changes in saline osmolarity due to the addition of these fractions were monitored with a Wescor vapour pressure osmometer (model 5500; Logan, Utah) and were found not to be significant. In a typical experiment, physiological saline (3–5 μl) was added to the haemocoel side of the ileal or rectal sacs for the first 2 h, and the sacs were placed in 50 ml of physiological saline to obtain control rates. At the end of the second hour, crude CC, ITP or physiological saline (control) was added to the sacs. The rate of fluid transport was then measured for the next 2 h and compared to control preparations over the same period and to rates during the previous control period for the same preparation.

Estimation of potassium permeability

To determine the effects of ITP on ileal and rectal K^+ conductance, a simple saline was used which contained (mmol l^{-1}): 70 NaCl, 10 KCl, 10 $MgCl_2.6H_2O$, 10 glucose, 100 sucrose, 10 proline, 5 glutamine, 5 $CaCl_2$, 10 3-(*N*-morpholino)propanesulphonic acid (Mops), adjusted manually to pH 7.0 with concentrated HNO_3 or NaOH using a Radiometer PHM 84 pH meter (Copenhagen), and bubbled with 100 % O_2. Ilea and recta were first allowed to attain a steady state. All external Cl^- was then replaced by gluconate on both sides to abolish electrogenic Cl^- absorption and basolateral Cl^- conductance.

Haemocoel K^+ concentration was then raised to 80 mmol l^{-1} to create a K^+ concentration difference of 80:10 across the epithelium, thereby inducing a transcellular K^+ diffusion current (I_K) to the lumen side. This reverse gradient avoided the known effects of high luminal K^+ concentration on apical K^+ channels in locust rectum (Hanrahan and Phillips, 1984*a*). ITP was then tested for its ability to stimulate I_K. After 30 min, 10 mmol l^{-1} Ba^{2+} was added to the haemocoel side of the tissue to determine whether this inhibitor of basal K^+ channels blocked the increase in conductance caused by ITP. This is the same protocol as that developed by Hanrahan *et al.* (1986).

Acid secretion

To measure acid secretion (J_H) to the lumen side, ilea were mounted as flat sheets in Ussing-type chambers (as previously described) under open-circuit conditions and allowed to come to steady state (60 min) under bilateral perfusion (5–8 ml min^{-1}) with saline under open-circuit conditions. The saline on both sides of the chamber was always mixed by bubbling with 100 % O_2.

Salines were based on the composition of locust haemolymph and contained (in mmol l^{-1}) 100 NaCl, 5 K_2SO_4, 10 $MgSO_4$, 10 sodium isethionate, 10 glucose, 100 sucrose, 5 $CaCl_2$, 2 Mops, and amino acids as described above for physiological saline, except that they were CO_2–HCO_3^- free. Salines were aerated with 100 % O_2 for 2–3 h before use, and perfusion reservoirs were continuously aerated throughout the experiments. The salines were filtered, and pH was manually titrated to 7 with concentrated HNO_3 or NaOH, using a Radiometer PHM 84 pH meter (Copenhagen) before each experiment. Bilateral perfusion (but not mixing) was stopped, and ITP (experimental) or nothing (control) was added to the haemocoel side of the ileum. Rates of luminal acidification (J_H) were determined using a pH-stat technique (PHM 84 research pH meter, TTT 80 titrator, ABU 80 autoburette; Radiometer, Copenhagen) as described by Thomson (1990). J_H was calculated as the rate of titrant addition (0.01 mol l^{-1} NaOH) required to maintain the initial pH, and is expressed as μequiv cm^{-2} h^{-1} over the second hour after mounting the tissue.

Sodium flux measurements

^{22}Na fluxes across ilea were determined by mounting the tissue as flat sheets in Ussing-type chambers containing physiological saline, which was stirred by vigorously bubbling with 95 % O_2:5 % CO_2 at 20 °C. Ilea were voltage-clamped at 0 mV and allowed to come to steady state under short-circuit current conditions. I_{sc} was monitored continuously throughout the experiment. ^{22}Na was purchased from New England Corporation as $^{22}NaCl$. Samples of stock solution were added to the lumen (for forward fluxes; L–H) or haemocoel (back fluxes; H–L) sides of the tissue, 30 min before the experimental period. A 50 μl sample was taken from the 'hot' side (in duplicate) and added to 500 μl of cold saline in polypropylene scintillation vials. Flux of ^{22}Na across ilea were determined at 15 min intervals, 2 h before and after stimulation with 5 CC equivalents ml^{-1} of ITP added on the haemocoel side of the ileum. Samples of saline (0.5 ml) were taken from the 'cold' side of the chambers every 15 min for determination of increase in radioactivity

and replaced with 0.5 ml of cold saline. If taken from the haemocoel side after stimulation, additional ITP was added back to keep the concentration of this peptide the same throughout the experiment. Samples (0.5 ml) were counted with an automatic well-type gamma counter (Nuclear Chicago model 1058). Unidirectional flux was calculated using the following formula (Williams *et al.* 1978):

$$J_{1-2}=a_2VC/a_1TA,$$

where J_{1-2} is the unidirectional flux (μequiv $cm^{-2}h^{-1}$), a_1 is the radioactivity of the 'hot' side (cts $min^{-1}ml^{-1}$), a_2 is the increase in radioactivity of the 'cold' side (cts $min^{-1}ml^{-1}$), C is the concentration of the unlabelled Na^+ in solution (mmol l^{-1}), V is the volume of solution in the chambers, A is the tissue area (0.196 cm^2) and T is the time interval between collection of samples (h).

Effect of ITP on rectal transport

To determine the effects of ITP on rectal I_{sc}, J_v and I_K, the fraction from the penultimate purification step was used (see Audsley *et al.* 1992). This contains ITP plus an unknown peptide P2 in a 5:1 ratio. This fraction was used because of the limited amount of pure peptide available, compounded by losses during the final purification step and also the much greater amounts of ITP required for stimulation of rectal I_{sc}. The impurity (P2) removed by the final purification of ITP had no effect on ileal I_{sc} at a concentration of 5 CC equivalents ml^{-1} (Audsley *et al.* 1992). Moreover addition of fractions containing P2 to those with pure ITP did not alter the actions of ITP on the ileum (Audsley, 1991). Presumably P2 also has no effect on the actions of ITP on the rectum.

Statistical treatment

Differences between treatments were considered significantly different when Student's t-test indicated a P value of less than 0.05.

Results

Dose–response relationship and time course of ileal I_{sc} with ITP

The time course of the ileal I_{sc} response to ITP is similar at the four concentrations tested (Fig. 1), rising to a maximum for each dose between 0.5 and 1.0 h, and remaining at this level for at least the next hour. This differs from the time course of the response to crude extracts (Audsley and Phillips, 1990), which varied with dose. The dose–response relationship of ITP on ileal I_{sc} is shown in Fig. 2. A linear dose–response relationship over the range 1.0–5.0 CC equivalents ml^{-1} was observed, which corresponds to 0.487–2.44 pmol ml^{-1} of pure peptide as determined from the amino acid analysis data (Audsley *et al.* 1992). At 5 CC equivalents ml^{-1} of ITP, the increase in I_{sc} of 9.96± 0.66 μequiv $cm^{-2}h^{-1}$ agrees well with the maximum response of 10.32±1.29 μequiv $cm^{-2}h^{-1}$ caused by 0.25 crude CC ml^{-1} (Audsley and Phillips, 1990). There is therefore a twentyfold difference in the concentrations of crude and pure extracts required for a maximum response, and most of this activity is lost during the final purification step

(Audsley *et al.* 1992). The maximum increase in I_{sc} caused by ITP is also quantitatively similar to that caused by 5 mmol l^{-1} cyclic AMP (13.0±0.7 μequiv $cm^{-2}h^{-1}$; Irvine *et al.* 1988).

Effect of ITP on ileal potassium permeability (I_K)

The effects of ITP on increases in ileal I_K are shown in Fig. 3. In the presence of control saline (i.e. with Cl^- present) I_{sc} fell to a steady level of 0.82±0.24 μequiv $cm^{-2}h^{-1}$ after 1 h. There was only a slight change in residual I_{sc} when all Cl^- was replaced bilaterally by gluconate (0.35 μequiv $cm^{-2}h^{-1}$) and when I_K was initiated by raising haemocoel $[K^+]$ to 80 mmol l^{-1} (1.67±0.22 μequiv $cm^{-2}h^{-1}$). Subsequently 5.0 CC equivalents ml^{-1} of ITP caused significant several-fold increases in potassium diffusion current to 4.6±0.93 μequiv $cm^{-2}h^{-1}$, which is qualitatively similar to that caused by crude CC stimulation (Audsley and Phillips, 1990). Addition of 10 mmol l^{-1} Ba^{2+} completely

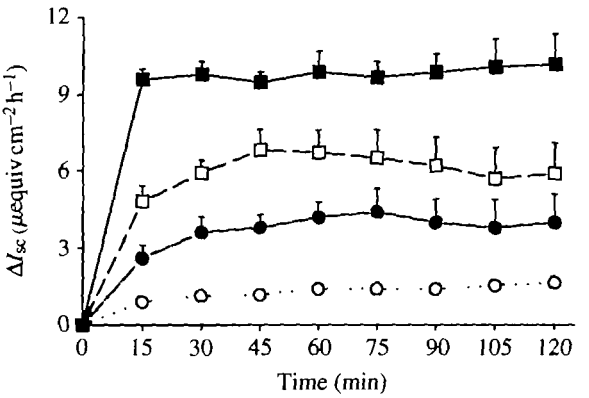

Fig. 1. Time course of the ileal I_{sc} response to various doses of ITP added to the haemocoel side (mean+S.E., N=4–8): open circles, 1.0 CC; filled circles, 2.0 CC; open squares, 2.5 CC; filled squares, 5.0 CC equivalents ml^{-1} of ITP. Error bars are within the size of the open circles.

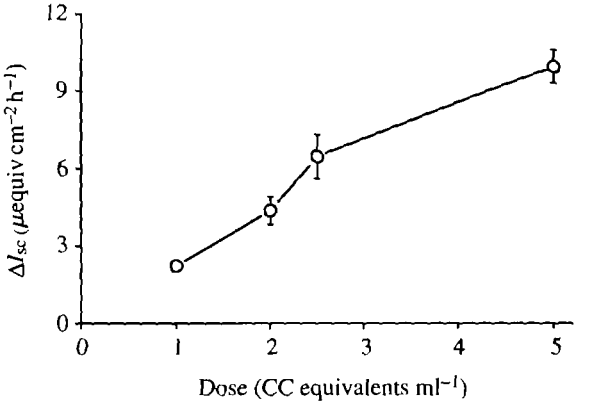

Fig. 2. Increase in I_{sc} 1 h after adding various doses of ITP to the haemocoel side of ilea (mean±S.E., N=4–8).

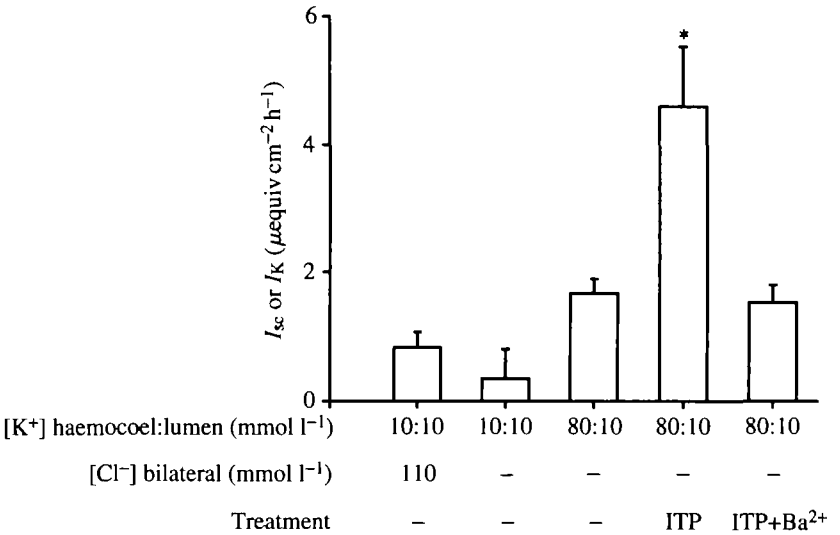

Fig. 3. Effect of ITP (5.0 CC equivalents ml^{-1}) on current (mean±S.E., N=6–12) required to clamp ileal V_t at 0 mV under different sequential external conditions. The bar on the left represents normal unstimulated I_{sc}, which was slightly reduced by bilateral replacement of all Cl^- (second bar). Imposition of a haemocoel-to-lumen (H:L) K^+ gradient had little effect on causing a potassium diffusion current (I_K, third bar) until ITP was added (fourth bar). The fifth bar shows the effect of adding barium to the haemocoel side. Means that are significantly different from the control value are marked with an asterisk (P<0.05).

abolished the I_K due to ITP when added to the haemocoel side of the tissue. These results are similar to those previously described for cyclic-AMP-induced I_K in both ileum and rectum (Irvine *et al.* 1988; Hanrahan *et al.* 1986).

Effect of ITP on ileal Na^+ reabsorption

The effect of ITP on unidirectional and net Na^+ fluxes is shown in Fig. 4A,B. Short-circuit current values in Fig. 4C indicate a net anion (Cl^-) movement from the lumen to the haemocoel side of the tissue of 1.12±0.05 μequiv cm^{-2} h^{-1} (unstimulated), increasing to 11.49±0.09 μequiv cm^{-2} h^{-1} on the addition of ITP. Under control conditions there is a mean back flux of 0.87±0.11, a forward flux of 1.51±0.12 and a net flux of 0.62±0.21 μequiv cm^{-2} h^{-1} of Na^+ to the haemocoel side over the first 2 h. After the addition of ITP, the back flux falls slightly to 0.31±0.11 and the forward flux increases significantly to 3.7±0.09 μequiv cm^{-2} h^{-1}, resulting in a significant increase (P<0.05) in net flux to 3.41±0.17 μequiv cm^{-2} h^{-1} to the haemocoel side. This is similar to results previously observed for the effects of cyclic AMP and crude CC extracts on ileal Na^+ transport (Lechleitner, 1988; Irvine *et al.* 1988).

Effect of ITP on ileal acid secretion

Table 1 shows the effect of ITP on ileal J_H. Under control conditions the rate of J_H to the lumen was 0.93 μequiv cm^{-2} h^{-1} and this was significantly (P<0.005) reduced by

75 % when 5 CC equivalents ml^{-1} ITP was present. ITP reduced J_H to the same extent as crude extracts of CC (Audsley, 1991). In contrast, cyclic AMP did not inhibit ileal J_H (Thomson *et al.* 1991).

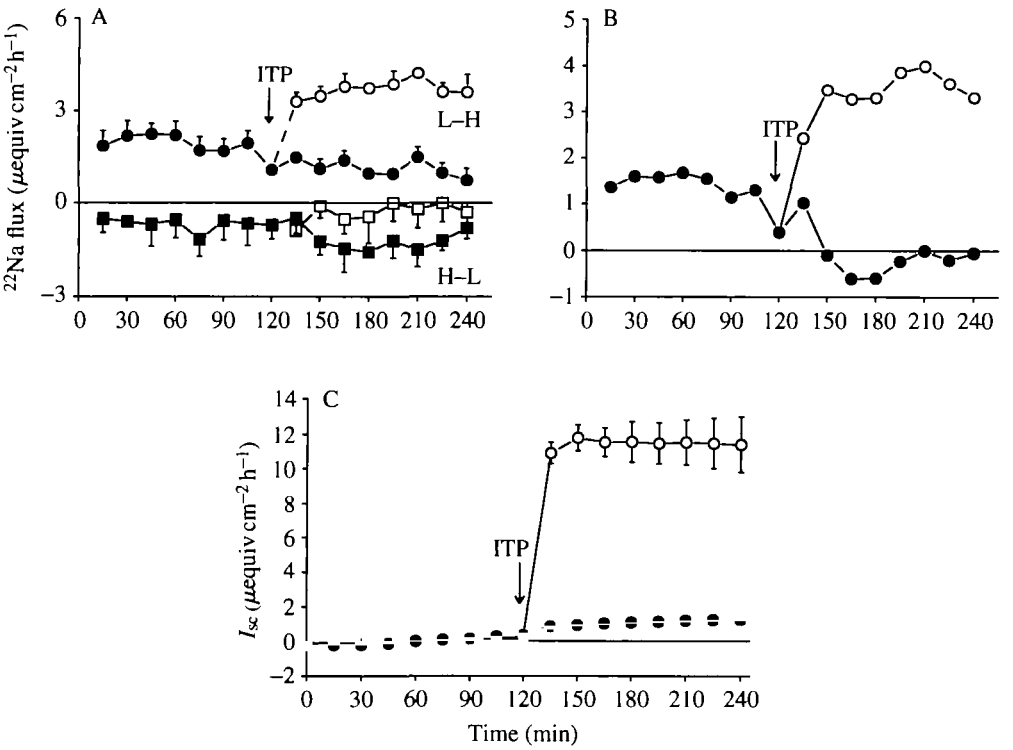

Fig. 4. (A) The time course of unidirectional ^{22}Na fluxes across short-circuited ilea under control and ITP-stimulated conditions. Time zero is 90 min after dissection. (L–H represents the forward flux from lumen to haemocoel side, and H–L the back flux of haemocoel to lumen side). Control preparations (filled circles, filled squares) were bathed in normal saline throughout and 5.0 CC equivalents ml^{-1} of ITP was added to the haemocoel side of experimental preparations (open circles, open squares) 2 h after the start of the experiment. (B) The net flux to the haemocoel side was calculated by subtraction from unidirectional fluxes in A. The differences are significantly different (P<0.05). (C) The short-circuit current across ilea during flux measurements (means±S.E., N=4–8).

Table 1. *Effect of ITP (5.0 CC equivalents ml^{-1}) on ileal acid secretion (J_H) and transepithelial potential difference (V_t) under open-circuit conditions*

Treatment	J_H (μequiv cm^{-2} h^{-1})	V_t (mV)
Control (no treatment)	0.93±0.02	5.25±1.31
ITP	0.23±0.04**	10.0±1.96*

Mean±S.E., N=4–6.
Significant difference from control: * P<0.05 and ** P<0.005.
Measured over the second hour after mounting the tissue.

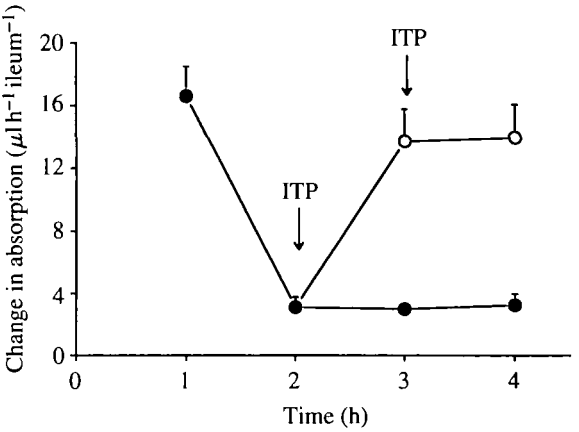

Fig. 5. Time course of ileal J_V under control (filled circles) and ITP-stimulated (open circles) conditions (mean±S.E., N=4).

Effect of ITP on ileal fluid transport

The effect of ITP on ileal fluid transport is shown in Fig. 5. At a dose of 0.1 CC equivalent μl^{-1}, ITP caused a fourfold increase in J_V over the third and fourth hours compared to controls over the same period. Thus, ITP caused as large an increase in J_V as does crude CC or exogenous cyclic AMP (Lechleitner *et al.* 1989*a*).

Effect of ITP on ileal ammonia secretion J_{Amm}

Not surprisingly, ITP had no effect on ileal J_{Amm}, given that crude CC extracts also had no effect (Audsley, 1991).

Effect of ITP on rectal transport

Fig. 6 shows the effect of ITP together with P2 on rectal I_{sc}. A maximum response of 3.63±0.29 μequiv $cm^{-2} h^{-1}$ is observed with 2 CC equivalents ml^{-1}, and I_{sc} could not be

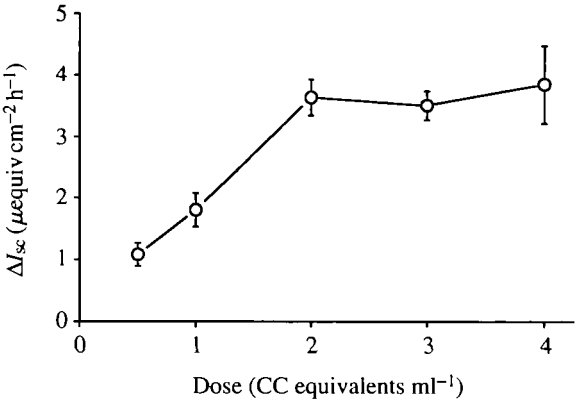

Fig. 6. Increases in rectal I_{sc} 1 h after adding various amounts of ITP+P2 to the haemocoel side (mean±S.E., N=6–8).

Table 2. *Effect of crude CC extracts (0.5 CC ml^{-1}) and ITP+P2 (4.0 CC equivalents ml^{-1}) on rectal fluid (J_v) and potassium (I_K) absorption*

Treatment	J_v (μl rectum h^{-1})	I_K (μequiv cm^{-2} h^{-1})
Control (no treatment)	10.3±0.4	1.3±0.2
Crude CC	14.2±0.9*	5.09±0.3**
ITP+P2	9.9±0.5	1.4±0.3

Mean±S.E., N=4–6.
Significant difference from control: *P<0.05, ** P<0.005.

increased further by doubling the dose. This value is only 41 % of the maximum rectal response to crude CC or cyclic AMP and could only be achieved at ITP concentrations four times greater than crude CC dosages. It appears that a maximum rectal I_{sc} response cannot be produced with the active factor (ITP+P2), suggesting that neither peptide in this fraction is CTSH. Phillips *et al.* (1980) observed that the active fraction (containing CTSH) obtained by gel filtration chromatography caused the same maximum stimulation of rectal I_{sc} as did crude CC.

Table 2 shows the effects of crude CC and ITP+P2 on rectal J_v and I_K. Crude CC caused significant increases in both rectal J_v and I_K whereas ITP+P2 had no measurable effect on either of these transport processes (Table 2). This provides further evidence that the factor in crude CC (CTSH) that stimulates rectal salt transport is not the same as ITP, which stimulates ileal salt transport.

Discussion

In the current study the physiological actions of ITP, a neuropeptide isolated from locust CC, on ileal salt transport are described. ITP had the same quantitative effects on all the transport processes in the locust ileum as did crude CC extracts (Audsley and Phillips, 1990; Lechleitner *et al.* 1989*a,b*). Although more CC equivalents of ITP are required (due to losses during purification), the same maximum stimulation of all these processes, and inhibition of J_H, is achieved with ITP as with crude CC. Neither crude CC extracts nor ITP affected ammonia secretion (results not shown), which could be stimulated by cyclic AMP (Audsley, 1991).

Crude CC causes large increases in rectal I_{sc} (10-fold), I_K and J_v. In contrast, ITP did not maximally stimulate rectal I_{sc} and had no effect on rectal J_v or potassium permeability (I_K). It is therefore unlikely that ITP and CTSH are the same peptide. This loss of CC stimulatory actions on the rectum may be due to the instability of CTSH during one of the purification steps described by Audsley *et al.* (1992). Trifluoroacetic acid (TFA) is strongly acidic and Phillips *et al.* (1980) reported that CTSH was unstable below pH 5. Earlier attempts to isolate CTSH by RP-HPLC, using rectal I_{sc} as the bioassay, were unsuccessful (J. Meredith, personal communication). The small effects of ITP on rectal I_{sc} may indicate that ITP is sufficiently similar to CTSH to activate some CTSH receptors or that the effects are pharmacological. CTSH must be characterized using rectal I_{sc} as the

bioassay to confirm whether it is different from ITP. The evidence at present is strongly against CTSH and ITP being the same peptide.

The time course of the ileal I_{sc} response to ITP was similar at all doses tested, unlike the time courses observed with crude CC extracts, which varied with dose (Audsley and Phillips, 1990). The reason for this difference is unknown, but crude CC does contain at least three factors, including ITP (the most active), that stimulate ileal I_{sc}. These other less potent CC stimulants may have a different time course of action on ileal I_{sc}. There may also be factors in the CC that act to inhibit the ileal response to ITP or modify the receptor affinity for ITP, all of which could explain the discrepancy in the time course of I_{sc} with different dosages of CC as compared to ITP.

Hanrahan and Phillips (1984*a*) developed an epithelial model for the organization of transport processes in the locust rectum and identified specific sites of control by CTSH acting through cyclic AMP. They proposed that CTSH elevates intracellular cyclic AMP level to stimulate active Cl^- entry, an apical membrane K^+ conductance and a basolateral membrane Cl^- conductance. Although intracellular recording of ion activities has not yet been carried out on locust ilea, all other observations on this segment are consistent with this rectal model with regard to transport mechanisms, their properties, their location and their control (Irvine *et al.* 1988). ITP may also act *via* cyclic AMP to stimulate active Cl^- and Na^+, and passive K^+, absorption in the ileum, because these processes are also all stimulated by cyclic AMP in a similar manner (Irvine *et al.* 1988). In support of this view, forskolin (10–50 μmol l^{-1}), which stimulates adenylate cyclase, and the phosphodiesterase inhibitor theophylline (5 mmol l^{-1}), also stimulate ileal Cl^--dependent I_{sc} (Audsley and Phillips, 1990). Intracellular levels of cyclic AMP must be monitored in parallel with the rise in ileal I_{sc} to confirm this prediction. Preliminary observations confirmed that intracellular cyclic AMP concentration increased 1 h after adding ITP (N. Audsley, unpublished observations), but the time course of changes in cyclic AMP levels after adding ITP remain to be studied.

H^+ secretion is inhibited by both crude CC extracts (Audsley and Phillips, 1990) and pure ITP, but not by cyclic AMP (Thomson *et al.* 1991). This suggests that ITP must act *via* a different intracellular messenger to mediate this specific response. Intracellular levels of Ca^{2+}, cyclic GMP, arachidonic acid and inositol phosphates will have to be measured before and after stimulation with ITP to determine their possible roles as second messengers controlling ileal transport functions. Fournier (1991) demonstrated the formation of inositol 1,4,5-trisphosphate during Nps stimulation of fluid reabsorption in the rectum of *L. migratoria*. However, phosphoinositides do not appear to mediate the stimulation of fluid and Cl^- reabsorption in the rectum of *S. gregaria* by crude CC extracts (L. Jeffs and J. E. Phillips, personal communication).

If several reabsorptive processes in the ileum can all be stimulated by cyclic AMP, it is to be expected that a factor (e.g. ITP) that elevates intracellular cyclic AMP concentration is also going to regulate those same processes. Since other stimulants of ileal I_{sc} were detected during HPLC separation of locust CC (Audsley, 1991) and other neuroendocrine tissues (e.g. ventral ganglia; Audsley and Phillips, 1990), the action of ITP may be enhanced and modified by these agents, which could act through other second messengers.

It still remains to be shown that ITP and other CC factors are normally released into the haemolymph to influence ileal transport activity *in situ*. However, the haemolymph does contain a stimulant(s) of ileal I_{sc} which elutes in the same fraction as ITP from a preparative C_4 cartridge separation (Audsley *et al.* 1992).

In summary, ITP has the same range of effects on ileal transport as do crude CC extracts, demonstrating that a single neuropeptide can account for all the known actions of crude CC on locust ileal transport. However, ITP has a reduced effect on rectal I_{sc} and no effect on rectal J_v or I_K, suggesting that a different factor (e.g. CTSH and/or neuroparsins) may regulate ion and fluid reabsorption in this hindgut segment.

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