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METABOLIC SUPPORT OF CHLORIDE-DEPENDENT SHORT-CIRCUIT CURRENT ACROSS LOCUST RECTUM*

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SUMMARY

1. Recta of desert locusts were short-circuited and depleted of endogenous substrates by exposing them to saline containing cyclic AMP but no metabolites. Individual substrates were then added to substrate-depleted recta and the change in short-circuit current (I_{sc}) monitored.
2. Proline or glucose (50 mM) caused by far the largest increase in I_{sc} of all substrates tested. Stimulation of the I_{sc} by proline was not dependent upon external sodium, but did require external chloride.
3. Physiological levels of proline also caused a large increase in I_{sc}, while physiological levels of glucose produced a much smaller stimulation. Over 90% of the proline-dependent I_{sc} stimulation can be produced by adding 15 mM proline solely to the lumen side of the tissue.
4. These results are discussed with regard to rectal oxidative metabolism and availability of metabolic substrates *in vivo*. High levels of proline in Malpighian tubule fluid are probably the major substrate source for rectal Cl^- transport.

INTRODUCTION

The locust rectum actively transports chloride and this transport can be stimulated by cyclic AMP (cAMP) *in vitro* (Spring & Phillips, 1980*a*, *b*; Spring, Hanrahan & Phillips, 1978). The mechanism of this chloride transport is different from those described from vertebrates (reviewed by Frizzel, Field & Schultz, 1979) in that it is stimulated by luminal potassium, but not sodium or bicarbonate (Hanrahan & Phillips, 1980). The locust rectum actively transports several other solutes *in vitro* but the rate of cAMP-stimulated chloride transport exceeds that of other transported species by several fold (reviewed by Phillips, 1981). Clearly, chloride transport must be a major consumer of metabolic energy in this tissue. This energy is supplied by oxidative metabolism because azide or cyanide completely abolishes chloride transport (Baumeister *et al.* 1981).

Studies by Chamberlin (1981) and M. Chamberlin & J. E. Phillips (in preparation) indicated that isolated rectal mitochondria of locusts preferentially oxidize proline

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over all other substrates tested, and a pathway of proline oxidation in the rectum has been proposed based upon measurements of enzyme activities in rectal tissue. Proline is a likely metabolic substrate *in vivo*, since proline is abundant in the haemolymph (12 mM) and is actively secreted at high concentrations (38 mM) by locust Malpighian tubules (Chamberlin, 1981; Chamberlin & Phillips, 1979, 1980). This secreted proline is subsequently actively and rapidly absorbed from the rectal lumen and is present at high levels (60–70 mM) in rectal tissue (Balshin, 1973). However, lipids, sugars and organic acids are also present in insect haemolymph (Altman, 1961), and locust Malpighian tubule fluid contains several amino acids and glucose, albeit at much lower levels than proline (Chamberlin, 1981; Chamberlin & Phillips, 1980). Any of these substrates might provide energy for chloride transport providing they can (1) cross the rectal cell membrane(s) and (2) be metabolized by the tissue. It is also unclear whether metabolic substrates are normally provided to rectal tissue from the haemolymph side or from the absorbate derived from tubular fluid entering the rectal lumen.

Very little is known about the metabolic support of ion transport in insect transporting epithelia in general. Berridge (1966) showed that exogenous alanine, glutamine, pyruvate and several sugars supported fluid secretion by the Malpighian tubules of *Calliphora*. Giordana & Sacchi (1978) reported that pyruvate or alanine supported the development of a transepithelial potential across the midgut of *Bombyx*.

Active chloride transport by the locust rectum *in vitro* can be easily monitored quantitatively because all of the increase in short-circuit current (I_{sc}) after cAMP addition is due to increased transport of this anion from lumen to haemolymph (Hanrahan, 1982; Spring & Phillips, 1980*a*, *b*; Spring *et al.* 1978). In the present study, potential metabolites were added at high concentrations to cAMP-stimulated recta previously depleted of endogenous substrates, and the subsequent changes in I_{sc} monitored. Experiments were also performed to see if proline or glucose alone fully stimulates the I_{sc} at physiological concentrations measured *in vivo*. In addition, proline was added to either the luminal or haemocoel side of the rectum at natural concentrations to see if the I_{sc} is stimulated preferentially by this substrate on just one side of the epithelium.

MATERIALS AND METHODS

Adult female *Schistocerca gregaria*, 2–4 weeks past their final moult, were used in all experiments. Animals were raised at 28 °C and 60% relative humidity on a 12:12 light:dark cycle and fed a mixture of dried grass, dried milk, bran and lettuce. Animals were denied lettuce 24 h prior to experiments.

The compositions of salines used in this study are given in Table 1. Sodium, potassium, chloride and magnesium concentrations in these salines mimic those measured in locust haemolymph (J. Hanrahan, personal communication). The amino acid concentrations in saline D (Table 1) are based upon levels in female locust haemolymph measured according to the methods outlined by Chamberlin (1981). Published values for glucose (Mayer & Candy, 1969) and trehalose (van der Horst, van Doom & Beenakkers, 1978) concentrations were used in saline D (Table 1).

The final osmotic concentration of 420 m-osmole was obtained by adding sucrose to the salines. Preliminary experiments demonstrated that ^{14}C-sucrose was not oxidized to $^{14}CO_2$ by the rectum.

Locust recta were mounted as flat sheets between two modified Ussing chambers (Williams *et al.* 1978) and bathed bilaterally with identical salines which were bubbled

Table 1. *Composition of salines used in this study**

	Saline							
Compound	A	B	C	D	E	F	G	H
NaCl	100·0	100·0	75·0	100·0	—	—	—	—
K_2SO_4	5·0	5·0	—	5·0	—	—	—	—
$MgSO_4$	10·0	10·0	—	10·0	—	—	—	—
NaH_2PO_4	1·9	1·9	—	1·9	1·9	1·9	—	—
Na_2HPO_4	3·1	3·1	—	3·1	3·1	3·1	—	—
$NaHCO_3$	25·0	25·0	25·0	25·0	25·0	25·0	—	—
$CaCl_2$	1·0	1·0	1·0	1·0	—	—	1·0	1·0
$NaCH_3SO_4$	—	—	—	—	100·0	100·0	—	—
KCH_3SO_4	—	—	—	—	5·0	5·0	—	—
$Mg(NO_3)_2$	—	—	—	—	10·0	10·0	—	—
$CaNO_3$	—	—	—	—	1·0	1·0	—	—
Choline Cl	—	—	—	—	—	—	80·0	80·0
$MgCl_2$	—	—	10·0	—	—	—	10·0	10·0
KH_2PO_4	—	—	1·9	—	—	—	1·9	1·9
K_2HPO_4	—	—	3·1	—	—	—	3·1	3·1
Choline HCO_3	—	—	—	—	—	—	25·0	25·0
KCl	—	—	5·0	—	—	—	—	—
Glutamine	—	—	—	5·0	—	—	—	—
Proline	—	—	—	18·0	—	—	—	—
Glycine	—	—	—	15·0	—	—	—	—
Serine	—	—	—	2·0	—	—	—	—
Alanine	—	—	—	2·0	—	—	—	—
Histidine	—	—	—	2·0	—	—	—	—
Glucose	—	—	—	1·0	—	—	—	—
Trehalose	—	—	—	20·0	—	—	—	—
Na Succinate	—	—	50·0	—	—	—	—	—
Sucrose	119·0	69·0	14·0	49·0	118·0	68·0	164·0	114·0

* All values are in mM. Sucrose was added to all salines to bring the final osmolarity to 420 mOsm. Salines were bubbled with 95 % O_2 and 5 % CO_2 and the pH adjusted to 7·4.

with 95% O_2 and 5% CO_2. Transepithelial potential was clamped at O mV (short-circuited condition) with compensation for series resistance of the external saline (circuitry described by Rothe, Quay & Armstrong, 1969). The I_{sc} was monitored with a Soltec 220 recorder.

To deplete the tissue of endogenous substrates, recta were bathed with saline A (Table 1) which lacked metabolic substrates. Recta were rinsed twice with this saline for 1–3 h and cAMP was then added to the haemolymph side of the tissue (final concentration of 1 mM), which caused an increase in I_{sc}. The I_{sc} then declined and when the I_{sc} dropped to 0·95–1·90 μequiv $cm^{-2}\,h^{-1}$ the rectum was considered 'substrate-depleted'. Preliminary experiments indicated that if the I_{sc} fell below this level before metabolite addition the preparation often died. If metabolites were added when the I_{sc} was well above this level, they often failed to affect the I_{sc}, presumably because of high levels of endogenous substrates. With the addition of

appropriate substrates to substrate-depleted recta, the I_{sc} could be restored to levels comparable to those observed at the time of dissection. Preparations which failed to decay to the desired low level after several hours were used in inhibitor studies.

After recta were substrate-depleted, the saline was changed bilaterally to saline B (Table 1) to which 50 mM of a single metabolite was added. The saline containing 50 mM succinate had a different composition (saline C, Table 1). These salines also contained 1 mM cAMP on the haemolymph side of the rectum. The high metabolite concentration of 50 mM was chosen in an attempt to overcome any barrier to the metabolite's entry into the tissue such as low permeability or low carrier affinity. However, diolein was added at the physiological concentration of 10 mg/ml (Jutsum & Goldsworthy, 1976). Bovine serum albumin (1% weight/volume, Sigma fraction V, essentially fatty acid free) was added with the diolein. For comparative purposes the I_{sc} was measured in a control saline which contained all the major amino acids and sugars at concentrations found in the haemolymph (saline D, Table 1). The rectum was exposed to this saline from dissection to the end of the experiment.

The results of experiments outlined above indicate that 50 mM proline caused by far the largest increase of all substrates tested. We then investigated whether this substrate stimulated the I_{sc} equally well when added to either the haemolymph or lumen side of the tissue. For these experiments a concentration (15 mM) similar to that found in haemolymph (Chamberlin & Phillips, 1979) was used. Experiments were also conducted in which physiological concentrations of glucose or proline (Chamberlain, 1981) were added to the substrate-depleted tissue.

To confirm that the proline stimulation of I_{sc} was due to an increase in chloride transport and not that of other ions, experiments with chloride-free salines were performed. The recta were depleted of endogenous substrates as described above and then the bathing saline was changed bilaterally to a chloride-free saline (Saline F, Table 1) which contained 1 mM cAMP but no metabolites. The tissue was exposed to several changes of this saline for one to two hours. Williams *et al.* (1978) showed that this treatment virtually depletes the rectal tissues of chloride. The bathing saline was then changed to saline F (Table 1) which contained no chloride, but contained 50 mM proline and 1 mM cAMP. Chloride was added at the end of the experiment in the form of NaCl so that the final chloride concentration was 100 mM.

Glycine transport in the locust rectum is partially coupled to sodium transport (Balshin, 1973; Balshin & Phillips, 1971). To see if sodium was required for the proline-stimulated I_{sc}, experiments using sodium-free salines were conducted. The protocol for these experiments was the same as that in the chloride-free experiments, except that salines G and H replaced E and F respectively (see Table 1). NaCl was added at the end of the experiment so that the final sodium concentration was 100 mM.

Inhibitors were added to short-circuit preparations to investigate the nature of the endogenous substrate and the pathway of proline oxidation in rectal tissue. An inhibitor of glycolysis, 2-deoxy-d-glucose, or an inhibitor of aminotransferases, amino-oxyacetate, was added (final concentration, 10 mM) to the saline bathing recta oxidizing endogenous substrates, or exogenous proline or alanine.

All experiments were conducted at room temperature (22–25 °C).

RESULTS

The mean time course of the I_{sc} stimulation by cAMP is shown in Fig. 1. In the absence of exogenous substrates, the maximum I_{sc} was much smaller than when recta were exposed to natural levels of sugars and amino acids. Moreover, the mean I_{sc} subsequently fell to very low values when exogenous substrates were absent. The time course of I_{sc} decay for individual recta was quite variable, presumably reflecting

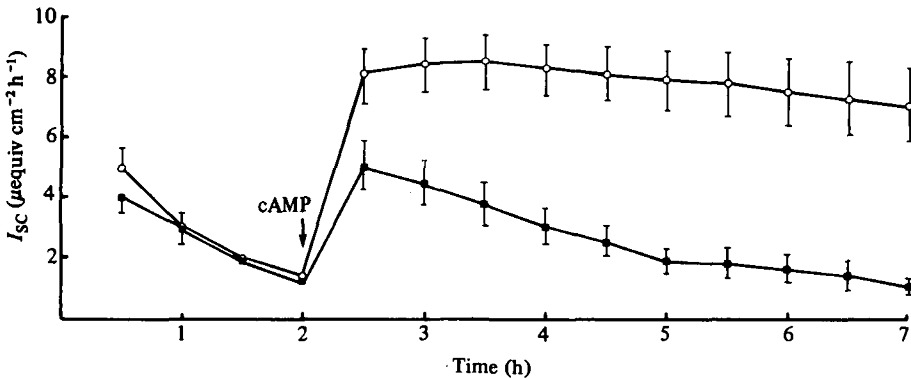

Fig. 1. Mean short-circuit current, I_{sc} ($\bar{x} \pm$ S.E., where larger than symbol) with time after dissection for recta bathed by different salines: ■, saline A (Table 1), containing no metabolites ($n = 7$); O, saline D (Table 1), containing physiological levels of the main amino acids and sugars ($n = 6$).

different intracellular amounts of endogenous substrates. In contrast, a full complement of natural exogenous substrates sustained the I_{sc} at high levels for many hours.

Figure 2 shows the effects of adding different substrates (50 mM) to both sides of substrate-depleted recta. Proline caused a 5-fold increase in I_{sc} which was 2·5–5-fold greater than I_{sc} stimulation produced by other amino acids (Fig. 2*a*). Glycine, the other major haemolymph amino acid besides proline (Chamberlin & Phillips, 1979), failed to stimulate the I_{sc}, which fell significantly below control levels (Fig. 2*a*). Glucose stimulated the I_{sc} 4 times better than trehalose, succinate or pyruvate (Fig. 2*b*), but to only 70% of the value after adding proline (Fig. 2*a*). Addition of diolein at natural haemolymph levels to substrate-depleted recta did not change the I_{sc} (Fig. 2*c*).

When physiological levels of substrates were added to substrate-depleted recta, glucose (2 mM haemocoel; 4 mM lumen) produced a much smaller stimulation than proline (12 mM haemocoel; 38 mM lumen; Fig. 3), which caused a 4-fold increase in I_{sc}. Indeed, natural levels of proline alone were almost as effective as a full compliment of all natural amino acids and sugars.

Figure 4 shows the typical effect of adding 15 mM proline first to the haemolymph and then to the lumen side of recta. Addition of proline to the haemolymph side caused only a small mean increase in I_{sc} of $2{\cdot}00 \pm 0{\cdot}41$ μequiv cm^{-2} h^{-1} ($n = 6$): subsequent addition of proline to the lumen caused a further and much larger increase in I_{sc} of $4{\cdot}81 \pm 1{\cdot}6$ μequiv cm^{-2} h^{-1} ($n = 6$). If 15 mM proline is added to the lumen

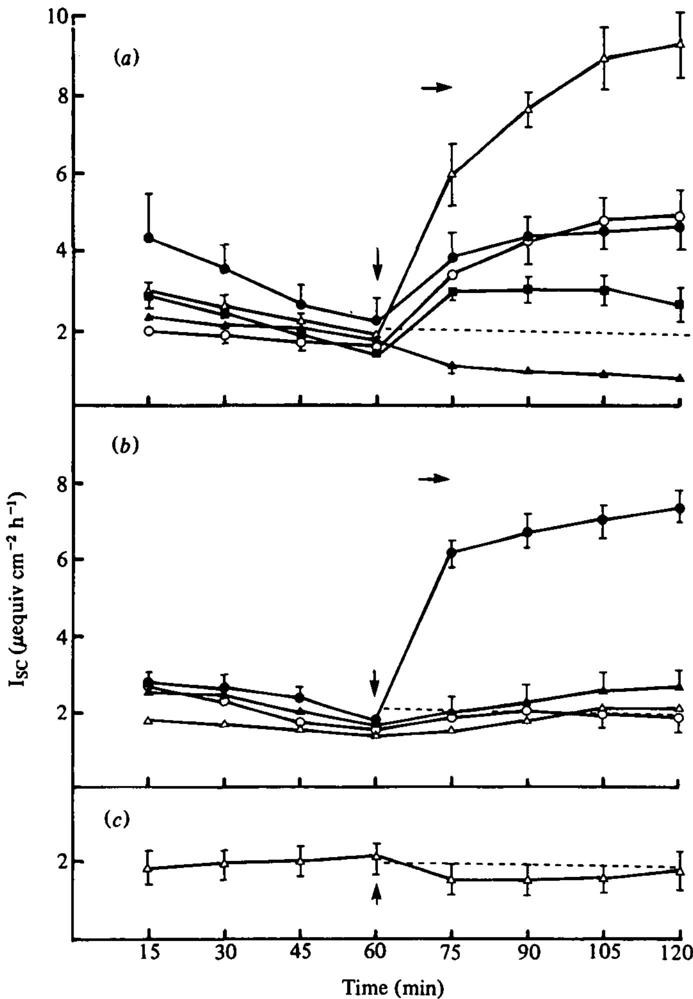

Fig. 2. The effects on I_{sc} ($\bar{x} \pm$ S.E., where larger than symbol) of adding individual metabolites to both sides of substrate-depleted recta. Vertical arrow indicates addition of metabolite and horizontal arrow indicates the maximal stimulation of I_{sc} by cAMP when recta are exposed to saline D (Table 1) which contains all the main amino acids and sugars (taken from Fig. 1). The dashed line represents the time course of the I_{sc} for substrate-depleted recta when no exogenous substrates are added (taken from Fig. 1). (*a*) Addition of 50 mM amino acid 5·3 ± 1·0 to 6·2 ± 0·2 h after dissection ($n = 6$): △, proline; ●, alanine; ▲, glycine; ○, glutamate; ■, glutamine. (*b*) Addition of 50 mM carbohydrate or organic acid 4·6 ± 0·6 to 6·1 ± 1·0 h after dissection ($n = 5$–6): ●, glucose; ○, succinate; ▲, trehalose; △, pyruvate. (*c*) Addition of 10 mg/ml diolein 10·3 ± 0·2 h after dissection.

side of substrate-depleted recta first, there is a large increase in I_{sc} (4·83 ± 0·77 μequiv cm^{-2} h^{-1}; $n = 6$), while subsequent additions of proline to the haemolymph side of the same preparations caused the I_{sc} to increase by only 0·34 ± 0·13 μequiv cm^{-2} h^{-1} ($n = 6$). These results indicate that 93 % of the total I_{sc} stimulation due to bilateral addition of 15 mM proline can be achieved by adding proline solely to the lumen side of the tissue.

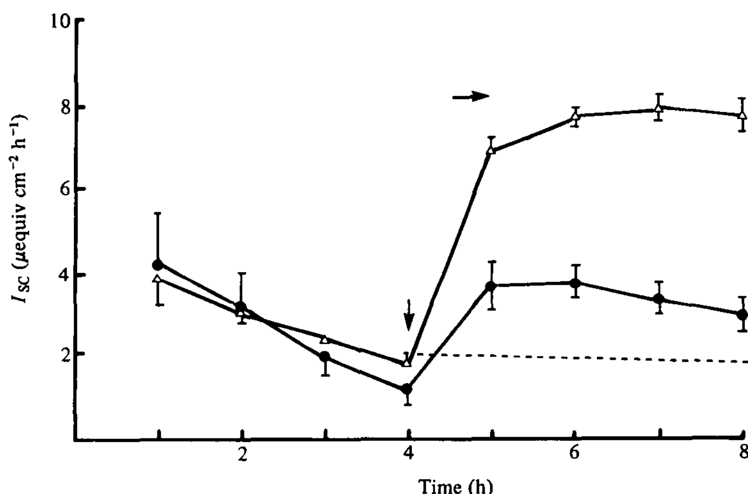

Fig. 3. The effects of adding physiological levels of proline or glucose on the I_{sc} ($\bar{x} \pm$ S.E., where larger than symbol; $n = 4$) of substrate-depleted recta: △, proline (38 mM lumen side; 12 mM haemocoel side); ●, glucose (4 mM lumen side; 2 mM haemocoel side). Metabolites were added 3·2 ± 0·3 to 3·9 ± 0·6 h after dissection. Arrows and dashed line are as described in Fig. 2.

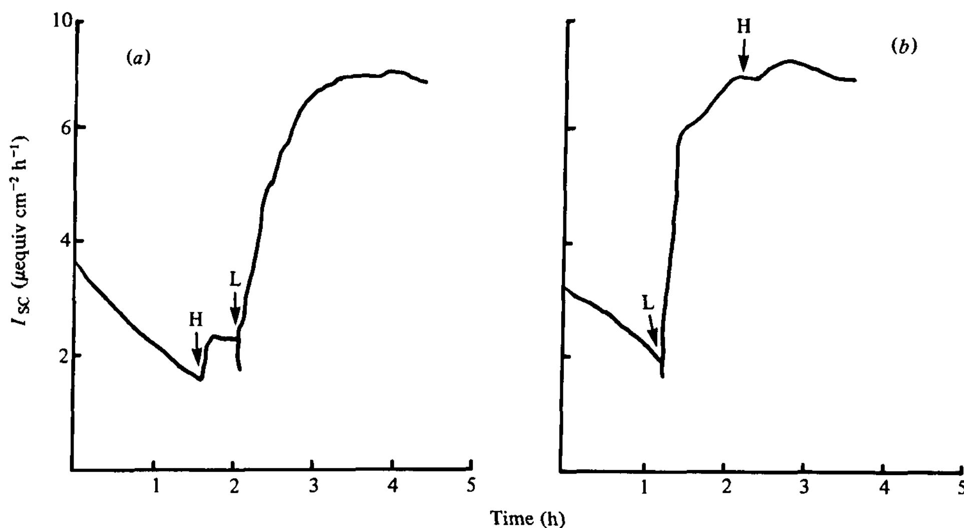

Fig. 4. Typical traces of I_{sc} with time for individual substrate-depleted recta after addition of 15 mM proline to one side of the tissue: (*a*) Proline added first to the haemocoel (H) side, then lumen (L) side of the tissue. (*b*) Proline added first to the lumen side, then haemocoel side of the tissue. Mean values for replicate experiments are given in the text.

Hanrahan (1982), Spring & Phillips (1980*b*) and Spring *et al.* (1978) showed that the cAMP-stimulated increase in I_{sc} was completely due to an increase in chloride transport. This observation is supported by the results in Fig. 5*a*. Proline addition does not stimulate the I_{sc} if chloride is not present in the saline. This indicates that the proline-stimulated I_{sc} is due to an increase in chloride absorption and not secre-

tion of cations into the lumen (i.e. sodium or hydrogen) or absorption of other anions (i.e. bicarbonate). The addition of NaCl at the end of the experiment produced an increase in the I_{sc} (Fig. 5*a*), but this stimulation was smaller than that seen in Fig. 2*a*. The recta used in the experiment shown in Fig. 5*a* were kept at a substrate-depleted level (0·95–1·90 μequiv cm^{-2} h^{-1}) longer than those in Fig. 2*a*. This may have damaged the tissue so that it became less responsive to stimulation.

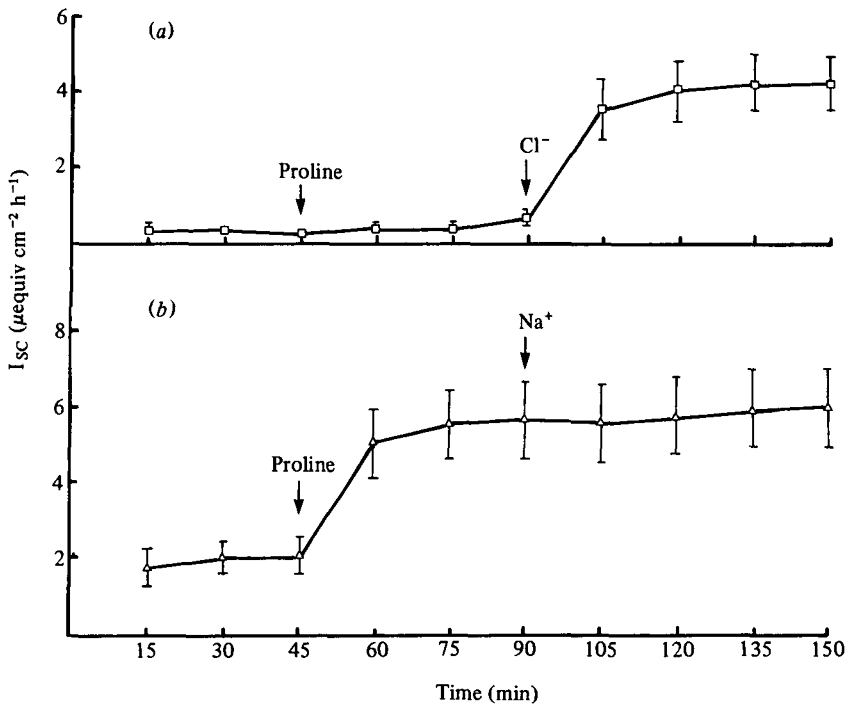

Fig. 5. The effect of proline on the I_{sc} ($\bar{x} \pm$ S.E., where larger than symbol; $n = 6$) of substrate depleted recta bathed in ion-free salines: 50 mM proline added bilaterally at the first arrow. NaCl added bilaterally to the final concentration of 100 mM at the second arrow. (*a*) Recta initially in chloride-free saline; NaCl added 5·5 ± 0·5 h after dissection. (*b*) Recta initially in sodium-free saline; NaCl added 8·2 ± 0·6 h after dissection.

Transport of glycine, and probably other amino acids, by the locust rectum *in vitro* is partially dependent upon luminal sodium (Balshin, 1973). Figure 5*b* indicates that 50 mM proline can stimulate the I_{sc} of substrate-depleted recta in the absence of external sodium; moreover, addition of external sodium does not potentiate the stimulation by proline. However, the stimulation produced by the addition of proline in Fig. 5*b* is not as great as in Fig. 2*a* for the reasons mentioned above.

Figure 6*a* shows that addition of 10 mM 2-deoxy-d-glucose, an inhibitor of glycolysis, caused an abrupt decrease in the I_{sc} of cAMP-stimulated recta oxidizing endogenous substrates. This indicates that the endogenous substrate is carbohydrate, probably non-diffusible glycogen, which has been seen in electron micrographs of locust rectal tissue (unpublished observations). The addition of this inhibitor to cAMP-stimulated recta exposed bilaterally to 50 mM proline causes a slight initial

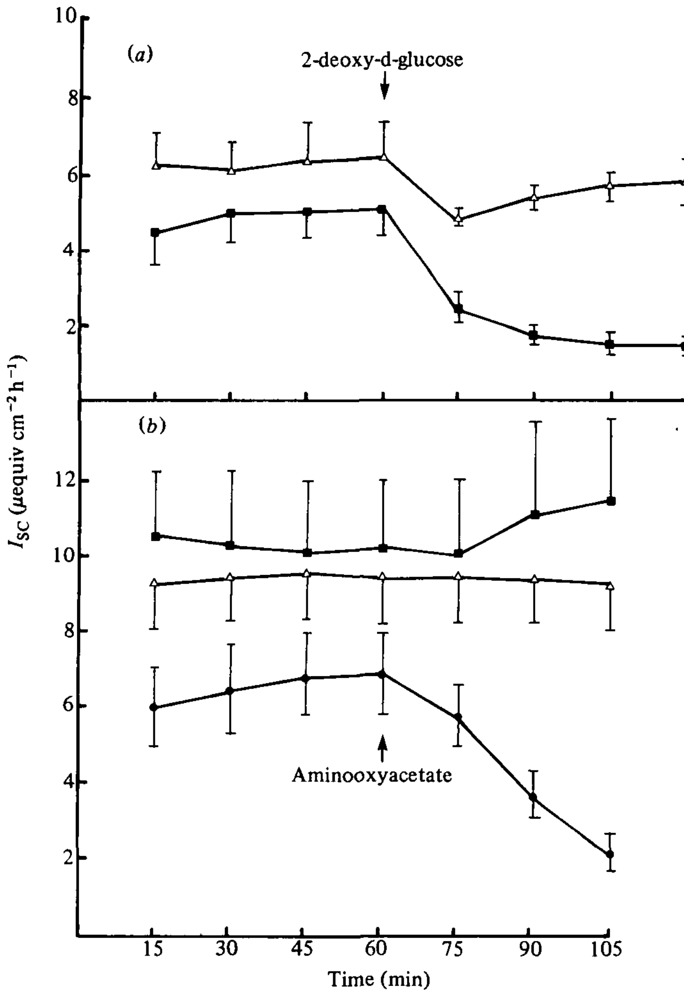

Fig. 6. The effects of 10 mM inhibitor on the I_{sc} ($\bar{x} \pm$ S.E., where larger than symbol): (*a*) 2-deoxy-d-glucose added to recta stimulated by 50 mM proline (△, $n = 4$) 10·4 ± 0·7 h after dissection or to recta with no exogenous substrates (■, $n = 6$) 6·2 ± 0·9 h after dissection. (*b*) Amino-oxyacetate added to recta with no exogenous substrate (■, $n = 4$) 4·6 ± 1·1 h after dissection; or to recta exposed to 50 mM alanine (●, $n = 6$) 6·4 ± 0·5 h after dissection; or to recta exposed to 50 mM proline (△, $n = 7$) 8·2 ± 0·3 h after dissection.

decrease in I_{sc}, but subsequently the I_{sc} rises toward the pre-inhibitor level. This result suggests that proline does not simply augment oxidation of endogenous carbohydrate, but rather that oxidation of proline alone can support the I_{sc}.

Aminooxyacetate inhibits amino transferases and the bilateral addition of 10 mM amino-oxyacetate greatly inhibits the I_{sc} of cAMP-stimulated recta exposed to 50 mM alanine (Fig. 6*b*). This indicates that alanine metabolism occurs *via* an amino-transferase, probably glutamate-pyruvate transaminase, which is present in the tissue (Chamberlin, 1981; M. Chamberlin & J. E. Phillips, in preparation). Recta oxidizing endogenous substrates or stimulated by 50 mM proline are not inhibited by

aminooxyacetate, indicating that aminotransferases may not be important in proline oxidation or oxidation of endogenous substrates.

DISCUSSION

The results of this study indicate that several exogenously applied amino acids and carbohydrates support the I_{sc} of the locust rectum, while the lipid, diolein, does not. The endogenous substrate, which is present after many hours in metabolite-free saline, is probably carbohydrate, since the I_{sc} of recta without supply of exogenous substrates is inhibited by a glycolytic inhibitor (Fig. 6*a*). The endogenous substrates at this time may not include amino acids, because an inhibitor of aminotransferases had no effect on I_{sc} (Fig. 6*b*). However, oxidation of amino acids by amino oxidases or deaminases cannot be ruled out. Finally, it is unlikely that the endogenous substrate is lipid because rectal tissue and isolated mitochondria have little capacity for lipid oxidation (Chamberlin, 1981; M. Chamberlin & J. E. Phillips, in preparation) and lipid deposits are not apparent in electron micrographs of the rectal tissue (Irvine, 1966).

In order for a metabolite to stimulate the I_{sc} of substrate-depleted recta, it must be able to first reach the cell membranes. A restrictive barrier exists on the apical side of the tissue in the form of the cuticular intima. The intima prevents compounds the size of disaccharides from reaching the rectal tissue from the lumen at significant rates (Phillips & Dockrill, 1968). Access to the basal membrane is restricted by a muscle layer and secondary cells (Phillips 1964*a*), as well as by a flow of fluid from the lumen to the haemolymph (reviewed by Phillips, 1970). Having reached the rectal epithelium, substrates must be able to enter the rectal cell *via* diffusion or a carrier-mediated process. High concentrations of glucose produce a 4-fold stimulation of I_{sc} in substrate depleted recta (Fig. 2*b*) and therefore must enter the cell. The glucose analogue, 3-o-methyl glucose is not transported by the rectum, but preliminary evidence suggests that transepithelial transport of ^{14}C-glucose does occur (Balshin, 1973). Glucose is found in low concentrations in locust haemolymph, Malpighian tubule fluid (2 and 4 mM respectively; Chamberlin, 1981) and midgut fluid (2 mM Dow, 1981). When glucose is added to substrate-depleted recta at these natural low concentrations, it still stimulates the I_{sc} but the stimulation is much smaller than with 50 mM glucose and the stimulation soon decays. J. W. Hanrahan (personal communication) also has observed that *in vitro* recta exposed to a saline containing only 10 mM glucose as an energy source fail to exhibit large, sustained I_{sc} after stimulation with cAMP. These results indicate that exogenous glucose *in vivo* probably supplies some energy for the rectum, but other compounds are more important in fuelling the rectum. The main blood sugar, trehalose, stimulated the I_{sc} of substrate-depleted recta only slightly when added at 50 mM, a concentration similar to that in locust haemolymph (Strang & Clement, 1980). Trehalose must enter the rectal cells from the haemolymph, since this sugar cannot penetrate the intima rapidly (Phillips & Dockrill, 1968).

Pyruvate and succinate failed to stimulate the I_{sc} of the locust rectum, although isolated rectal mitochondria readily oxidize these organic acids (Chamberlin, 1981; M. Chamberlin & J. E. Phillips, in preparation). Both of these compounds are nega-

tively charged at physiological pH and may not be able to diffuse into the rectal cells. Baumeister *et al.* (1981) showed that other organic acids (oxalocaete, fumarate, malate, citrate, propionate) are not transported by the locust rectum *in vitro*, and this may also be the case for pyruvate and succinate.

Diolein failed to stimulate the I_{sc}. This was not surprising since the rectum appears to have limited lipid oxidizing capabilities (Chamberlin, 1981; M. Chamberlin & J. E. Phillips, in preparation). Ketone bodies were not tested in this study, but it is possible that they may be reabsorbed and oxidized by the rectal tissue as suggested by Baumeister *et al.* (1981). However, levels of ketone bodies in locust haemolymph are quite low (Baumeister *et al.* 1981).

All amino acids tested, except glycine, stimulated the I_{sc} of substrate-depleted recta to some extent. Balshin (1973) and Balshin & Phillips (1971) showed that glycine is accumulated in rectal cells of locusts but not metabolized by the rectal tissue. Therefore it was not unexpected that glycine did not stimulate the I_{sc}. In fact, the addition of glycine to substrate-depleted recta caused a decrease in the I_{sc}. Active transport of glycine across the locust rectal wall is at least partially coupled to sodium transport (Balshin, 1973; Balshin & Phillips, 1971) so the transport of neutral glycine molecules from the lumen to the haemolymph side of the tissue would be accompanied by a movement of sodium in the same direction. In the presence of cAMP, the I_{sc} is due to active chloride absorption (Spring *et al.* 1978; Spring & Phillips, 1980*b*); therefore this glycine-stimulated movement of positive charge (sodium) would result in a decrease in I_{sc} which was observed in Fig. 5*b*.

Balshin (1973) reported that glutamate was not transported across the rectal epithelium. However, 50 mM glutamate stimulates the I_{sc} of substrate-depleted recta suggesting that at this concentration it can at least enter the cell and be metabolized. Since glutamate occurs at trace levels in the haemolymph and is very low (1·1 mM) in the Malpighian tubule fluid (Chamberlin, 1981), the contribution of exogenous glutamate to rectal metabolism *in vivo* is probably negligible.

Glutamine is found in the rectal tissue at high concentration (Chamberlin, 1981; 'amides', Balshin, 1973) and may be metabolized by the rectum. Although glutaminase has not been measured in the locust rectum, it is likely that this enzyme is present because glutamine stimulated the I_{sc}.

Alanine, at high levels (50 mM), caused a two-fold increase in the I_{sc} of substrate-depleted recta. This amino acid is found in haemolymph (1·2 mM) and Malpighian tubule fluid (1·0 mM; Chamberlin, 1981) and is actively reabsorbed by the rectum (Balshin, 1973). The alanine-dependent stimulation is blocked by aminooxyacetate (Fig. 6*b*), suggesting that an aminotransferase, probably glutamate–pyruvate transaminase, is involved in alanine metabolism. Alanine, itself, is not oxidized by isolated rectal mitochondria (Chamberlin, 1982; M. Chamberlin & J. E. Phillips, in preparation), but if ammonia from alanine could be transferred to endogenous α-ketoglutarate *via* glutamate-pyruvate transaminase, then the product, pyruvate, could be oxidized by the mitochondria. Alanine is also thought to stimulate the transepithelial potential of *Bombyx mori* midgut *via* a metabolic effect (Giordana & Sacchi, 1978; 1980; Sacchi & Giordana, 1980).

Unlike alanine-dependent I_{sc} stimulation, the proline-stimulated I_{sc} is unaffected

by aminooxyacetate (Fig. 5*b*). This indicates that proline metabolism in the rectum probably does not occur *via* glutamate-pyruvate transaminase, as in some insect flight muscles (Bursell, 1977; Sacktor and Childress, 1967), but may occur *via* glutamate dehydrogenase.

By far, the greatest stimulation of the I_{sc} was observed when 50 mM proline was added to the saline bathing the recta. Even at physiological concentrations, proline alone is as effective as a complete saline containing natural levels of major haemolymph amino acids and sugars. Proline is actively accumulated from the lumen by the rectal tissue *in vitro* (Balshin, 1973), where it is found in high concentration intracellularly (over 60 mM, Chamberlin, 1981; Balshin, 1973).

There is no evidence in this study that proline transport is dependent upon external sodium (see Fig. 5*b*) as is glycine transport (Balshin, 1973; Balshin & Phillips, 1971). However, addition of proline to the lumen side of the tissue caused a transient drop in I_{sc} (Fig. 4) consistent with an inward flow of sodium ions accompanying the entry of proline.

Over 90% of the maximum proline-dependent I_{sc} stimulation can be achieved by adding proline solely to the lumen side of the rectum (see Results). *In vivo*, the locust tubules secrete a fluid containing 38 mM proline (Chamberlin, 1981) at a rate of 8 μl h^{-1} (Phillips, 1964*b*), giving a total secretion of 304 nmol proline h^{-1}. Active transport of proline by the rectum *in vitro* (356 nmol h^{-1}; Balshin, 1973) is sufficient completely to reabsorb secreted proline. This luminal supply of proline has the advantage of presenting the apically located mitochondria (Irvine, 1966) with a substrate they can readily oxidize (Chamberlin, 1981; Chamberlin & Phillips, in preparation). We have calculated that proline is oxidized at a rate which is sufficient to energize chloride transport by cAMP-stimulated, short-circuited recta (Chamberlin, 1981).

In summary, proline absorbed from the lumen is apparently the major substrate sustaining chloride transport in locust recta. This organ is also capable of oxidizing a wide variety of carbohydrate and other amino acids (Chamberlin, 1981; M. Chamberlin & J. E. Phillips, in preparation), but these appear less important in supporting chloride transport. By oxidizing primarily amino acids and carbohydrates, the locust rectum does not have to compete for the same substrate with the more metabolically demanding, lipid-burning flight muscle (Weiss-Fogh, 1952).

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