J. exp. Biol. **116**, 153–167 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

FURTHER OBSERVATIONS ON THE REGULATION OF KCl ABSORPTION ACROSS LOCUST RECTUM

BY J. W. HANRAHAN* AND J. E. PHILLIPS

Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 2A9, Canada

Accepted 17 September 1984

SUMMARY

1. Electrophysiological and tracer flux techniques were used to study regulation of KCl reabsorption across locust recta. Physiologically high K^+ levels (100 mmol l^{-1}) on the lumen side stimulated net ^{36}Cl flux and reduced the theoretical energy cost of anion transport under open-circuit conductions.

2. The stimulation of short-circuit current (I_{sc}, i.e. active Cl^- absorption) by crude corpora cardiaca extracts (CC) was not dependent on exogenous Ca^{2+}. Stimulations of I_{sc} were greatly enhanced in the presence of theophylline, indicating that the rate of synthesis of cAMP is increased by CC extracts. High CC levels lowered transepithelial resistance (R_t), suggesting that chloride transport stimulating hormone (CTSH) regulates both active Cl^- absorption and counter-ion (K^+) permeability.

3. High mucosal osmolarity or K^+ concentration decreased I_{sc} and caused a disproportionately large increase in R_t, consistent with a decrease in the shunt (K^+) conductance. Measurements of relative mucosal-to-serosal membrane resistance confirmed that high mucosal K^+ levels reduced apical membrane conductance. Lowering mucosal pH to values observed *in vivo* at the end of resorptive cycles also inhibited I_{sc}, apparently without affecting K^+ permeability.

INTRODUCTION

Active reabsorption of chloride in locust rectum is regulated by a neuropeptide hormone (CTSH) (Spring, Hanrahan & Phillips, 1978; Spring & Phillips, 1980*a*,*b*; Hanrahan & Phillips, 1983) and also by luminal K^+ concentration (Hanrahan & Phillips, 1984*a*). However, KCl absorption depends not only on the rate of active Cl^- transport (J^{Cl}_{net}) but also on the transepithelial conductance to the major counter-ion, which passively follows Cl^- to maintain electroneutrality. Hanrahan & Phillips (1984*a*) have shown that K^+, which enters the rectal lumen at high concentrations *in vivo* (140 mmol l^{-1}), is the predominant counter-ion (i.e. shunt pathway) for electrogenic Cl^- absorption in this tissue. CTSH, acting *via* intracellular cAMP,

* Present address: Department of Physiology, Yale University Medical School, New Haven, Conn. 06510, U.S.A.

Key words: Locust rectum, potassium chloride absorption, regulation.

enhances transepithelial K^+ permeability (P_K), but this action is inversely proportional to luminal K^+ concentration so that excessively high luminal [K^+] actually limits KCl absorption rate (Hanrahan & Phillips, 1984*a*).

In this paper we examine hormonal regulation further, providing evidence that CTSH acts by stimulating cAMP synthesis and that extracellular Ca^{2+} is not required for CTSH stimulation. *In vitro* studies reveal that luminal pH, osmotic concentration and K^+ levels within the physiological range influence P_K and net transport of chloride (J^{Cl}_{net}). These results suggest that local conditions in the lumen exert some control over the rate of KCl absorption *in situ* in addition to that exerted by CTSH. Finally, by measuring open-circuit $^{36}Cl^-$ fluxes under different conditions, we show that the theoretical energy cost of Cl^- reabsorption is reduced by exploiting the large electrochemical gradient for K^+ (>70 mV) generated by the Malpighian tubules. In this sense, the tubules could be argued to be driving a component of rectal reabsorption.

METHODS

Experimental animals, physiological salines, voltage-clamp methods for studying recta mounted as flat sheets in Ussing chambers, and radiotracer flux measurements are described by Hanrahan & Phillips (1983, 1984*a*). In some experiments, sucrose, which is not metabolized by this tissue (Chamberlin & Phillips, 1982), was used to raise the total osmotic concentration of the normal saline which resembled locust haemolymph. Phosphate (20 mmol l^{-1}) was used to buffer normal saline when testing the influence of external pH on electrical parameters across locust rectum. Bicarbonate-free saline was used during microelectrode experiments to reduce intracellular levels of bicarbonate, which interferes with measurement of intracellular Cl^- activity. Bathing salines were stirred with 95 % O_2:5 % CO_2, except that 100 % O_2 was used with bicarbonate-free salines. In some experiments, Ca-ionophore (A23187, a gift of Dolman, Eli-Lilly Canada) dissolved in ethanol was added in small amounts (< 0·25 % v/v) to Ca-free normal saline containing 2·5 mmol l^{-1} EGTA to achieve a final concentration of 1 μg ml^{-1}. Experiments were conducted at 22 ± 1 °C. Maximum stimulation of recta was achieved with 1 mmol l^{-1} cAMP on the serosal side when required.

Intracellular microelectrode measurements

For microelectrode studies, recta were mounted in a Plexiglas chamber (Hanrahan, 1982; see Hanrahan, Meredith, Phillips & Brandys, 1983), and each side was perfused independently by gravity feed. Transepithelial potential (V_t) was measured using a high input impedance amplifier connected to agar bridges through Ag/AgCl wires. Constant-current pulses (20 μA) were passed transepithelially using silver foil electrodes at either end of the chamber connected to a wave form/pulse generator (Type 160, Tektronix, Beaverton, Ore.). Cells were impaled from the mucosal side (cuticle cut) at an angle of 30–40° by a double-barrelled microelectrode mounted on a

Leitz micromanipulator (Wetzler, F.R.G.). Compensation for series resistance of the saline was made by focusing the microscope just below the tissue surface, then removing the tissue and repositioning the microelectrode in the plane of focus. Deflections in the potential (recorded differentially) between mucosal agar bridge and microelectrode, and between serosal agar bridge and microelectrode, were subtracted from measurements made with the microelectrode located intracellularly to calculate apical and basal membrane potentials (V_a and V_b, respectively). To prevent mixing when calibrating the series resistance compensation with high K-solutions present only on the mucosal side, a piece of rectal cuticle was mounted on the chamber opening. The ratio of deflections in apical and basal membrane potentials (V_a and V_b respectively) produced by transepithelial current pulses was used to calculate the voltage divider ratio, α, as:

$$\Delta V_a/\Delta V_b = R_a/R_b = \alpha, \quad (1)$$

where R_a and R_b are apical and basal membrane resistances respectively, and transepithelial resistance (R_t) in these experiments was calculated as:

$$R_t = (\Delta V_a + \Delta V_b)/I_t = 1/G_t, \quad (2)$$

where I_t and G_t are transepithelial current and conductance respectively.

Intracellular Cl^- and K^+ activities were also measured under these conditions and are reported in detail elsewhere (Hanrahan, 1982; Hanrahan & Phillips, 1984*b*).

RESULTS

Observations related to hormonal control mechanisms

We have reported evidence that the neuropeptide, CTSH, from locust corpus cardiacum (CC) acts *via* elevation of cAMP levels in rectal tissue to stimulate electrogenic Cl^- transport and passive absorption of K^+ (reviewed by Phillips & Hanrahan, 1984). In most cells, cAMP levels are maintained through the actions of two enzymes, adenylate cyclase (synthesis) and phosphodiesterase (degradation). Either enzyme might be controlled by CTSH. The phosphodiesterase inhibitor, theophylline, is by itself a potent stimulant of rectal I_{sc} (Spring *et al.* 1978), which suggests that the rate of increase in rectal I_{sc} following addition of theophylline reflects the normal rate of cAMP synthesis when CTSH is absent. If CTSH acts on adenylate cyclase to enhance cAMP synthesis, then the rate of increase in rectal I_{sc} should be enhanced after adding CTSH when phosphodiesterase is inhibited by theophylline. As shown in Fig. 1, adding CTSH to recta bathed in normal saline containing theophylline caused the rate of rise in I_{sc} (i.e. dI_{sc}/dt) to increase five-fold from 448 ± 57 to 2640 ± 374 nA cm^{-2} min^{-1} ($\bar{x} \pm$ S.E., $N = 4$), indicating that CTSH stimulates adenylate cyclase to increase cAMP levels. However, we cannot exclude an additional action of CTSH on phosphodiesterase.

We found that stimulation of rectal I_{sc} by 1 mmol l^{-1} cGMP ($\Delta I_{sc} = 9{\cdot}9 \pm 0{\cdot}7$ μequiv cm^{-2} h^{-1}) and 1 mmol l^{-1} cAMP ($8{\cdot}8 \pm 1{\cdot}4$ μequiv cm^{-2} h^{-1}) were not

significantly different ($P > 0{\cdot}2$). Because the second messenger action of cGMP is often intimately associated with that of intracellular Ca^{2+} (Berridge, 1979), we attempted to alter intracellular Ca^{2+} levels by bathing short-circuited recta in Ca-free saline containing 2·5 mmol l^{-1} EGTA and the Ca^{2+} ionophore (A23187) for 1 h before stimulation with CC extract. As shown in Table 1B, this treatment had no effect on the normal stimulation of chloride-dependent I_{sc} by CC containing CTSH. Either CTSH action on I_{sc} is not influenced by changes in external or internal Ca^{2+} levels, or the intracellular Ca^{2+} pool in rectal tissue is not easily perturbed by methods which have been successful in other tissues. Further studies with intracellular Ca-sensitive microelectrodes are needed to distinguish between these two possibilities.

Stimulation of recta with cAMP causes a substantial drop in transepithelial resistance, which is K-dependent (Hanrahan & Phillips, 1983, 1984*a*,*b*). However,

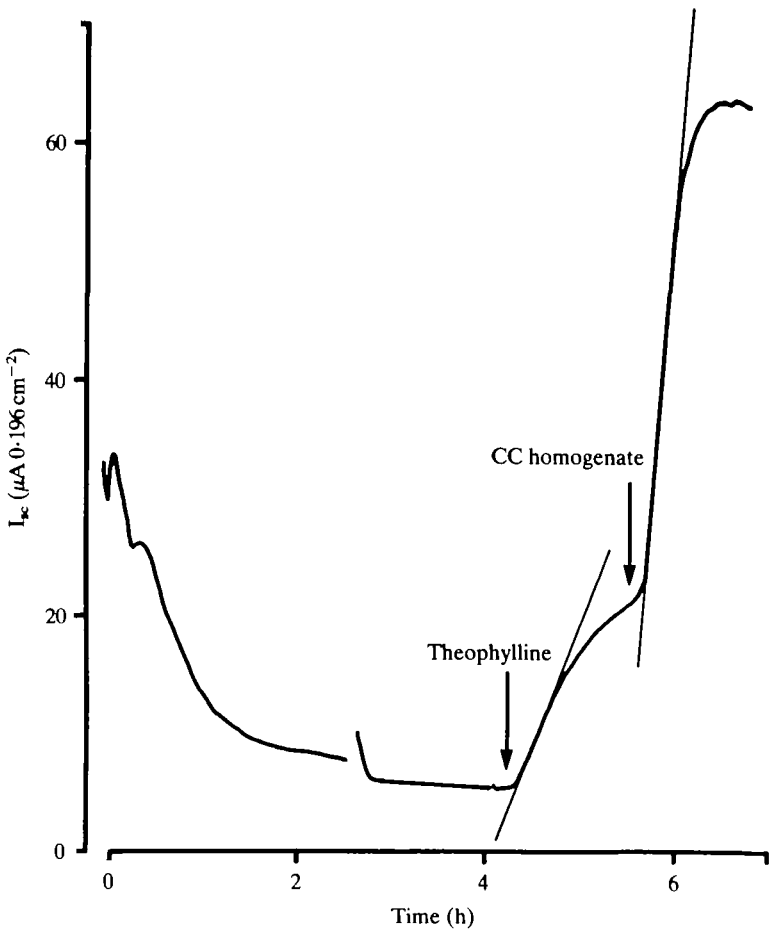

Fig. 1. Effects of sequential serosal addition of 4 mmol l^{-1} theophylline and corpus cardiacum homogenate (CC: 1 gland pair/5 ml) on I_{sc} across a typical rectal preparation (mean ± S.E. in text). Straight lines indicate initial rate of rise in I_{sc}.

Spring & Phillips (1980*a*) reported only a slight change in rectal R_t during stimulation with CC extracts. We therefore reinvestigated this anomaly using a more adequate physiological saline and with correction for series resistance of the saline (not done by Spring & Phillips, 1980*a,b*).

As shown in Table 1A, we observed only a small decrease in R_t when the dosage of CC was the same as that used by Spring & Phillips (1980*a*), i.e. 0·016 CC pairs ml^{-1}. However, increasing the CC dosage beyond that required for near maximum stimulation of I_{sc} caused increasingly large decreases in R_t (Table 1A).

Effects of high external osmotic concentrations

Fig. 2 shows the effects of varying mucosal and serosal osmotic concentrations over a wide range (364–1220 mosmol l^{-1}) on stimulated recta. The lower of these values is somewhat less than that of Malpighian tubule fluid collected *in situ* (420 mosmol l^{-1}; Phillips, 1964*a,c*) and the normal physiological saline used in these studies (440 mosmol l^{-1}). The higher value is still below that measured for rectal contents in dehydrated desert locusts (Phillips, 1964*a*). Perfusion with hyposmotic saline (364 mosmol l^{-1}) on both sides had no significant effect on I_{sc} and R_t; however, both I_{sc} and transepithelial conductance (G_t) declined by about 40 % when hyperosmotic saline (1220 mosmol l^{-1}) was present on the mucosal side only, or on both sides. In normal saline, chloride activity was 81·9 mmol l^{-1}, as measured using an ion-sensitive microelectrode. When mucosal osmotic concentration of the saline was increased to 1220 mosmol l^{-1} by adding sucrose, saline Cl^- activity decreased to 75·4 mmol l^{-1}. Based on the kinetics of Cl^- transport across locust rectum described previously (Hanrahan & Phillips, 1984*a*), the 40 % inhibition of rectal I_{sc} at high osmotic concentrations cannot be attributed simply to a reduction in the external Cl^- activity coefficient, which would only reduce I_{sc} by 5 %. Similarly, additional passive backflux

Table 1. *Influence of corpora cardiaca homogenates (CC) on electrical parameters across recta in normal and Ca-free salines*

Treatment	I_{sc} (μequiv cm^{-2} h^{-1})	V_t(mV)†	R_t(Ωcm^2)
Normal saline unstimulated control	1·9 ± 0·4	8·9 ± 2·0	177 ± 9·8
(A) CC added to normal saline (gland pair ml^{-1})			
0·016	7·1 ± 2·0	29·0 ± 7·0	163 ± 14·4
0·20	9·6 ± 2·2	34·0 ± 7·1	133 ± 13·0
0·40	9·3 ± 2·0	31·0 ± 6·6	124 ± 11·7
(B) Ca-free saline with 2·5 mmol l^{-1} EGTA and Ca-ionophore* unstimulated	2·0 ± 0·1	8·6 ± 1·6	146 ± 11·0
CC added (0·20 gland pair ml^{-1})	10·0 ± 0·5	26·7 ± 1·9	100 ± 8·1

Means ± S.E. ($N = 7$ in A, $N = 4$ in B).
* A23187 (1 μg ml^{-1}).
† V_t, the lumen side positive.

of Cl^- due to small differences in ion activities across the rectal wall under these conditions was too small to alter I_{sc}. These considerations suggest that the reduction in active Cl^- transport (i.e. ΔI_{sc}) is due directly to exposure of recta to high mucosal osmolarity in the range that occurs *in situ*. Moreover, a large decrease in leak or 'shunt' conductance must also occur under these hyperosmotic conditions, because R_t is 70 % higher than the value predicted by the relationship between I_{sc} and G_t during cAMP stimulation (dotted line in Fig. 2). This change is not due to a decrease in saline

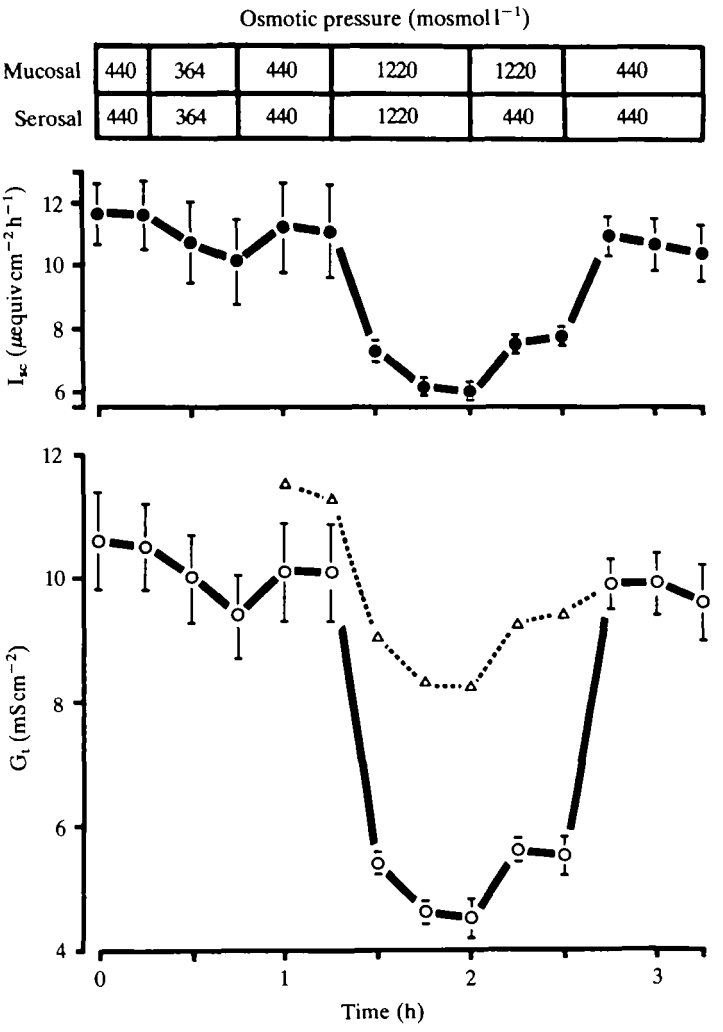

Fig. 2. Influence of mucosal and serosal osmotic pressure on I_{sc} and transepithelial conductance (G_t). Mucosal and/or serosal osmotic concentration was elevated by adding sucrose. Dotted line shows values of G_t which are predicted from the normal relationship between I_{sc} and G_t due to changes in saline conductivity. The much lower values of G_t observed at high osmotic pressures indicate that passive permeability of the epithelium is reduced under these conditions. Means ± S.E.; $N = 7$.

conductivity because compensation was made for saline resistance. Since K^+ is the counter-ion for Cl^- transport and is the main ion contributing to the shunt conductance (Hanrahan & Phillips, 1983, 1984*a*), this large decrease in G_t caused by high osmotic pressure is probably due to a decline in K^+ conductance.

Inhibition of transepithelial K permeability by high [K]: localization of the K concentration effect

In recta of salt-depleted hydrated locusts, luminal K^+ concentration declines during reabsorption from about 150 to 0·5 mmol l^{-1} (Phillips, 1964*b*,*c*; Hanrahan,

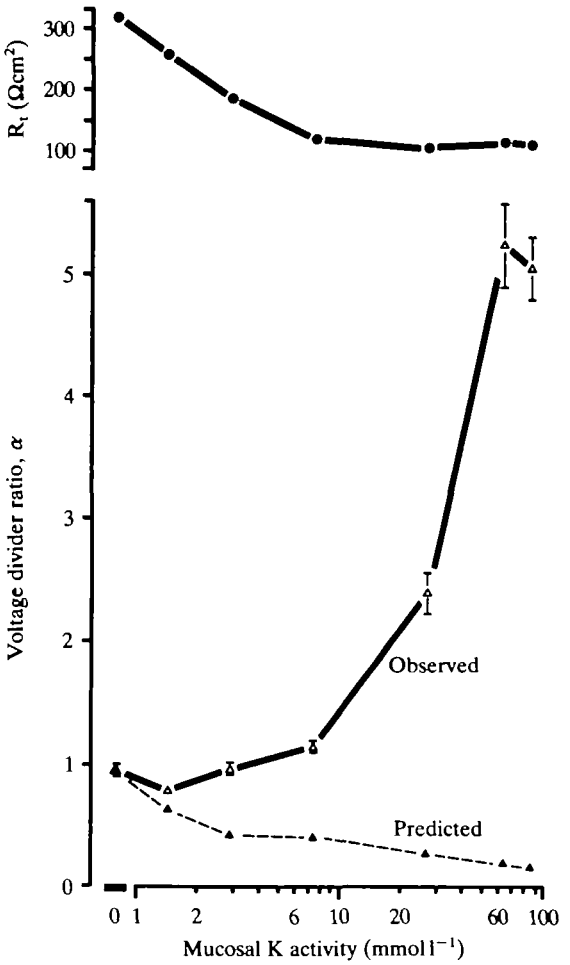

Fig. 3. Relationship between mucosal K^+ concentration and the voltage divider ratio (α) measured in cAMP-stimulated recta during serosal perfusion of one rectal preparation with normal saline ($\bar{x}$ + S.E.; 9–10 impalements at each mucosal K activity). For five preparations, α increased 3·5-fold after K addition ($P \ll 0{\cdot}005$). Voltage divider ratios under these conditions were also predicted as explained in the text, assuming no change in apical or basal Cl^- and K^+ conductances.

1982). The apparent potassium permeability (P_K) was calculated from the mucosal-to-serosal flux of $^{42}K^+$ across stimulated recta under I_{sc} conditions (Hanrahan & Phillips, 1984*a*). The calculated P_K was approximately 5×10^{-5} cm s^{-1} when external K^+ was 0–10 mmol l^{-1}; however, P_K declined drastically when $[K^+]$ was increased above this range. The decrease in P_K was half-maximal between 10 and 40 mmol l^{-1} K^+, and was maximal at 100 mmol l^{-1} K^+. These results were consistent with other findings, e.g. that high K^+ addition does not reduce R_t as anticipated (Hanrahan & Phillips, 1984*a*). This decline in P_K could occur at the apical or basal membrane or both, since K was added to both sides of the tissue in these earlier experiments.

In this section, we derive a simple equation that allows comparison of the measured values for the voltage divider ratio (α) with those predicted if K^+ and Cl^- permeability coefficients remain constant at both membranes. Deviation from the predicted behaviour can then be used as an indication of changes in ionic permeability. Our method assumes that locust rectum is a tight epithelium. In this regard, flat-sheet cable analysis has revealed that locust rectum is indeed a very tight epithelium: during cAMP stimulation, 96% of transepithelial ionic diffusion occurs transcellularly (Hanrahan, Phillips & Steeves, 1982; Hanrahan & Phillips, 1984*b*). Also, sodium is ignored in these calculations because Na^+ conductance is a minor fraction of apical and basal membrane conductance (less than 15% and 7%, respectively, according to cable analysis; Hanrahan, 1982). Our estimation follows from the fact that ionic conductance (G_i) is proportional to the product of membrane permeability (P_i), logarithmic mean ionic activity ($\bar{a}_i$), and a constant (Z^2F^2/RT):

$$G_i = P_i\bar{a}_i\frac{Z^2F^2}{RT}. \tag{3}$$

Logarithmic mean activities were calculated as $(\bar{a}_i^c-\bar{a}_i)/\ln(\bar{a}_i^c-\bar{a}_i)$, where $\bar{a}_i^c$ is the intracellular ionic activity and $\bar{a}_i$ is the external ionic activity. Potassium and chloride activities (a_K, a_{Cl}) were measured under these conditions using ion-sensitive microelectrodes (see Hanrahan & Phillips, 1984*b*). For each mucosal [K],

$$\alpha=\frac{G_b}{G_a}=[(\bar{a}_K^bP_K^b+\bar{a}_{Cl}^bP_{Cl}^b)/(P_K^b+P_{Cl}^b)]/[(\bar{a}_K^aP_K^a+\bar{a}_{Cl}^aP_{Cl}^a)/(P_K^a+P_{Cl}^a)], \tag{4}$$

where P_K^a and P_K^b are the K permeabilities of apical and basal membranes respectively, P_{Cl}^a and P_{Cl}^b are the corresponding Cl permeabilities, and G_a and G_b are the conductances of the two membranes.

Fig. 3 shows the effects of mucosal K^+ addition on measured α and on the values of α predicted using equation 4. When mucosal K^+ activity was elevated, α increased five-fold instead of decreasing as predicted if permeability coefficients (i.e. P_K^a and P_{Cl}^a) remained constant. It might be argued that higher values of α could result from a decline in R_b rather than an increase in R_a (see equation 1). Certainly some decline in R_b is expected since intracellular ion activities increase when mucosal K^+ level is elevated (Hanrahan, 1982). Nevertheless, there must still be an increase in R_a when mucosal K^+ is increased, even if R_b does decline, because R_t remains constant at 110–120 Ω cm^2 when K^+ concentration is varied between 6 and 100 mmol l^{-1} (Fig.

3). Since most of the apical membrane conductance is due to K^+ (90% during cAMP stimulation, Hanrahan, 1982), the increase in R_a during mucosal K^+ addition indicates a decline in K^+ permeability of the apical membrane. This would also explain the large decline in transepithelial K^+ permeability, calculated from ^{42}K fluxes, which was reported previously after raising [K] (Hanrahan & Phillips, 1984*a*).

Effects of external pH

The pH of luminal contents can be as low as 4·5 in the locust rectum *in situ* (Phillips, 1964*b*; Speight, 1967). Fig. 4 shows the effects of varying mucosal pH on stimulated recta. Reductions of mucosal pH over the range 7·0 to 4·0 reversibly reduced I_{sc} and V_t, and increased R_t. In contrast, V_t, R_t and I_{sc} were surprisingly insensitive to similar changes in pH (3·0–8·0) on the serosal side, even though haemolymph pH was relatively constant *in vivo* (7·1 ± 0·04, $\bar{x} \pm$ S.E., $N = 6$). Possible explanations for the effects of mucosal pH on I_{sc} are given in the Discussion.

Effects of cAMP and mucosal K^+ addition on open-circuit $^{36}Cl^-$ fluxes

All previous measurements of $^{36}Cl^-$ fluxes from our laboratory were for short-circuited recta bathed bilaterally in identical salines, whereas there is a large K^+ concentration difference (140:10 mmol l^{-1}) across this epithelium *in vivo* (i.e. open-circuit state). To evaluate the influence of luminal K^+ on Cl^- absorption under more physiological conditions, we measured $^{36}Cl^-$ fluxes across recta mounted in Ussing chambers in the open-circuit state and initially in normal saline (10 mmol l^{-1} K^+). We then sequentially stimulated with cAMP and increased luminal K^+ to 100 mmol l^{-1} (haemocoel side still 10 mmol l^{-1}). The results are shown in Fig. 5, which also compares the observed flux ratios with those predicted for passive diffusion of $^{36}Cl^-$ using the Ussing flux-ratio equation. Addition of cAMP caused a three-fold increase in V_t and large increases in the mucosal-to-serosal flux of $^{36}Cl^-$ and in the flux ratio, which exceeded the value predicted for diffusion by about 10-fold. After adding cAMP, the back flux (J_{sm}^{Cl}) under open-circuit conditions (1·34 ± 0·06 μequiv $cm^{-2} h^{-1}$; Fig. 5) was not very different from that under short-circuit conditions (1·71 ± 0·12 μequiv $cm^{-2} h^{-1}$, Hanrahan & Phillips, 1983); however, J_{ms}^{Cl} was significantly lower in the open-circuit state (4·75 ± 0·7 μequiv $cm^{-2} h^{-1}$ *vs* 11·04 ± 0·64 μequiv $cm^{-2} h^{-1}$; $P < 0·001$). When K methylsulphate was added to the mucosal side to raise K^+ from 10 to 100 mmol l^{-1} and thereby mimic *in vivo* conditions, V_t decreased by 92% (i.e. by 23 mV), J_{ms}^{Cl} increased by 38%, but J_{sm}^{Cl} remained unchanged ($P > 0·2$), and the $^{36}Cl^-$ flux ratios were even higher than when luminal K^+ was 10 mmol l^{-1}.

DISCUSSION

The dose-response curves for the actions of cAMP on I_{sc} (Cl^- transport increase) and on resistance across locust rectum are quantitatively similar (Hanrahan & Phillips, 1984*b*). Since there is good evidence that cAMP is the second messenger for

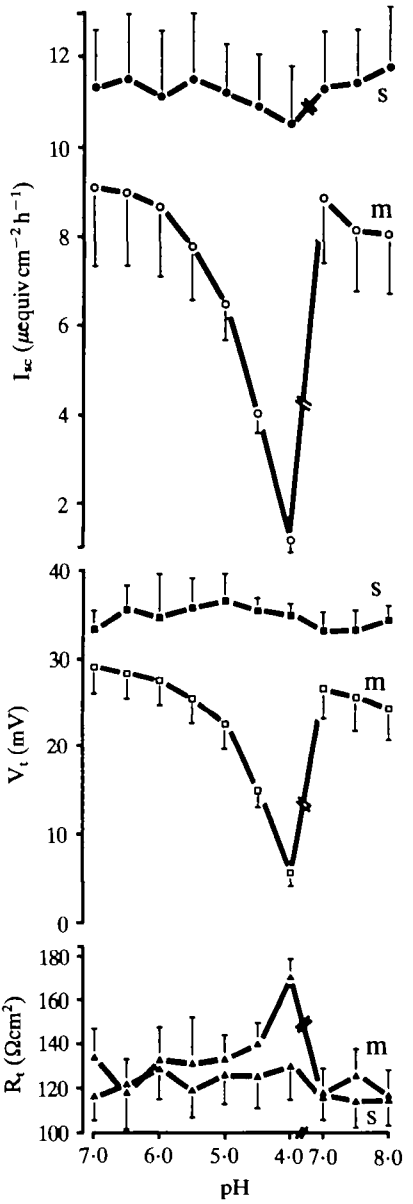

Fig. 4. Effects of external pH on transepithelial electrical parameters in cAMP-stimulated recta. Mucosal (m) and serosal (s) pH were varied in separate experiments. I_{sc}, V_t and R_t were determined after 30 min exposure to each external pH. Tissues were bathed bilaterally in normal saline containing 20 mmol l^{-1} phosphate. Means ± S.E.; $N = 8$ (pH on mucosal side), $N = 6$–8 (pH on serosal side).

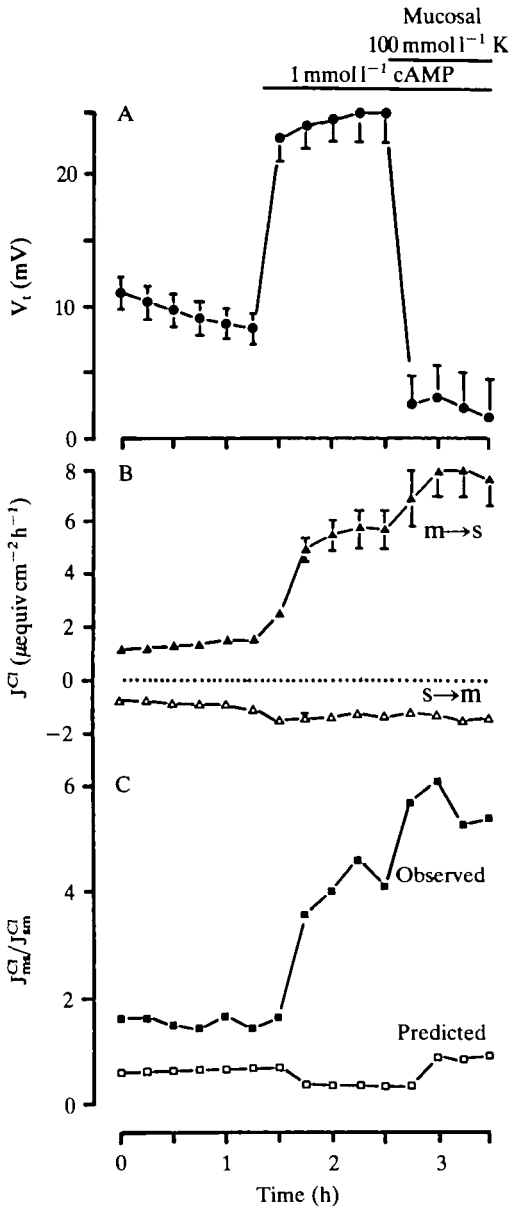

Fig. 5. Effects of sequential addition of 1 mmol l^{-1} cAMP to the serosal side, and mucosal addition of potassium, on $^{36}Cl^-$ fluxes under open-circuit conditions. Potassium concentration was increased from 10 to 100 mmol l^{-1} by adding K-methylsulphate to the mucosal side. (A) Transepithelial potential (● V_t); (B) unidirectional $^{36}Cl^-$ fluxes from mucosa to serosa (▲m→s), and serosa to mucosa (△s→m; $N = 6$); and (C) $^{36}Cl^-$ flux ratios (■) observed and (□) predicted from V_t using the Ussing flux ratio equation. Means ± S.E.; $N = 12$ (V_t), $N = 6$ (J^{Cl}_{ms}) and (J^{Cl}_{sm}).

CTSH, it was therefore surprising to observe (Table 1) that rectal I_{sc} is considerably more sensitive to crude CC homogenates than is rectal conductance. This result suggests that CTSH may have other actions in addition to that of increasing intracellular cAMP levels, or there may be other factors in crude CC homogenates that suppress the stimulatory action of CTSH on P_K. Studies with purified CTSH will be required to decide between these explanations.

Towards the end of a reabsorptive cycle in intact dehydrated locusts, the rectal contents are strongly hyperosmotic and sometimes quite acidic (Phillips, 1964*a*,*b*). Experiments in this paper indicate that both low pH and high osmolarity in the lumen directly inhibit Cl-dependent I_{sc} (i.e. active chloride transport) and hence would lower the electrical potential available to drive passive cation absorption *in vivo*. High osmolarity also increases rectal R_t, indicating a reduction in transepithelial P_K. Moreover, the high K^+ concentrations commonly associated with high osmolarity of the rectal contents in dehydrated locusts has an additional action in reducing P_K of the mucosal membrane. Thus, the reduction in rectal KCl reabsorption previously observed *in situ* when locusts were salt loaded (Phillips, 1964*b*) could be due in part to luminal [K], pH and osmolarity. Indeed these three luminal factors may be part of a positive feedback mechanism to amplify reductions in KCl reabsorption caused by lower CTSH levels in the haemolymph in non-feeding locusts (Phillips *et al.* 1982). The osmotic permeability of the rectal wall (P_{osm}) also exhibits rectification which reduces water movement into the lumen once an osmotic gradient is established across this epithelium (Goh & Phillips, 1978). The probable result of all these processes, i.e. reduced P_{osm}, P_K and chloride transport, would be a reduction in the metabolic cost of maintaining large ionic and osmotic gradients across the rectal wall once they are developed in dehydrated locusts.

Effect of high mucosal osmolarity

Geometrical changes (e.g. collapse of lateral intercellular spaces) are thought to be one of the reasons for rectification of P_{osm} in other epithelia (e.g. Bentzel, Parsa & Hare, 1969). They might also be the basis for reduction of G_t when the rectal lumen is hyperosmotic, since KCl probably still has to move down the very long lateral intracellular spaces to the haemocoel in locust rectum after crossing the cell membranes. Other possibilities include a direct effect of hyperosmolality on apical membrane conductance, as suggested for amphibian gallbladder (Reuss & Finn, 1977), or indirect effects mediated by changes in cell volume. Cell swelling is thought to increase the K^+ conductance of the basolateral membrane in toad urinary bladder (Lewis *et al.* 1984), *Necturus* small intestine (Lau, Hudson & Schultz, 1984) and turtle colon (Germann & Dawson, 1984), and volume-sensitive K channels have recently been reported in amphibian red cells (Hamill, 1983). Thus, cell shrinkage induced by luminal hypertonicity in locust rectum might cause K channels that are normally open to close. Cell shrinkage is extreme during bilateral exposure of locust recta to hyperosmotic saline (normal saline + 600 mmol l^{-1} sucrose), as indicated by measurements of ^{14}C-mannitol space (Hanrahan & Phillips, 1984*b*).

Effects of low mucosal pH

Several possible reasons for the inhibitory effect of mucosal acidity on rectal I_{sc} must be considered. (i) The applied pH gradient might have resulted in a net flux of protons to the serosal side, leading to a reduction in the apparent I_{sc}. It is unlikely that proton conductance of the rectal wall is high enough to reduce I_{sc} substantially, because reversal of the pH difference (serosa acidic) did not enhance I_{sc} as predicted by this hypothesis (Fig. 4). (ii) Competition of protons with K^+ for the cation-activating site on the mucosal Cl^- 'pump' (Hanrahan & Phillips, 1984*a*) cannot alone explain the reduction in I_{sc}, because very low mucosal acidity reduced I_{sc} even more than did K-free conditions (Hanrahan & Phillips, 1984*a*). (iii) A more likely mode of action is through the lowering of intracellular pH, which apparently mediates the inhibitory effects of low external pH on Na^+ transport across frog skin (e.g. Funder, Ussing & Weith, 1967; Mandel, 1978). Ion-sensitive microelectrode experiments are needed to test whether the intracellular or the extracellular surface of the mucosal Cl^- pump is sensitive to acidity, or whether changing pH might increase the electrical gradient opposing Cl^- transport by lowering mucosal P_K. Unlike the effects of hyperosmotic solutions, the increase in R_t caused by extreme mucosal acidity (Fig. 4) does not suggest any large changes in shunt conductance. Transepithelial conductance at low pH agrees with the G_t/I_{sc} relationship observed during cAMP stimulation in normal saline at pH 7 (Hanrahan & Phillips, 1984*a*); in other words, at pH 4, all of the increase in R_t can be explained by the parallel decrease in I_{sc}.

Experiments in this paper (i.e. change in voltage divider ratio, α, after stimulation) and those reported elsewhere (mucosal K^+ conductance measurements, Hanrahan & Phillips, 1984*b*) indicate that the K^+ conductance change responsible for much of ΔR_t after stimulation resides in the mucosal membrane. The five-fold increase in α when external K^+ levels are raised cannot be attributed to a decrease in serosal resistance alone because, if that were true, R_t should decline with increasing [K], but it does not (Fig. 3). In summary, high luminal K^+ levels apparently inhibit mucosal P_K directly.

Energetic efficiency of the locust excretory system

The minimum metabolic energy requirement for active transport of Cl^- under each of the experimental conditions shown in Fig. 5 can be calculated by the following equation (Zerahn, 1956):

$$W = 0{\cdot}239\,(V_tF + RT\ln\frac{J^{Cl}_{ms}}{J^{Cl}_{sm}}),$$

where W is work (J equiv^{-1} Cl transported; J = joules), V_t in transepithelial potential (in V), and R, T and F have their usual meanings. Under open-circuit conditions, unstimulated recta in normal saline must expend more than $0{\cdot}741\times10^{-4}$ J cm^{-2} h^{-1} to transport Cl^- (taking $V_t = 0{\cdot}008$ V, $J^{Cl}_{ms}/J^{Cl}_{sm} = 2{\cdot}1$ and $J^{Cl}_{net} = 0{\cdot}5$ μequiv cm^{-2} h^{-1}), and during cAMP stimulation this increases by 20-fold to $1{\cdot}43\times10^{-3}$ J cm^{-2} h^{-1} (V_t = 0·025 V, $J^{Cl}_{ms}/J^{Cl}_{sm} = 4{\cdot}1$ and $J^{Cl}_{net} = 4{\cdot}3$ μequiv cm^{-2} h^{-1}). However, these calculations for

10 mmol l^{-1} K saline are not representative of the Cl^- transport work *in vivo*, where the lumen contains more than 100 mmol l^{-1} K. After raising mucosal K^+ to a more physiological value of 100 mmol l^{-1}, both the $^{36}Cl^-$ flux ratio and net Cl^- flux increased by 42% (Fig. 5), but the theoretical cost of Cl^- transport rises only 5% to $1{\cdot}51\times10^{-3}$ J cm^{-2} h^{-1} due to the much reduced transepithelial potential ($V_t = 0{\cdot}002$ V, $J^{Cl}_{ms}/J^{Cl}_{sm} = 5{\cdot}4$ and $J^{Cl}_{net} = 6{\cdot}1$ μequiv cm^{-2} h^{-1}). These results suggest an interesting consequence of high mucosal K^+ for rectal Cl^- transport. The energy stored in the K^+ gradient between Malpighian tubule fluid and haemolymph can be partially utilized during reabsorption in the rectum to drive indirectly a component of active Cl^- transport as a result of the reduced V_t.

This work was supported by grants from NSERC, Canada.

REFERENCES

BENTZEL, G. J., PARSA, B. & HARE, D. K. (1969). Osmotic flow across proximal tubule of *Necturus*: correlation of physiological and anatomical studies. *Am. J. Physiol.* **217**, 570–580.

BERRIDGE, M. J. (1979). Relationship between calcium and cyclic nucleotides in ion secretion. In *Mechanisms of Intestinal Secretion*, (ed. J. Binder), pp. 65–81. New York: A. R. Liss.

CHAMBERLIN, M. & PHILLIPS, J. E. (1982). Metabolic support of chloride-dependent short-circuit current across locust rectum. *J. exp. Biol.* **99**, 349–361.

FUNDER, J., USSING, H. H. & WEITH, J. O. (1967). The effects of CO_2 and hydrogen ions on active Na transport in the isolated frog skin. *Acta physiol. scand.* **71**, 65–76.

GERMANN, W. J. & DAWSON, D. C. (1984). Two types of potassium channel in the basolateral membrane of turtle colon. *Biophys. J.* **45**, 302a.

GOH, S. L. & PHILLIPS, J. E. (1978). Dependence of prolonged water absorption by *in vitro* locust rectum on ion transport. *J. exp. Biol.* **72**, 25–41.

HAMILL, O. P. (1983). Potassium and chloride channels in red blood cells. In *Single Channel Recording*, (eds B. Sakmann & E. Neher), pp. 451–471. London: Plenum Press.

HANRAHAN, J. W. (1982). Cellular mechanism and regulation of KCl transport across an insect epithelium. Ph.D. thesis, University of British Columbia, Vancouver, Canada.

HANRAHAN, J. W., MEREDITH, J., PHILLIPS, J. E. & BRANDYS, D. (1983). Methods for the study of transport and control in insect hindgut. In *Methods for the Measurement of Ion Transport and Metabolic Rate in Insect Epithelia*, (eds T. J. Bradley & T. A. Miller), pp. 19–67. New York: Springer-Verlag.

HANRAHAN, J. W. & PHILLIPS, J. E. (1983). Mechanisms and control of salt absorption in locust rectum. *Am. J. Physiol.* **244**, R131–R142.

HANRAHAN, J. W. & PHILLIPS, J. E. (1984*a*). KCl transport across an insect epithelium: characterization of K-stimulated Cl absorption and active K transport. *J. exp. Biol.* **111**, 201–223.

HANRAHAN, J. W. & PHILLIPS, J. E. (1984*b*). KCl transport across an insect epithelium. II. Electrochemical potentials and electrophysiology. *J. Membrane Biol.* **80**, 27–47.

HANRAHAN, J. W., PHILLIPS, J. E. & STEEVES, J. D. (1982). Electrophysiology of Cl transport across locust rectum. *Fedn Proc. Fedn Am. Socs exp. Biol.* **41**, 1492.

LAU, K. R., HUDSON, R. & SCHULTZ, S. G. (1984). Cell swelling increases a barium-inhibitable potassium-conductance in the basolateral membrane of *Necturus* small intestine. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3591–3594.

LEWIS, S. A., BUTT, A. G., BOWLER, M. J., LEADER, J. P. & MACKNIGHT, A. D. C. (1984). Anions modulate the basolateral membrane potassium conductance in toad urinary bladder. *Biophys. J.* **45**, 303a.

MANDEL, L. J. (1978). Effects of pH, Ca, ADH and theophylline on the kinetics of Na entry in frog skin. *Am. J. Physiol.* **235**, C35–C48.

PHILLIPS, J. E. (1964*a*). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskål. I. Water. *J. exp. Biol.* **41**, 15–38.

PHILLIPS, J. E. (1964*b*). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskål. II. Sodium, potassium and chloride. *J. exp. Biol.* **41**, 39–67.

PHILLIPS, J. E. (1964*c*). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskål. III. The nature of the excretory process. *J. exp. Biol.* **41**, 69–80.

PHILLIPS, J. E. & HANRAHAN, J. (1984). Chloride transport in insects: a novel K-stimulated electrogenic pump. In *Chloride Coupling in Biological Membranes and Epithelia*, (ed. G. A Gerencser), pp. 151–181. Amsterdam: Elsevier Science Publ.

PHILLIPS, J. E., SPRING, J., HANRAHAN, J., MORDUE, W. & MEREDITH, J. (1982). Hormonal control of salt reabsorption by the excretory system of an insect: isolation of a new protein. In *Neurosecretion: Molecules, Cells, Systems,* (eds D. S. Farner & K. Lederis), pp. 371–380. New York: Plenum Press.

REUSS, L. & FINN, A. L. (1977). Effects of luminal hyperosmolality on electrical pathways of *Necturus* gallbladder. *Am. J. Physiol.* **232**, C99–C108.

SCHULTZ, S. G. & FRIZZELL, R. A. (1976). Ionic permeability of epithelial tissues. *Biochim. biophys. Acta.* **443**, 181–189.

SPEIGHT, J. (1967). Acidification of rectal fluid in the locust *Schistocerca gregaria*. M.Sc. thesis, University of British Columbia, Vancouver, Canada.

SPRING, J., HANRAHAN, J. & PHILLIPS, J. (1978). Hormonal control of chloride transport across locust rectum. *Can. J. Zool.* **56**, 1879–1882.

SPRING, J. H. & PHILLIPS, J. E. (1980*a*). Studies on locust rectum. I. Stimulants of electrogenic ion transport. *J. exp. Biol.* **86**, 211–223.

SPRING, J. H. & PHILLIPS, J. E. (1980*b*). Studies on locust rectum. II. Identification of specific ion transport processes regulated by corpus cardiacum and cAMP. *J. exp. Biol.* **86**, 225–236.

ZERAHN, K. (1956). Oxygen consumption and active sodium transport in the isolated and short-circuited frog skin. *Acta physiol. scand.* **36**, 300–318.