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CELLULAR MECHANISMS AND CONTROL OF KCl ABSORPTION IN INSECT HINDGUT

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SUMMARY

The hindgut of the desert locust possesses an unusual chloride transport system. The isolated locust rectum absorbs chloride from the mucosal (lumen) to the serosal (haemolymph) side at a rate which is equal to the short-circuit current (I_{sc}). Net chloride transport (J^{Cl}_{net}) persists in nominally Na-free or $HCO_3(CO_2)$-free saline, is insensitive to normal inhibitors of NaCl co-transport and anion exchange, and is independent of the net electrochemical gradient for sodium across the apical membrane. However, active chloride transport is strongly dependent on mucosal potassium ($K_a = 5{\cdot}3$ mM-K). Chloride entry across the apical membrane is active, whereas the net electrochemical gradient across the basal membrane favours passive Cl exit from the cell. Although mucosal potassium directly stimulates 'uphill' chloride entry, there is no evidence for coupled KCl co-transport, nor would co-entry with potassium be advantageous energetically.

Net chloride absorption and I_{sc} are stimulated by a peptide hormone from the central nervous system which acts *via* cyclic-AMP. Cyclic-AMP increases I_{sc} and J^{Cl}_{net} approximately 1000 % and transepithelial conductance (G_t) ~100 %. Approximately half of the ΔG_t during stimulation results from increased Cl conductance at the basal cell border. This increase is also reflected in a shift of the basal membrane e.m.f. towards the Nernst potential for chloride. The remainder of the cAMP-induced ΔG_t is due to an elevation of apical membrane K conductance, which causes a 400 % increase in transepithelial potassium permeability as estimated by radiotracer diffusion. Because of this stimulation of K conductance, potassium serves as the principal counterion for active chloride transport under open-circuit conditions. Very high luminal levels of K oppose the stimulatory actions of cAMP on active Cl transport and K conductance. These and other results have been incorporated into a cellular model for KCl absorption across this insect epithelium.

INTRODUCTION

There is evidence for active chloride transport across six epithelia from 17 different insect species; however, little is known regarding the mechanisms which are responsible, or how these are controlled at the cellular level. In epithelia from vertebrate

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animals, models for active chloride transport generally involve uphill entry into the cell by co-transport with sodium or entry of chloride in exchange for internal bicarbonate or hydroxyl ions. However, insect epithelia are unusual in that they often absorb chloride from external fluids that contain little sodium but which are extraordinarily rich in potassium [e.g. the hindgut of the desert locust (140 mM-K; Phillips, 1964; Hanrahan, 1982), proximal segment of blowfly salivary gland (190 mM-K; Oschman & Berridge, 1970), proximal segment of *Rhodnius* Malpighian tubules (70 mM-K; Maddrell & Phillips, 1975) and pupal integument of lepidopterans (164 mM-K; Jungreis, 1978; Cooper, Deaton & Jungreis, 1980)]. Recently, we studied active Cl absorption across the locust rectum by testing predictions of vertebrate Na- and HCO_3-coupled mechanisms (Hanrahan & Phillips, 1982*a*). We consistently obtained negative or quantitatively unfavourable results and concluded that locust hindgut has an electrogenic, K-dependent mechanism for Cl transport. Both active Cl absorption and epithelial K conductance are apparently stimulated by a peptide hormone *via* cAMP. This paper briefly reviews the function of the rectal epithelium in locusts, describes evidence for this unusual Cl transport mechanism, and then discusses the regulation of KCl absorption with reference to a simple equivalent circuit model.

REVIEW OF TRANSPORT IN LOCUST RECTUM

As in most other insects, the hindgut of the desert locust is an important renal organ which functions in ionic and osmotic regulation (see Phillips, 1981). The locust excretory system is shown diagrammatically in Fig. 1. Approximately 200 blind-ended Malpighian tubules secrete a K-rich, primary urine into the hindgut lumen. The rate of tubular secretion is stimulated during feeding and flight by diuretic hormone, a small peptide (M_r about 2000) either from the corpus cardiacum (Mordue, 1970, and personal communication) or the suboesophageal ganglion (Proux, Rougon & Cupo, 1982). Most of the secreted fluid, containing wastes, ions and metabolites, moves posteriorly to the rectum, where it is modified by selective reabsorption of ions, amino acids and water (Phillips, 1964, 1981; Balshin & Phillips, 1971).

Locust rectum is a columnar epithelium consisting mainly of large cells (~15 μm diameter, ~100 μm length) organized into six discrete pads. A thin layer of cuticle loosely covers the luminal (mucosal) surface and attaches to the tissue at the margins of each pad. The apical (lumen-facing) membrane does not form a brush-border as such; however, both apical and lateral membranes are highly infolded and are associated with numerous mitochondria. The rectal epithelium is richly supplied with tracheoles for gas exchange. Ion transport strongly depends on aerobic metabolism, and preferentially oxidizes proline which is available in Malpighian tubule fluid (Chamberlin & Phillips, 1982).

Phillips (1964) showed that chloride, sodium and potassium are actively reabsorbed from the lumen of ligated recta *in situ*. Transepithelial potential (V_t) and the rate of Cl transport in tissues mounted in Ussing-type chambers are similar to those measured in intact locusts (Williams, Phillips, Prince & Meredith, 1978). Williams *et al.* found that net chloride flux from the lumen (mucosal) side to haemocoel (serosal) side (J_{net}^{Cl}), and short-circuit current (I_{sc} – uncorrected for saline resistance)

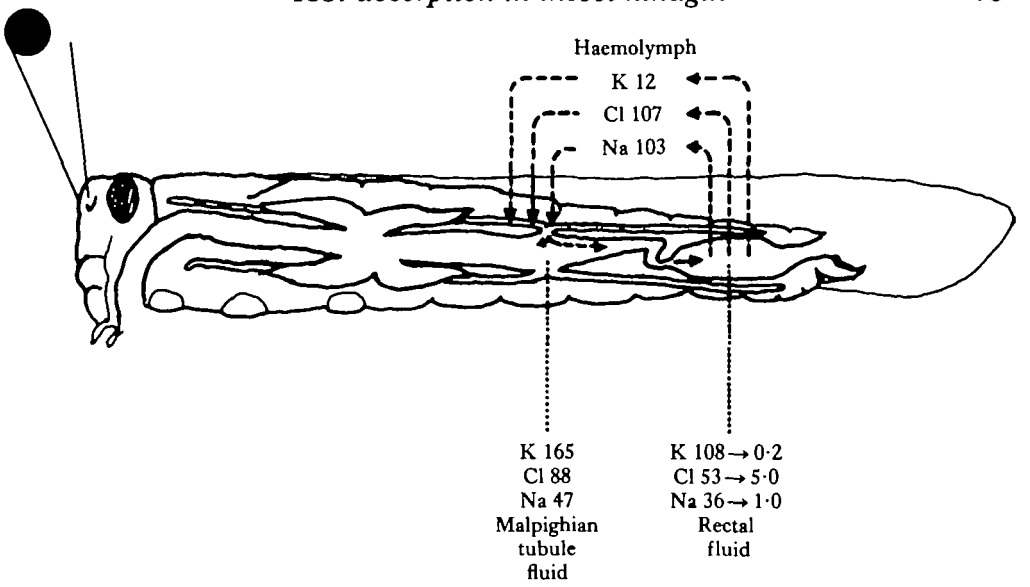

Fig. 1. Diagram of renal function in the desert locust. According to the recycling hypothesis (dashed lines), most K-rich fluid which is secreted by Malpighian tubules flows posteriorly to the rectum, where Cl and Na are actively reabsorbed and most K is recovered passively. Concentrations of K, Cl, and Na (mM) in the haemolymph, Malpighian tubule fluid (collected between ligations around the proctodeal valve), and hindgut fluid (collected as it enters the rectum) are also shown (Hanrahan, 1982 and unpublished). Final low concentrations in rectal fluid are from Phillips (1964) for hydrated, salt-depleted animals.

both declined exponentially from approximately 6·0 μequiv cm^{-2} h^{-1} (after 30 min) to 3·5 μequiv cm^{-2} h^{-1} and 3·0 μequiv cm^{-2} h^{-1}, respectively after 3 h *in vitro*. In contrast, Na absorption was ~4 μequiv cm^{-2} h^{-1} and did not change with time. The early decay of I_{sc} was attributed to a decline in electrogenic Cl transport because high initial values of I_{sc} were not observed when recta were bathed by chloride-free salines in which chloride was replaced by nitrate or sulphate. Further *in vitro* evidence for the electrogenicity of chloride absorption was obtained by Spring (Spring, Hanrahan & Phillips, 1978; Spring & Phillips, 1980*a*,*b*) who found that adding homogenates of the corpus cardiacum (CC) 1·5 h after dissection restored both I_{sc} and J^{Cl}_{net} to their initial values. Similar stimulations were later observed using haemolymph from recently-fed locusts (Hanrahan, 1978; Spring & Phillips, 1980*c*). A peptide hormone named CTSH (Chloride Transport-Stimulating Hormone; $M_r = 8000$) has been isolated from corpus cardiacum and haemolymph (Phillips, Mordue, Meredith & Spring, 1980; Phillips *et al.* 1981). The actions of CTSH are apparently mediated by the second messenger cAMP because; (i) addition of cAMP increases I_{sc} and J^{Cl}_{net} (Spring & Phillips, 1980*a*; Hanrahan & Phillips, 1982*a*,*b*,*c*), (ii) exposure of recta to CTSH causes a three-fold elevation of tissue cAMP levels (Spring & Phillips, 1980*a*) and (iii) theophylline (which inhibits phosphodiesterase activity and thus cAMP degradation) is also stimulatory (Hanrahan, 1978, 1982). Fig. 2 shows the response of I_{sc} (corrected for saline resistance) and mucosa-to-serosa Cl flux (J^{Cl}_{ms}) after addition of 1 mM-cAMP to the serosal side.

These studies provide strong evidence that Cl is actively absorbed and that transport

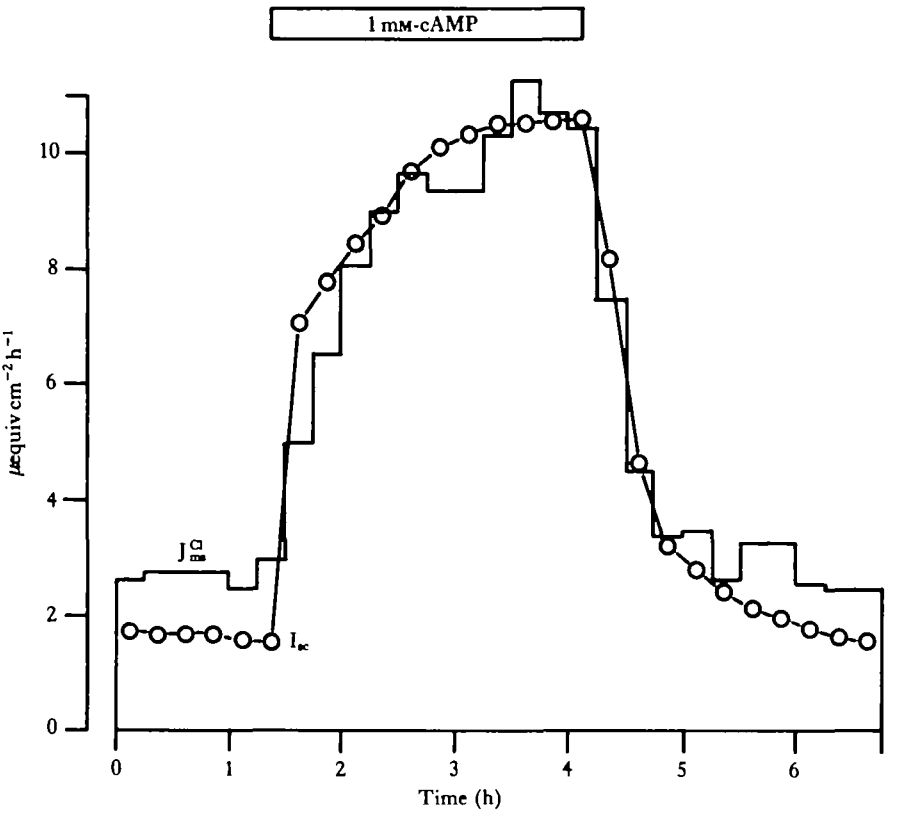

Fig. 2. Effects of 1 mM-cAMP on the mucosa-to-serosa ^{36}Cl flux (J_{ms}^{Cl}) and short-circuit current (I_{sc}). Instantaneous I_{sc} recordings (—O—) were integrated by planimetry for comparison with J_{ms}^{Cl} (histogram). Time 0 h was preceded by 4 h equilibration in normal saline resembling normal haemolymph under I_{sc} conditions.

is stimulated *in vitro* by CTSH and cAMP, but they give little information regarding the cellular mechanism of transport or its regulation. Before describing recent findings in locust rectum, it would be useful first to outline some models that have been proposed for active chloride transport in other tissues.

VERTEBRATE MODELS FOR EPITHELIAL CHLORIDE TRANSPORT

Many models of chloride absorption and secretion in vertebrates propose that 'uphill' entry of Cl into the cell is driven by entry of Na down its net electrochemical gradient through co-transport with Na (see Frizzell, Field & Schultz, 1979) or Na and K (Gregor, 1981; Musch *et al.* 1982). The inward sodium gradient is maintained by the ubiquitous Na/K exchange pump at the basal membrane. In those instances where transepithelial chloride transport is electrogenic, sodium that co-enters with Cl may recycle back to the mucosal side through a Na-selective, paracellular pathway (Field *et al.* 1978). Alternatively, Cl may enter *via* Cl/OH or Cl/HCO_3 exchange mechanisms, driven by primary active transport through the action of a membrane-bound ATPase (Bornancin, DeRenzis & Naon, 1980; Herrera *et al.* 1978; Humphreys

[redacted]hou, 1979; Komnick, Schmitz & Hinssen, 1980; but see Bonting, de Pont, van Amelsvoor & Schrijen, 1980) or by secondary active transport, driven by exit of HCO_3 or OH down their respective electrochemical gradients, or perhaps by parallel Na/H exchange. The anion exchange mechanism postulated for vertebrate epithelia is usually inhibited by thiocyanate (Epstein, Maetz & DeRenzis, 1973), disulphonic stilbenes such as SITS (4-acetamide-4′-isothiocyanostilbene-2,2′-disulphonic acid, e.g. Perry, Haswell, Randall & Farrell, 1981), and may be blocked indirectly by acetazolamide (e.g. Maetz & Garcia-Romeu, 1964), which inhibits carbonic anhydrase activity so that HCO_3 supply becomes rate-limiting.

Some predictions of vertebrate models

If chloride co-enters with sodium, then (i) I_{sc} and J^{Cl}_{net} should be abolished or greatly reduced in nominally Na-free saline and (ii) they should be correlated with the level of Na contamination. (iii) There should be a 1:1 relationship between unidirectional influxes of ^{22}Na and ^{36}Cl, and (iv) the net electrochemical gradient for Cl across the apical membrane ($\Delta\bar{\mu}^a_{Cl}$) should vary with $\Delta\bar{\mu}^a_{Na}$ and perhaps reverse when $\Delta\bar{\mu}^a_{Na}$ favours Na exit from cell to mucosa. (v) J^{Cl}_{net} determined by measuring tracer fluxes, should depend strongly on $\Delta\bar{\mu}^a_{Na}$. (vi) Finally, the diuretic furosemide should inhibit Cl transport by blocking NaCl co-entry (Candia, 1973). Ouabain should reduce J^{Cl}_{net} by inhibiting the basal membrane Na pump which would lead to a reduction $\Delta\bar{\mu}^a_{Na}$.

If rectal Cl absorption involves anion exchange entry (which seems unlikely in view of the electrogenicity of transepithelial transport), then (i) removing exogenous CO_2 and HCO_3 should inhibit Cl transport unless metabolic CO_2 is generated and converted to HCO_3 at a sufficiently high rate. In this case, (ii) metabolic CO_2 production must equal or exceed the rate of Cl entry. (iii) Because of the efficiency which would be required for CO_2 hydration, one would expect involvement of carbonic anhydrase and thus sensitivity of Cl transport to the inhibitor acetazolamide. (vi) Finally, Cl entry should be blocked by anion exchange inhibitors such as SITS.

Testing the predictions of vertebrate models

Sodium

Recta were mounted in Ussing-type chambers and stimulated with 1 mM-cAMP. Bilateral replacement of normal saline (containing 115·3±0·9 mM-Na) with nominally Na-free saline (0·049 mM-Na, *N*-methyl-D-glucamine substituted) had no effect on I_{sc} during the first 75 min exposure (Fig. 3A; $P >> 0{\cdot}2$). After 5·5 h equilibration in nominally Na-free saline, serosal addition of 1 mM-cAMP caused large increases ($>5\ \mu equiv\ cm^{-2}\ h^{-1}$) in I_{sc} and J^{Cl}_{net}. Results of longterm exposure to Na-free conditions were complicated by other side effects: stimulations after 5·5 h exposure were variable and not different statistically from those observed in normal saline, although the mean values of I_{sc} and J^{Cl}_{net} during maximal stimulation were approximately 20% and 40% lower, respectively.

A gradual loss of transport under Na-free conditions would be expected because the prolonged viability of locust rectum *in vitro* depends on amino acids, and amino acid absorption is Na-coupled in this tissue (Balshin & Phillips, 1971). Consistent with this [redacted]pothesis, lower J^{Cl}_{ms}, I_{sc} and R_t are also observed when recta are exposed for similar

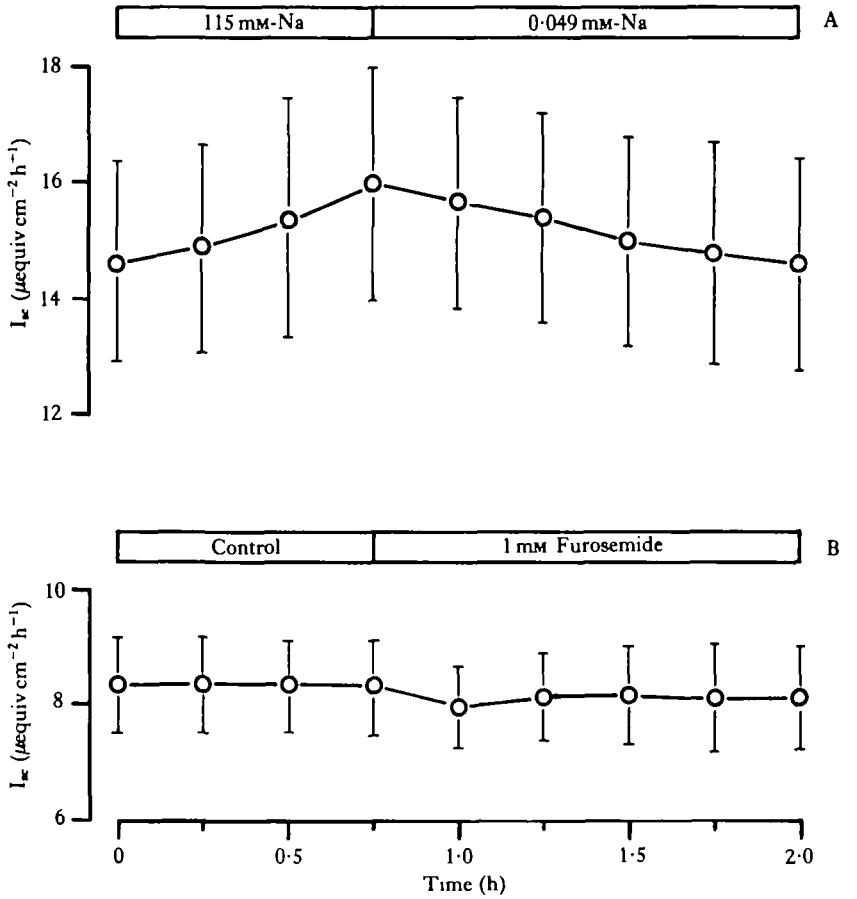

Fig. 3. (A) Effect on I_{sc} of replacing normal saline (115 mM-Na) with nominally Na-free saline (0·049 mM-Na, measured) during cAMP stimulation (means ±S.E.; $N = 6$ recta; from Hanrahan & Phillips, 1982*a*). (B) Effect of adding 1 mM furosemide in normal saline to both sides of cAMP-stimulated locust recta, from which the cuticular intima was removed from the mucosal surface (means ±S.E.; $N = 8$).

periods to amino acid-free saline containing normal levels of Na (114 mM) and glucose (10 mM; Hanrahan, 1982). Other explanations for poor viability during longterm exposure to Na-free conditions might include disruption of cell volume or intracellular pH regulation. No correlation was observed between final trace levels of sodium and cAMP-stimulated J^{Cl}_{net} and I_{sc} (5–9 μequiv cm^{-2} h^{-1}) which were measured at the end of flux experiments under nominally Na-free conditions (<1–200 μM-Na).

Initial rates of ^{36}Cl and ^{22}Na influx into the tissue of everted rectal sacs (mucosal side out) were measured with corrections for extracellular radioactivity using ^{3}H-mannitol space. Injection of 10 mM-cAMP into sacs had no effect on the low rate of ^{22}Na influx (<0·8 nequiv mg^{-1} h^{-1}). In contrast, ^{36}Cl influx increased from 0·8 to 4·9 ± 1·6 nequiv mg^{-1} h^{-1}. This six-fold elevation in ^{36}Cl influx without a concomitant stimulation of ^{22}Na influx is not consistent with obligatory NaCl co-entry across the apical cell border.

In a separate series of experiments, intracellular Cl and Na activities and potentials were measured using double-barrelled ion-sensitive microelectrodes while sodium concentration was varied on the mucosal side. Transepithelial potential was clamped at 0 mV during these experiments and the net electrochemical potentials for Na ($\Delta\bar{\mu}^{a}_{Na}/F$) and Cl ($\Delta\bar{\mu}^{a}_{Cl}/F$) across the apical membrane were calculated. When mucosal Na concentration was lowered from 115 mM to 49 μM, the calculated $\Delta\bar{\mu}^{a}_{Na}/F$ declined, reversing when mucosal Na concentration was less than 1 mM (see Hanrahan & Phillips, 1982*b*). In contrast, $\Delta\bar{\mu}^{a}_{Cl}/F$ remained relatively constant and was 38 mV above equilibrium when $\Delta\bar{\mu}^{a}_{Na}/F$ favoured Na exit rather than entry into the cell. Clearly, NaCl co-entry from the mucosal side is not required in order to maintain intracellular Cl above equilibrium.

To test directly the effects of varying $\Delta\bar{\mu}^{a}_{Na}/F$ on transepithelial chloride transport, recta were mounted in Ussing-type chambers and V_t was clamped at 0 mV. Unidirectional fluxes of ^{36}Cl were measured at 15-min intervals, first in normal saline (115·3 ± 0·9 mM-Na) and then when Na was reduced stepwise on the mucosal side to 2·18 ± 0·74 mM ($N = 14$) by replacement with *N*-methyl-D-glucamine. In other recta, double-barrelled microelectrodes were used to measure intracellular Na activity and potential under conditions that were identical to those during tracer flux experiments. Reducing the net inward gradient for sodium across the apical membrane from 118 ± 0·9 mV to 16 ± 1·3 mV had no significant effect on J^{Cl}_{ms} (from 8·97 ± 1·03 to 9·02 ± 1·36 μequiv cm^{-2} h^{-1}; $\bar{x}\pm$S.E.; $N = 7$; $P > 0{\cdot}2$) and J^{Cl}_{sm} decreased slightly (from 2·06 ± 0·48 to 1·39 ± 0·24 μequiv cm^{-2} h^{-1}; $\bar{x}\pm$S.E.; $N = 7$; $P < 0{\cdot}1$).

Mucosal addition of the inhibitor furosemide had no effect on I_{sc} after 1·5 h exposure ($P > 0{\cdot}2$; Fig. 3B). Note that the cuticular intima was dissected away from the luminal surface to ensure access of the furosemide to the apical membrane in these experiments. Exposure to 1 mM ouabain for 1 h did not reduce the stimulation of J^{Cl}_{net} and I_{sc} by cAMP and actually caused a small but significant stimulation of I_{sc} and J^{Cl}_{net} (0·34 and 0·28 μequiv cm^{-2} h^{-1}; $P < 0{\cdot}01$). This small, ouabain-induced change in I_{sc} is in the direction expected for inhibition of electrogenic Na absorption. Many insect epithelia are relatively insensitive to ouabain (see Harvey *et al.* 1983, this volume); however, homogenates of locust rectum have considerable ouabain-sensitive Na/K ATPase activity ($K_i = 10^{-6}$ M; Peacock, 1981), and intracellular Na and K activities under I_{sc} conditions in the presence of cAMP (8·01 ± 0·41 and 74·8 ± 1·2 mM, respectively) are similar to those for most other cells that possess a basolateral Na/K exchange pump (Hanrahan & Phillips, 1982*b*). These results provide no evidence for a Na-dependent Cl transport mechanism in locust rectum.

Bicarbonate

Transepithelial ^{36}Cl fluxes were measured across short-circuited recta after equilibration in normal saline containing 10 mM-HCO_3 (stirred with 95 % O_2/5 % CO_2), or nominally HCO_3-free saline (stirred with 100 % O_2). Fig. 4 shows that Cl fluxes were not affected by exposure of recta to HCO_3,CO_2-free saline after 7 h. Moreover, there is no reason to propose anion exchange because ΔI_{sc} equals ΔJ^{Cl}_{net} after stimulation.

Could enough HCO_3 be derived from metabolic CO_2 to supply an anion exchange mechanism? Chamberlin (1981) measured O_2 consumption by cAMP-stimulated

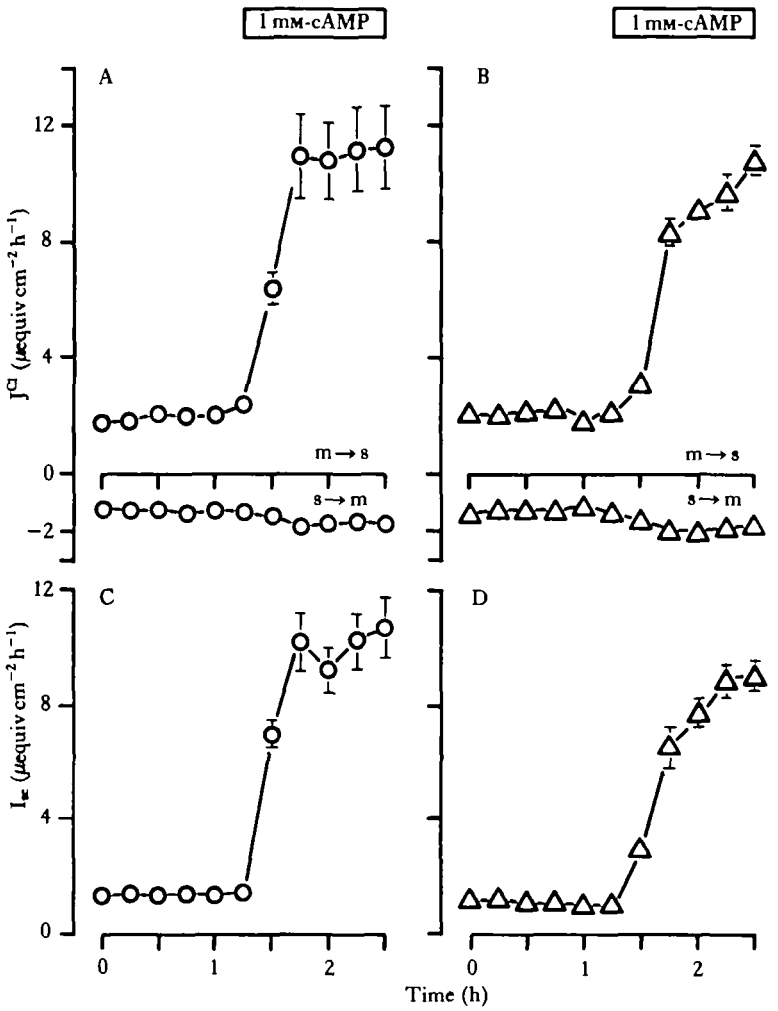

Fig. 4. Effects of 1 mM-cAMP on unidirectional ^{36}Cl fluxes (J^{Cl}_{ms} and J^{Cl}_{sm}) and short-circuit current (I_{sc}) in normal saline containing 10 mM-HCO_3 stirred with 95 % O_2/5 % CO_2 (A, C) and in nominally HCO_3,CO_2-free saline (B, D). There was no difference between cAMP stimulations in the presence or absence of exogenous bicarbonate ($P < 0{\cdot}01$).

recta under virtual short-circuit conditions. Assuming that locust rectum has a maximal respiratory quotient of 1·0 and that all metabolically-derived CO_2 is converted to HCO_3 and is made available to an apical membrane exchange process, the supply of HCO_3 would be ~3·2 μequiv cm^{-2} h^{-1} or only 40 % of J^{Cl}_{net} under nominally HCO_3,CO_2-free conditions (Fig. 4).

Finally, exposure of cAMP-stimulated recta to 1 mM-acetazolamide for 1 h in normal saline had no effect on I_{sc}. Cyclic AMP-stimulated I_{sc} was also insensitive to 1 mM-SITS when added to both sides (Hanrahan & Phillips, 1982*b*).

In summary, data obtained from a variety of different techniques suggest that the major fraction of transrectal Cl transport does not involve a standard Na- or HCO_3-coupled mechanism.

ACTIVE CHLORIDE TRANSPORT IS K-DEPENDENT IN LOCUST RECTUM

Serosal addition of 1 mM-cAMP to recta equilibrated in nominally K-free saline (5·5 h) stimulated J_{net}^{Cl} and I_{sc} to 30 % of controls (Fig. 5A). When K was restored to both sides by bilateral addition of 10 mM-K-methylsulphate, I_{sc} and J_{net}^{Cl} increased to normal levels. We measured the effects of adding K-methylsulphate to only one side of locust rectum and then corrected for potassium diffusion by measuring currents produced by adding K-methylsulphate asymmetrically under nominally Cl-free conditions and to azide-poisoned tissues. Low levels of K (0–10 mM) were stimulatory only when added on the mucosal side. The concentration-dependence of K-stimulated I_{sc} was measured by equilibrating recta for 4 h in K-free saline under I_{sc} conditions, stimulating Cl transport with 1 mM-cAMP on the serosal side, and then adding K-methylsulphate bilaterally. The K-dependent component of I_{sc} was determined by comparing the effects of bilateral K-methylsulphate addition with those of Na-methylsulphate addition. A Lineweaver-Burke plot revealed that ~5·3 mM external K caused half-maximal stimulation of I_{sc}. Fig. 5B shows the net electrochemical

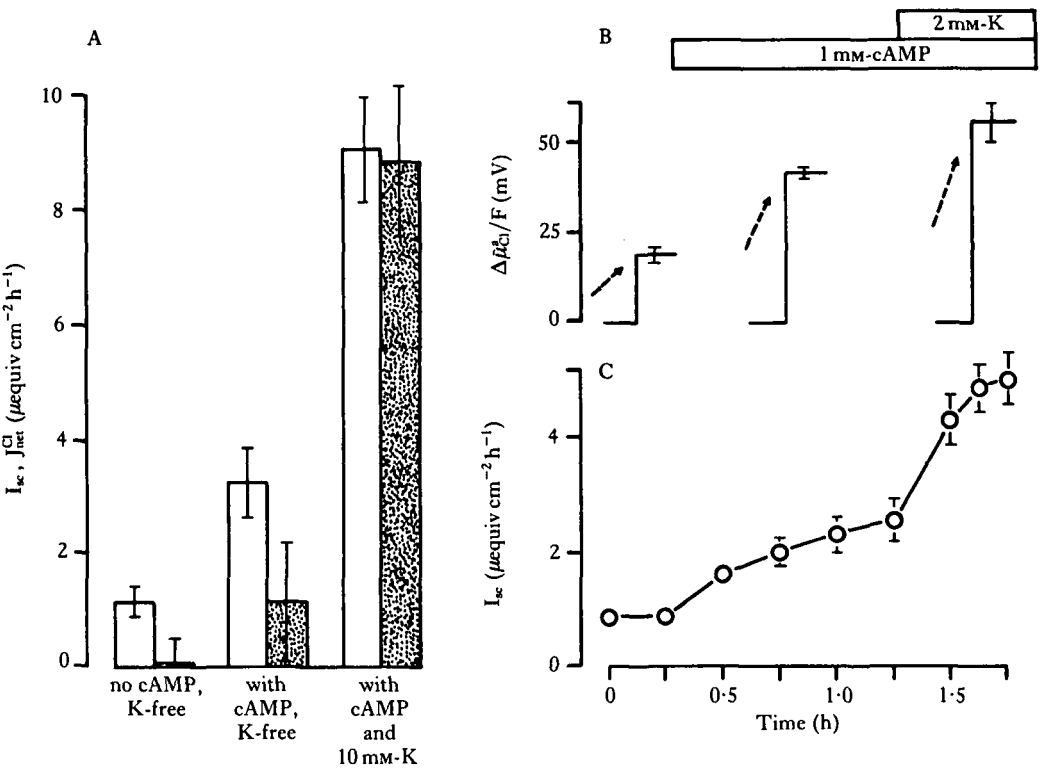

Fig. 5. (A) Short-circuit current (I_{sc}, open bars) and net ^{36}Cl flux (J_{net}^{Cl}, shaded bars) under three sequential conditions: (i) K- and cAMP-free saline, (ii) K-free but with 1 mM-cAMP, and (iii) after addition of 10 mM-K-methylsulphate to both sides in the presence of 1 mM-cAMP. (B) The net electrochemical gradient opposing Cl entry ($\Delta\bar{\mu}_{Cl}^{a}/F$) across the apical membrane was calculated from double-barrelled ion-sensitive microelectrode data and (C) I_{sc} was measured in parallel experiments under the same conditions as in (A). Cyclic-AMP and K apparently both stimulate active Cl entry since they cause simultaneous increases in steady-state I_{sc} and $\Delta\bar{\mu}_{Cl}^{a}/F$ (Hanrahan & Phillips, 1982*a*,*b*).

gradient for chloride across the apical membrane ($\Delta\bar{\mu}^{a}_{Cl}/F$) as calculated fr[redacted] intracellular potential and Cl activity measured (i) in the absence of cAMP and K, (ii) after addition of 1 mM-cAMP and (iii) after addition of 2 mM-K to both sides in the presence of cAMP. Short-circuit current measured in parallel experiments under identical conditions is shown in Fig. 5C. Net Cl flux and $\Delta\bar{\mu}^{a}_{Cl}/F$ opposing Cl entry both increase during exposure to cAMP and are further stimulated by addition of 2 mM-K. Thus, both these agents apparently stimulate active Cl entry directly. While Cl transport is dependent on mucosal K, there is no evidence for KCl co-entry: Firstly, $\Delta\bar{\mu}^{a}_{K}/F$ is only 0·1 mV at 10 mM external K (compared to an opposing $\Delta\bar{\mu}^{a}_{Cl}/F$ of 40 mV) and is in the wrong direction to permit passive K recycling back to the mucosal side at higher concentrations (40–140 mM-K). Recycling must be postulated if KCl co-transport occurs to explain why J^{Cl}_{net} is several times J^{K}_{net} under short-circuit conditions (Hanrahan & Phillips, 1982*a*). Secondly, co-transport with potassium would not supply energy for 'uphill' Cl entry (i.e. although an electroneutral co-transport step would effectively remove the electrical potential opposing chloride entry, KCl influx would then be opposed by an equally large potassium gradient). Lastly, due to the very high rate of Cl transport during cAMP stimulation (~10 μequiv $cm^{-2}h^{-1}$), a large K flux should pass through the postulated KCl pathway if Cl entry occurs by KCl co-transport: thus transepithelial K permeability would be partially Cl-dependent. However, chloride removal had no effect on transepithelial ^{42}K fluxes under I_{sc} conditions (see below and Fig. 7B). At present, the simplest explanation for the effects of potassium on transepithelial Cl transport is that external K enhances electrogenic Cl entry, perhaps by activating an ATPase in the apical membrane, without being co-transported. This scheme would be analagous to enzyme activation in which the K_a for mucosal potassium is 5·3 mM.

Transepithelial chloride absorption is describable by Michaelis-Menten kinetics, but K_t and J^{Cl}_{max} depend on external K concentration. In nominally K-free saline, the transepithelial K_t was 22·7 ± 4·0 mM-Cl and $J^{Cl}_{max} = 3·5 \pm 0·7$ μequiv $cm^{-2}h^{-1}$. When the external K concentration was raised to 100 mM, K_t increased 4·5-fold to 99·6 ± 13·4 mM-Cl and J^{Cl}_{max} was 23·1 ± 5·3 μequiv $cm^{-2}h^{-1}$. Locust rectum may possess a single Cl transport mechanism that is stimulated by K but which also operates slowly under K-free conditions. Alternatively, there may be two systems, one which is K-insensitive and the other made up of pump sites that respond to external K in a graded way or are gradually recruited as [K] is elevated. Regardless of the molecular details, these results show that the major fraction of Cl absorption across locust rectum occurs by an unusual mechanism which is stimulated by mucosal potassium.

CYCLIC-AMP REGULATES PASSIVE PERMEABILITY IN LOCUST RECTUM

There is evidence that locust rectum is a tight epithelium having low electrical resistance (Hanrahan, Phillips & Steeves, 1982). Qualitative evidence for tightness was obtained by briefly exposing tissues to transepithelial single-salt gradients and recording the deflection in transepithelial potential (ΔV_t). If locust rectum is leaky, ΔV_t would be similar regardless of whether the gradient was directed from mucosa-to-serosa or serosa-to-mucosa, because the paracellular pathway does not norma[redacted]

tify. Isosmocity was maintained by the addition of sucrose to low-salt solutions, and all salines were vigorously oxygenated, and contained 1 mM-cAMP and amino acids at their haemolymph concentrations. Results were consistent with high cation selectivity at the mucosal surface and only slight selectivity for K over Cl on the serosal side. This asymmetry would be consistent with transcellular diffusion across apical and basal membranes having different ionic permeabilities rather than through a single paracellular pathway.

The absence of non-selective shunts is further suggested by very low transrectal permeability to $^{35}SO_4$ ($<3{\cdot}5 \times 10^{-7}$ cm s^{-1}). This value is less than 1 % of that expected from measurements of ^{42}K fluxes after correcting for K and Cl mobilities in free solution, on the assumption that K and SO_4 diffuse through the same non-selective shunt pathway (i.e. edge damage). Voltage scanning the epithelial surface while passing large transepithelial currents did not reveal significant shunts between cells, rectal pads or at the edge of the tissue where it attaches to the chamber; however, holes made by deliberately damaging the epithelium were easily detected.

Finally, membrane and paracellular resistances were measured by flat-sheet cable analysis after demonstrating cell–cell coupling with fluorescent dye and electrical methods (Hanrahan *et al.* 1982). Intraepithelial spread of current was measured by injecting current pulses into one cell and measuring the resulting deflections in apical membrane potential at variable distances using a second microelectrode. The data were plotted semi-logarithmically against interelectrode distance and fitted to a Bessel function (see Frömter, 1972). Assuming that locust rectum may be treated as a syncytium (which is supported by demonstration of electrical coupling between cells; Hanrahan, 1982) and may be modelled as a thin flat sheet, apical and basal membrane resistances were calculated to be $220 \pm 30{\cdot}3$ and $193 \pm 12\ \Omega cm^2$, respectively, and paracellular resistance was $742 \pm 141\ \Omega cm^2$. The low macroscopic resistance of rectal cell membranes probably results from extensive infolding, which, according to electron micrographs, should cause between 9- and 200-fold amplification of surface area in various parts of the cell. During exposure to 1 mM-cAMP in normal saline, both apical and basal membrane resistances declined by ~80 % to 44 and 36 Ωcm^2, respectively, without significant change in paracellular resistance. Consequently, the fraction of transepithelial ionic diffusion through paracellular pathways declines from 40 % to ~5 % during exposure to cAMP so that locust rectum effectively becomes 'tighter' during stimulation.

Fig. 6A shows the effects of adding 1 mM-cAMP on transepithelial potential (V_t), and apical and basal membrane potentials (V_a and V_b, respectively) when bathed by normal saline. Deflections in V_t, V_a and V_b were produced by passing constant-current pulses from mucosa-to-serosa: they suggest that transepithelial resistance declines during stimulation with cAMP as a result of reductions in both apical and basal membrane resistances as described above. The decline in basal membrane resistance during cAMP exposure (Fig. 6B) was abolished under Cl-free conditions (compare with Fig. 6C), suggesting that cAMP increases basal membrane Cl conductance. The increase in apical membrane conductance was not dependent on chloride and therefore was not associated with electrogenic ion transport. Furthermore, cAMP reduced R_t under nominally Cl-free conditions when no change in I_{sc} was observed (see below, Fig. 7A).

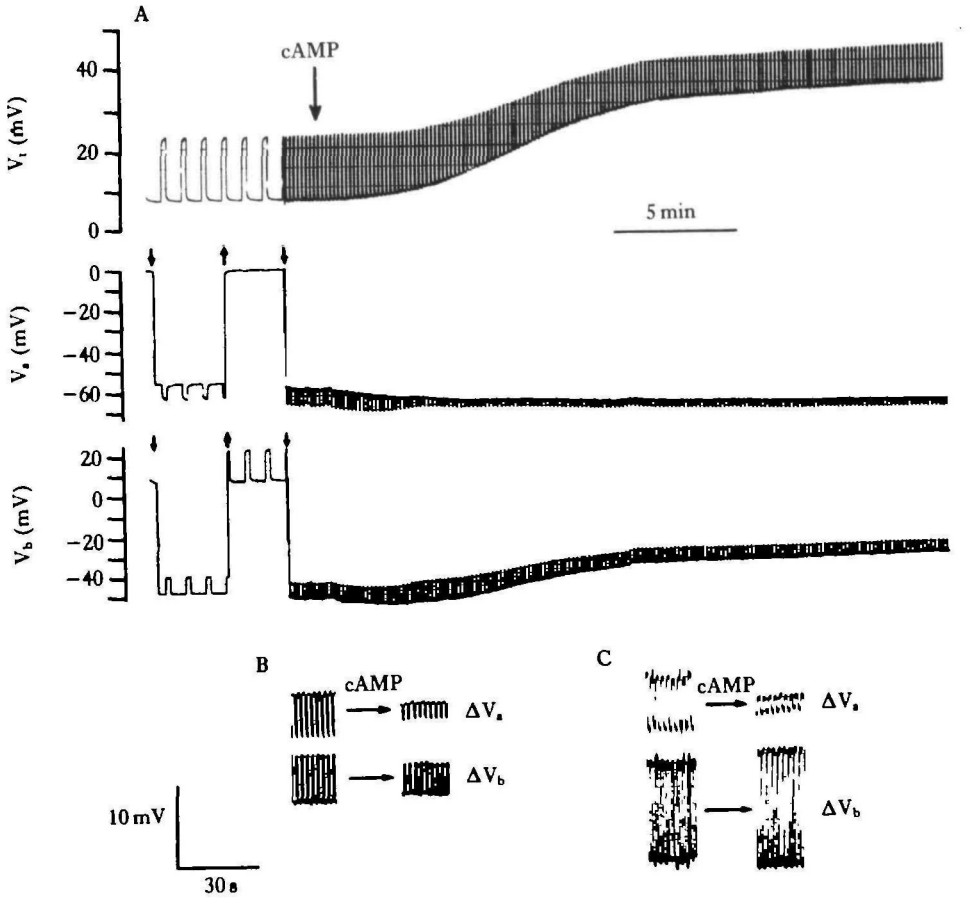

Fig. 6. Effect of 1 mM-cAMP on (A) transepithelial potential (V_t), and apical and basal membrane potentials (V_a and V_b, respectively). Transepithelial resistance declined during cAMP exposure as indicated by the voltage deflections produced during passage of transepithelial constant-current pulses. Deflections in both membrane potentials (ΔV_a and ΔV_b) declined during cAMP exposure in normal saline (B); however, cAMP reduced only the apical deflections under nominally Cl-free conditions (C).

We examined the possibility that cAMP increases apical membrane K permeability. Under Cl-free conditions, the cAMP-induced decline in R_t coincided with ~400% stimulation of transepithelial ^{42}K fluxes (Fig. 7A, B). Restoration of chloride did not stimulate J^K_{ms} or J^K_{sm} although I_{sc} increased ten-fold to control levels. These results indicate that cAMP enhances rectal K permeability in addition to stimulating basal membrane Cl conductance. Consistent with this notion, cAMP did not reduce R_a when K was omitted from 'Cl-free' saline (Hanrahan, 1982).

Hormonal stimulation of potassium conductance would allow K to serve as a more effective counterion for electrogenic Cl absorption. Net Cl absorption is ten-fold higher than J^K_{net} under I_{sc} conditions; however, they are similar under open-circuit conditions ($4{\cdot}3 \pm 0{\cdot}9$ and $4{\cdot}5 \pm 0{\cdot}4$ μequiv cm^{-2} h^{-1}, respectively; N.S., $P > 0{\cdot}2$). The actions of cAMP on electrogenic Cl transport and passive K conductance are thus complementary, and would provide much tighter control over KCl absorption und

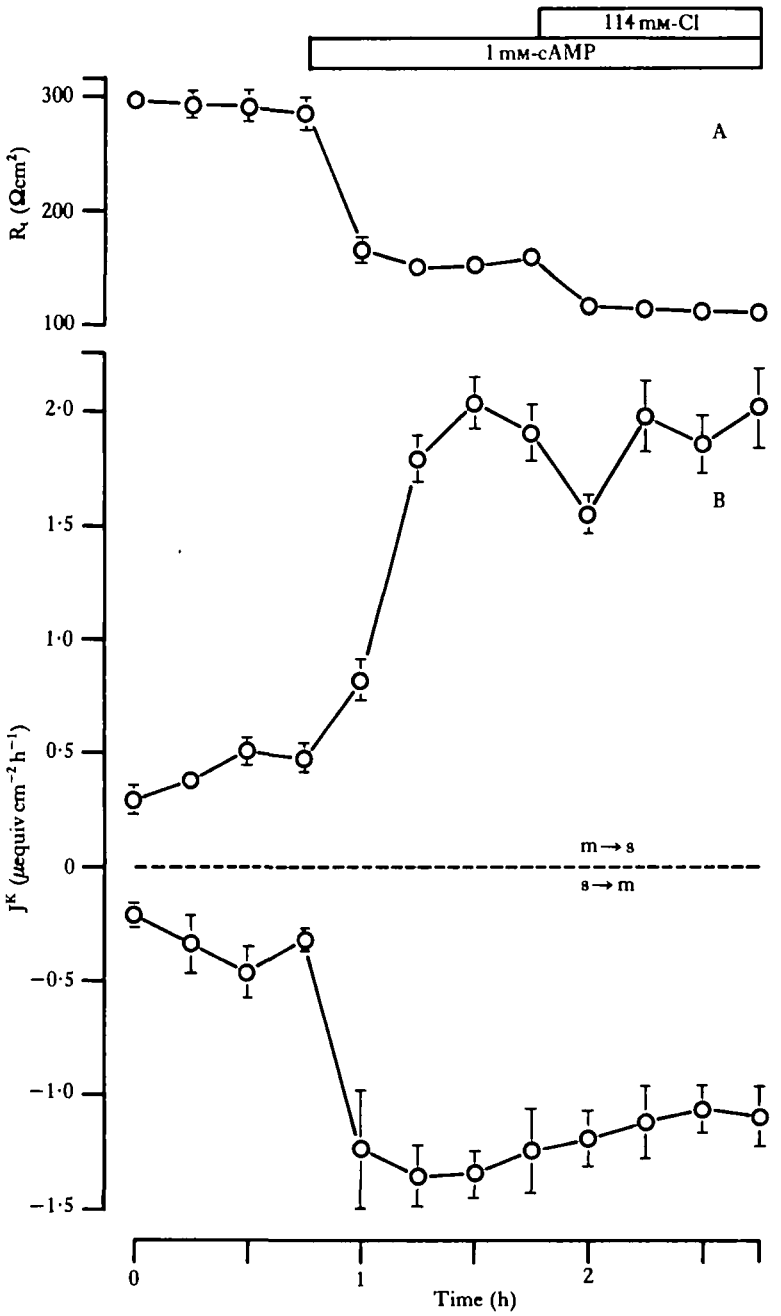

Fig. 7. (A) Transepithelial resistance (R_t) and (B) unidirectional ^{42}K fluxes under I_{sc} conditions in Cl-free saline, and the effects of sequentially adding 1 mM-cAMP and 114 mM-Cl. Tissues were equilibrated in nominally Cl-free saline (methylsulphate substituted); Note that cAMP caused a decrease in R_t in the absence of chloride, and four-fold stimulations of forward and back fluxes of ^{42}K, which were not influenced significantly by addition of Cl.

open-circuit (*in vivo*) conditions than would be possible by stimulating active transport alone.

There is some net absorption of K from mucosa-to-serosa during cAMP stimulation under I_{sc} conditions, but this is low compared to net chloride absorption (J^{K}_{net} = 0·8 μequiv cm^{-2} h^{-1}, J^{Cl}_{net} = 10 μequiv cm^{-2} h^{-1}). It was not possible to determine whether the active step in K transport occurred at the apical or basal membranes because net electrochemical gradients across both membranes favour K flux in the mucosa-to-serosa direction under open-circuit conditions, whereas potassium is near equilibrium across both cell borders under I_{sc} conditions. In any case, it should be emphasized that the major effect of cAMP on K transport is the stimulation of apical membrane K permeability. Under identical conditions, the measured rate of Na transport is low (1 μequiv cm^{-2} h^{-1}) and is unaffected by cAMP (K. Black & J. E. Phillips, unpublished observations).

Local control mechanisms in this epithelium are discussed in detail elsewhere (Hanrahan & Phillips, 1982*b*) but deserve brief mention to complete this summary of our present understanding of locust rectum. Above 100 mM and in the presence of cAMP, K has an increasingly inhibitory effect on Cl transport, and apical membrane K permeability varies inversely with luminal concentration of this cation. Thus excess reabsorption of KCl is automatically prevented should K levels be high in midgut and Malpighian tubule fluid entering the rectum. Finally, the rectal contents can become acidic (pH ~ 4·5) and strongly hyperosmotic (1200 mosmol l^{-1}) *in situ* (Phillips, 1964) and both these conditions inhibit Cl-dependent I_{sc} across cAMP-stimulated locust rectum (Hanrahan & Phillips, 1982*b*; Hanrahan, 1982).

AN EQUIVALENT CIRCUIT MODEL FOR THE LOCUST RECTAL EPITHELIUM

The model shown in Fig. 8A is based on results obtained using a variety of techniques. It features three cAMP-regulated elements: (i) an electrogenic Cl pump at the apical membrane, (ii) an apical membrane K conductance and (iii) a basal membrane Cl conductance. Also shown is a K conductance pathway in the basal membrane which is presumably the route of K exit for transepithelial K absorption under open-circuit conditions and for most K entering the cell from the serosal side through the Na/K exchange pump. Fluctuation analysis has shown this conductance to result from K channels (~150 × 10^6 channels per cm^2 macroscopic area) which are blocked by barium on the serosal side (K_i = 2·9 mM-Ba; Hanrahan, Wills & Lewis, 1983).

If this model is to be consistent with previous ion-sensitive microelectrode and tracer flux data, it should predict the electrical behaviour of locust rectum; for example, the effect of cAMP on V_t under open-circuit conditions, and the intracellular potential of cAMP-stimulated recta under I_{sc} conditions.

Table 1 summarizes the effects of adding 1 mM-cAMP to the serosal side on apical and basal membrane and junctional conductances (G_a, G_b and G_j, respectively) and potentials (V_a, V_b and V_t), intracellular K and Cl activities (a^c_{Cl} and a^c_K, respectively) and electromotive forces (E_a and E_b) calculated as

$$E_a = V_a - V_t G_j / G_a$$

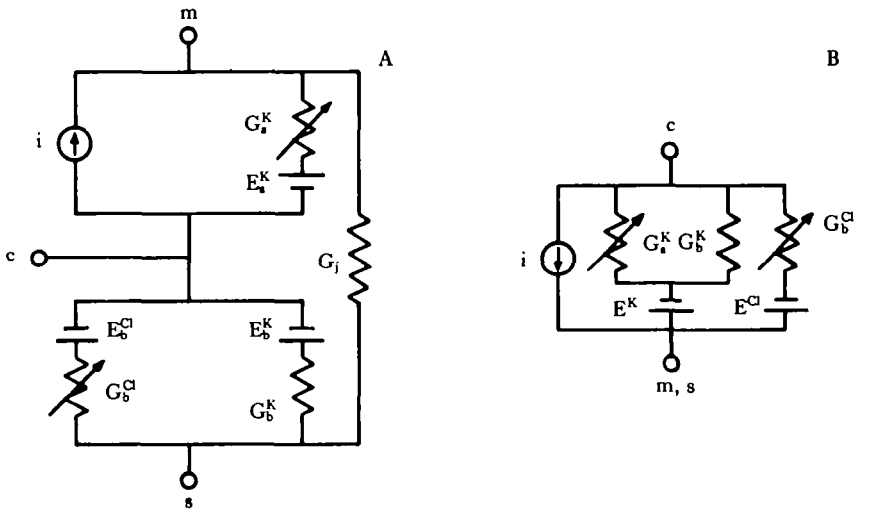

Fig. 8. Equivalent circuit model of locust rectum under (A) open-circuit and (B) short-circuit conditions. m, s and c are the mucosal, serosal and intracellular compartments. An apical electrogenic Cl pump is represented as a current source (i), and cAMP-stimulated conductances (see Table 1 for values) are shown as variable resistors.

Table 1. *Effects of cAMP on electrical properties of locust rectum in normal saline under open-circuit conditions (mean values)*

	Control	1 mM-cAMP
Conductances (mS cm^{-2})		
G_a	4·5	23
G_b	5·2	28
G_j	1·3	1·8
Potentials* (mV)		
V_a	58	70
V_b	51	38
V_t	7	32
Intracellular ion activities (mM)		
K	61	70
Cl	31	47
Calculated equivalent electromotive forces (mV)		
E_a	56·0	67·5
E_b	52·8	40·1

* Values of V_a and V_b are shown referenced to the intracellular compartment and V_t is lumen-positive.

$$E_b = V_b + V_t G_j / G_b \quad (2)$$

The effects of cAMP on E_a and E_b are probably due to stimulation of active Cl transport rather than K permeability because E_a reaches a value during cAMP stimulation that is 12 mV *more negative* than the Nernst potential for K across the apical membrane ($E_a^K = 58{\cdot}2$ mV, $V_a = 70$ mV). Furthermore, Cl-removal in the continued presence of cAMP restores E_a and E_b to approximately their pre-cAMP values (56·3

and 53 mV, respectively). Finally, cAMP stimulations of V_t during these experiments were similar to those observed previously, when ten-fold stimulations of J^{Cl}_{net} were measured using tracers and V_t was obtained from short-circuited preparations during brief interruptions in I_{sc} (from 6·4 ± 0·6 mV to 27·1 ± 1·4 mV; $\bar{x}\pm$S.E., $N = 20$ recta) or from continuously open-circuited preparations (from 8·3 ± 1·2 mV to 24·7 ± 2·2 mV, $\bar{x}\pm$S.E., $N = 12$ recta).

By inspection of Fig. 8A,

$$E_a = E^K_a + i(G_j + G_b)/[G_a(G_j + G_b) + G_jG_b] \quad (3)$$

$$E_b = (E^{Cl}_b G^{Cl}_b + E^K_b G^K_b)/G_b + iG_j/[G_a(G_j + G_b) + G_jG_b] \quad (4)$$

where $i \sim F(J^{Cl}_{net}+J^K_{net}) = 28\ \mu A\ cm^{-2}$ before and 280 $\mu A\ cm^{-2}$ after addition of cAMP, $G_a \sim G^K_a$, and G^{Cl}_b/G_b increases from 0·2 to 0·5 during cAMP exposure. Note that K and Cl are within 23 mV of equilibrium across both membranes; therefore, there should be little difference between slope and chord conductances, at least as a result of Goldman-type rectification (Helman & Thompson, 1982).

From Table 1 and equations 3 and 4, we calculate that E_a would increase from 59·8 mV to 69·5 mV and E_b would decrease from 54·1 mV to 37·0 mV during exposure to cAMP. These values are in good agreement with those based solely on electrical measurements (Table 1).

Transepithelial potential (V_t) was predicted using E_a and E_b from equations 3 and 4 according to:

$$V_t = (E_a - E_b)(G_aG_b)/[G_j(G_a + G_b) + G_aG_b]\,. \quad (5)$$

Using this equation, V_t was calculated to be 3·7 mV before addition of 1 mM-cAMP and 29·7 mV after addition, in agreement with the values obtained experimentally (see Table 1).

Under I_{sc} conditions, apical and basal membranes are effectively in parallel and may be represented as shown in Fig. 8B. Intracellular voltage during cAMP stimulation (V_i) is then:

$$V_i = [E^{Cl}G^{Cl}_b + E^K(G^K_a + G^K_b) + i]/(G_a + G_b) \quad (6)$$

where $i = J^{Cl}_{net}F = 280\ \mu A\ cm^{-2}$, $E^{Cl} = 41{\cdot}4$ mV and $E^K = 57{\cdot}4$ mV as calculated from the Nernst equation using the intracellular activities of Cl (40·3 mM) and K (74·8 mM) which were measured experimentally under I_{sc} conditions (Hanrahan, 1982). Assuming that conductances are similar under open- and short-circuit conditions and that J^{Cl}_{net} approximates the pump current, the intracellular potential predicted from equation 6 under I_{sc} conditions (58·4 mV) is also in good agreement with that measured experimentally (57·5 ± 0·4 mV, $\bar{x}\pm$S.E., 66 cells, 4 recta; Hanrahan, 1982).

In summary, the circuit models shown in Fig. 8 are internally consistent in that e.m.f. values calculated from ion activities and tracer fluxes agree well with those calculated strictly from electrical data. These models also seem realistic since they accurately predict the effects of cAMP on transepithelial potential and the intracellular potential in short-circuited tissues.

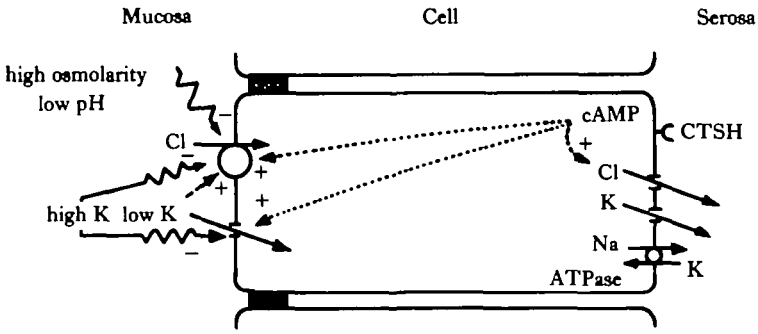

Fig. 9. Proposed mechanism of KCl absorption and regulation in locust rectum. Active Cl entry is enhanced directly by low mucosal K and by intracellular cAMP (+), which is elevated by the peptide hormone CTSH (chloride transport-stimulating hormone). Cyclic-AMP also stimulates passive K entry across the apical membrane, passive Cl exit, and active K absorption, although the location of the active step in K transport is not known. High K and osmolarity, and low pH, in the lumen all inhibit (−) Cl transport and/or K conductance (⇝).

CONCLUSIONS

Fig. 9 shows the mechanism of KCl absorption proposed for locust rectum. The rate of KCl transport depends on mucosal K concentration because K directly stimulates active Cl entry across the apical membrane (Fig. 5A, B; Hanrahan & Phillips, 1982*b*). Interestingly, mucosal potassium indirectly enhances its own passive reabsorption by stimulating electrogenic Cl transport, since K is the counterion for active Cl absorption under open-circuit conditions (Hanrahan & Phillips, 1982*a*). Simultaneous hormonal control of active Cl transport and passive K permeability by the hormone CTSH would provide remarkably efficient control over salt reabsorption and presumably ionic regulation in the desert locust.

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