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METABOLIC STIMULATION OF TRANSEPITHELIAL POTENTIAL DIFFERENCE ACROSS THE MIDGUT OF THE TOBACCO HORNWORM (*MANDUCA SEXTA*)

BY M. E. CHAMBERLIN

Department of Zoological and Biomedical Sciences and College of Osteopathic Medicine, Ohio University, Athens, OH 45701, USA

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Summary

1. The maintenance of *in vitro* transepithelial potential difference (PD) across the larval midgut of *Manduca sexta* is dependent upon the presence of metabolic substrates. Deletion of exogenous substrates from the bathing saline results in a rapid decline in PD. The PD can be restored by the bilateral addition of a saline containing physiological levels of amino acids and sugars. This stimulation is largely dependent upon the presence of potassium.

2. The following substrates stimulate the PD more than twofold: citrate, succinate, malate, fructose, sucrose, trehalose, glucose, hexanoate and octanoate. Acetate and some amino acids stimulate the PD, but to a lesser extent. Of the substrates tested, only proline, glycine and 3-methyl-*O*-glucose fail to stimulate the PD.

3. To determine if the observed stimulation of PD is due to absorption of anions, negatively charged substrates were added to either the haemolymph or luminal side of the tissue and the PD was monitored. The results indicate that diffusion of anions cannot account for the stimulation of PD.

4. These results are discussed with regard to midgut oxidative metabolism and the metabolic support of active potassium secretion.

Introduction

The midgut of the *Manduca sexta* larva actively secretes potassium *via* an apically located potassium pump (reviewed by Dow, 1986) which may be identical to the K^+-ATPase identified in apical membrane vesicles of the midgut (Wieczorek *et al.* 1986). The ATP for active potassium transport is supplied by oxidative metabolism as demonstrated by the parallel decline in potassium-dependent short-circuit current and cellular [ATP] during anoxia (Mandel *et al.* 1980*b*). Measurements of enzyme activities and mitochondrial metabolism indicate that the midgut tissue oxidizes lipids and carbohydrates, whereas amino acids are oxidized to a lesser extent (Chamberlin, 1987). Oxidative metabolism is ultimately dependent upon substrates transported into the cells from the

Key words: tobacco hornworm, midgut, transepithelial potential difference, metabolism.

haemolymph or midgut lumen. However, transport and oxidation of exogenous substrates have not been studied in this epithelium.

Previous transport studies on tobacco hornworm midgut have employed a simple saline which allows researchers to equate short-circuit current with potassium flux. However, these *in vitro* studies have been hampered by a continuous decline in PD and short-circuit current (Cioffi & Harvey, 1981; Dow *et al.* 1984; Moffett, 1979). Schultz & Jungreis (1977) and Thomas & May (1984) have suggested that an inadequate saline may be responsible for the decay in performance during *in vitro* experiments. In support of this view, Thomas & May (1984) demonstrated that *M. sexta* midgut maintained high rates of short-circuit current and sustained a high PD when the tissue was bathed with a saline containing citrate and haemolymph levels of inorganic ions. Since citrate is oxidized by midgut mitochondria (Chamberlin, 1987), this result suggests that tobacco hornworm midgut can transport and oxidize this carboxylic acid to support electrogenic ion transport. However, the effects of other potential metabolites on midgut function have not been examined.

The PD and short-circuit current across the larval midgut of *Manduca sexta* are dependent upon the presence of extracellular potassium. Transepithelial current and PD are linearly related (Moffett, 1980) and the deletion of potassium from the bathing medium (sodium substitution) leads to a 90% reduction in short-circuit current (from approximately 1140 to 114 $\mu A\,cm^{-2}$) and PD (from approximately 70 to 7 mV; estimated from fig. 4 in Moffett, 1980). Therefore, most of the PD is generated by electrogenic potassium transport. In this study, the effects of various metabolites on PD across posterior sections of tobacco hornworm midgut were monitored. Initial studies involved monitoring the PD when the tissue was bathed in a saline which mimics the ion and organic solute content of larval haemolymph. In addition, potential metabolic substrates were added to substrate-depleted midguts and the changes in PD monitored. Finally, the metabolic pathways operating in substrate-depleted, as well as substrate-stimulated, midguts were probed by the addition of metabolic inhibitors.

Materials and methods

Insects

Eggs or larvae of *Manduca sexta* were purchased from Carolina Biological Supply Company (Burlington, North Carolina). The larvae were maintained at 25–30°C under continuous lighting and fed an artificial diet (Carolina Biological Supply Co.). Fifth-stage larvae weighing 4·5–7·8 g were used for haemolymph amino acid and carboxylic acid analyses. Larvae weighing 3–5 g were used for measurements of haemolymph osmolality and midgut PD.

Measurement of haemolymph organic solutes and osmolality

Haemolymph was collected in a test tube after puncturing the larval integument with a pin. Samples of haemolymph were immediately deproteinized in 3·75%

sulphosalicylic acid or 6% perchloric acid. Extracts in sulphosalicylic acid were centrifuged, filtered and used for amino acid analyses. Extracts in perchloric acid were centrifuged and the supernatant neutralized with KOH. The perchlorate was removed by centrifugation and the supernatant used in the enzymatic analyses described below.

Amino acids were measured on a Beckman 119C automatic amino acid analyser using lithium citrate buffers. Since the resin and gradient system used did not resolve glutamate from glutamine and aspartate from asparagine, these amino acids were measured enzymatically (Lund, 1985; Moellering, 1985*a*). Haemolymph levels of malate (Gutmann & Wahlefeld, 1974), citrate (Moellering, 1985*b*) and succinate (Beutler, 1985) were also measured by enzymatic analysis.

The osmolality of untreated haemolymph samples was measured in a Wescor vapour pressure osmometer.

Salines

The compositions of salines used in this study (Table 1) are based upon haemolymph solute concentrations. Concentrations of sodium, potassium and chloride in control saline (saline A, Table 1) approximate the haemolymph values reported by Dow *et al.* (1984). Concentrations of glucose and trehalose are those reported for *Manduca sexta* by Dahlman (1975). Concentrations for other organic solutes are based upon values reported in this study (Table 2). The pH of all salines except 32K saline is that reported for haemolymph pH (Dow *et al.* 1984). The pH of 32K saline was adjusted to 8·3 (Cioffi & Harvey, 1981). At this point the osmolality of the salines did not equal that of larval haemolymph (332 mosmol kg^{-1}, see Table 2). The osmotic deficit was made up (except in 32K saline) with the addition of polyethylene glycol (MW 400). This compound was chosen because it is metabolically inert and does not affect the short-circuit current or PD of the midgut (Moffett, 1979).

Measurement of transepithelial potential

Larvae were decapitated and a dorsal incision made to expose the midgut. The posterior midgut was then cleaned of adhering tracheae and Malpighian tubules, opened, removed from the insect, and stretched over the collar of a modified Ussing chamber (Hanrahan *et al.* 1984). The opening of the collar was backed with fine nylon screen to support the tissue and the midgut was secured to the collar with a cotton thread. The tissue was bathed bilaterally with identical salines which were bubbled with 100% oxygen. The voltage-sensing electrodes were calomel electrodes connected to KCl–agar bridges (NaCl–agar bridges in K^+-free experiments) which were inserted into the saline on either side of the midgut. The PD was measured with a high-impedance voltmeter contained within a custom-made voltage clamp (Duke University Physiology Department Technical Shop) and monitored on a Soltec recorder.

Initial experiments involved bathing the midguts in control saline (saline A; Table 1), substrate-free saline (saline B; Table 1) or 32K saline (saline E; Table 1)

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

	Saline				
Compound	A	B	C	D	E
Na_2HPO_4	6·0	6·0	6·0	6·0	
$MgCl_2$	5·0	5·0	5·0	5·0	1·0
$CaCl_2$	1·0	1·0	1·0	1·0	1·0
NaCl		5·6	5·6		
KCl		28·9			32·0
N-methyl glucamine			28·9	28·9	
KOH	5·8				
HCl		3·0	28·9		1·5
Tris base					5·0
Potassium citrate	7·7				
Citric acid				7·7	
Sodium succinate	2·8			2·8	
Glucose	2·0			2·0	
Trehalose	27·0			27·0	
Glutamine	9·4			9·4	
Serine	8·9			8·9	
Proline	7·4			7·4	
Glycine	12·8			12·8	
Histidine	9·7			9·7	
Malic acid	5·6			5·6	
Threonine	4·6			4·6	
Alanine	3·6			3·6	
Sucrose					166·0
Polyethylene glycol	140·0	208·0	208·0	140·0	
pH	6·7	6·7	6·7	6·7	8·3

All values are in mmol l^{-1}.

A, control saline; B, substrate-free saline; C, K^+-free/substrate-free saline; D, K^+-free/control saline; E, 32K saline.

and recording the PD over several hours. The results of these experiments revealed that the PD fell rapidly when exogenous substrates were absent (see Results), presumably because endogenous substrate oxidation could not support electrogenic transport. In the next set of experiments, the effects of substrate reintroduction on the PD of substrate-depleted midguts were examined. The tissue was bilaterally bathed in substrate-free saline until the PD had fallen to 15–25 mV. At this point the midguts were considered to be 'substrate-depleted'. Preliminary experiments indicated that if the PD fell below this level, the tissue was not very responsive to addition of metabolites. If the PD was much higher than 25 mV, the tissue responded poorly to substrate additions, presumably because of the presence of endogenous substrates. The PD was then monitored after adding control saline or substrate-free saline to which substrate had been added.

It is possible that some of the stimulation of PD by the addition of exogenous substrates is simply due to reabsorption of anions. To test this hypothesis, only one side of the tissue was exposed to the organic anion by the addition of a concentrated stock. The pH values of the added solutions were adjusted with NaOH or HCl when necessary. The additional sodium or chloride did not affect the PD, since unilateral addition of 20 mmol l^{-1} NaCl caused no change in PD (see Table 3).

To determine if the increase in PD elicited by the addition of substrates (see Results) is dependent upon the presence of potassium, substrates were added to midguts bathed in substrate- and potassium-free salines. These tissues were initially bathed in substrate-free saline until the PD reached 30 mV. The tissue was then rinsed twice with a potassium-free/substrate-free saline (saline C; Table 1). This saline was similar in composition to substrate-free saline (saline B) except that potassium was replaced with *N*-methyl-D-glucamine. The PD was allowed to level off and then potassium-free control saline (saline D; Table 1) was added. This saline contained the same substrates as control saline (saline A) but potassium was replaced with *N*-methyl-D-glucamine. Finally, KCl was added bilaterally to the saline to bring the potassium concentration up to 28·9 mmol l^{-1}, the concentration found in control saline (saline A). The potassium concentration of the saline, prior to adding KCl, was measured with a potassium-sensitive electrode (Ionetics, Inc., Costa Mesa, California) attached to a Corning pH meter.

The nature of the metabolic pathways involved in sustaining the PD was investigated by exposing the midgut to the metabolic inhibitors, aminooxyacetate, 2-deoxy-D-glucose or 3-mercaptopropionic acid. Some midguts bathed in substrate-free saline failed to reach 25 mV after 2 h. At this time these midguts were exposed to metabolic inhibitors to determine what endogenous substrates are oxidized in the absence of exogenous substrates. In other experiments these inhibitors were added to midguts which had been depleted of substrates and subsequently exposed to sucrose, alanine or glutamate.

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

Results

The haemolymph concentrations of amino acids and organic acids are shown in Table 2. Malate, succinate and citrate as well as the seven most concentrated amino acids were included in the control salines (salines A and D; see Table 1).

The mean time courses for the PD of midguts bathed in control and 32K saline are shown in Fig. 1. Midguts in both salines showed an initial increase in PD, but after 20 min the PD began to decline. Midguts bathed in 32K saline showed a continuous decline over the following 4 h. In contrast, midguts in control saline displayed an initial rapid decline in PD, but this then declined much more slowly. Between 1 and 4 h in 32K saline the PD fell by 40 %. During this same period, the PD across midguts bathed in control saline declined by only 16·0 %. In Fig. 2 it can be seen that the absence of exogenous substrates leads to a dramatic fall in PD,

Table 2. *Analysis of haemolymph contents from tobacco hornworm*

Compound	Concentration (mmol l^{-1})	N
Taurine	2·0 ± 0·4	6
Aspartate	1·7 ± 0·7	6
Asparagine	1·6 ± 0·6	6
Glutamate	0·4 ± 0·1	6
Glutamine	9·4 ± 2·3	4
Threonine	4·6 ± 1·9	6
Serine	8·8 ± 0·8	6
Proline	7·4 ± 1·8	6
Glycine	12·8 ± 0·8	6
Alanine	3·6 ± 0·6	6
Cysteine	1·0 ± 0·3	4
Methionine	0·7 ± 0·1	6
Isoleucine	1·3 ± 0·2	6
Leucine	2·0 ± 1·3	6
Tyrosine	3·5 ± 0·4	6
Phenylalanine	1·3 ± 0·3	6
Ornithine	1·8 ± 0·5	6
Lysine	1·2 ± 0·4	5
Histidine	9·6 ± 0·3	6
Arginine	1·9 ± 0·4	6
Malate	5·6 ± 0·5	6
Citrate	7·7 ± 2·0	6
Succinate	2·8 ± 0·5	6
Osmolality*	332·4 ± 5·2	10

* Expressed in mosmol kg^{-1}. All values are $\bar{x} \pm$ S.E. with N being the number of larvae.

whereas reintroduction of control saline produced a 191 % stimulation of PD (Fig. 3).

The increase in PD observed in Fig. 3 was dependent, in large part, upon the presence of potassium. Midguts depleted of substrates and bathed in potassium-free saline had a PD not significantly different from zero ($-1{\cdot}8 \pm 1{\cdot}5$ mV, $N = 6$). Addition of substrates transiently decreased the PD and it then rose to $21{\cdot}8 \pm 2{\cdot}7$ mV ($N = 6$). The potassium concentration of the saline at this point was $0{\cdot}20 \pm 0{\cdot}02$ mmol l^{-1} ($N = 4$). Subsequent addition of KCl to a final concentration of 28·9 mmol l^{-1} stimulated the PD to a maximum of $71{\cdot}2 \pm 5{\cdot}9$ mV ($N = 6$) (see Fig. 4).

Figs 5–8 demonstrate the effects of adding individual substrates to substrate-depleted midguts. The addition of four amino acids, glutamate, glutamine, alanine and aspartate, stimulated the PD by 71 %, 53 %, 48 % and 8 %, respectively. Two amino acids, glycine and proline, depressed the PD (Fig. 5). Addition of the sugars, fructose, trehalose, glucose and sucrose stimulated the PD by 198 %, 149 %, 132 % and 103 %, respectively. In contrast, addition of the non-metaboliz-

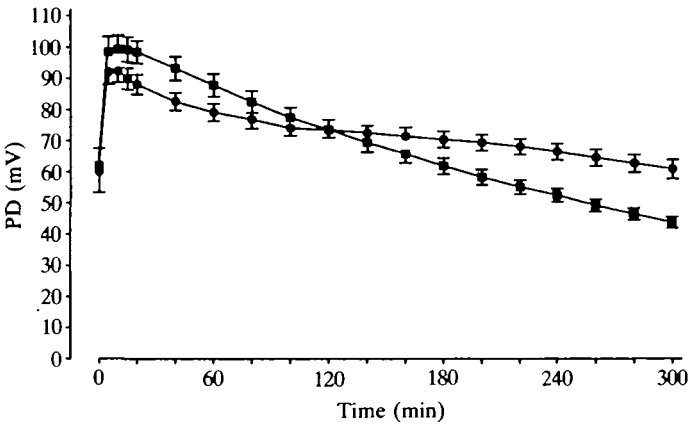

Fig. 1. Transepithelial potential ($\bar{x} \pm$ S.E.) with time after dissection for midguts bathed in different salines: ●, control saline (A, Table 1), containing physiological levels of ions, amino acids and sugars ($N = 8$); ■, 32K saline (E, Table 1), a KCl-based saline used in previous studies on *Manduca sexta* (see Cioffi & Harvey, 1981; $N = 9$).

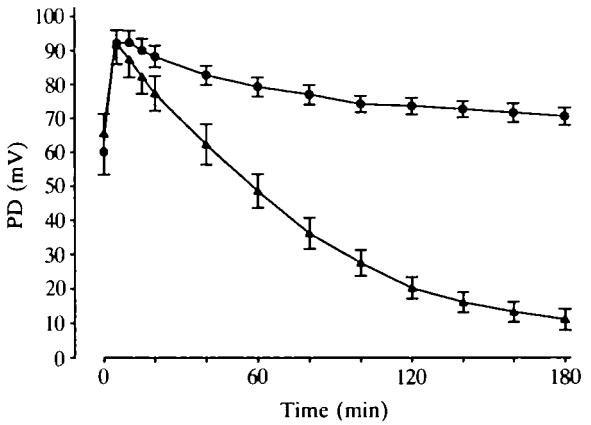

Fig. 2. Transepithelial potential ($\bar{x} \pm$ S.E.) with time after dissection for midguts bathed in different salines: ●, control saline ($N = 8$); ▲, substrate-free saline (B, Table 1) containing no metabolites ($N = 8$).

able sugar, 3-methyl-*O*-glucose, did not stimulate the PD (Fig. 6). The addition of the dicarboxylic acids succinate and malate stimulated the PD by 130% and 114%, respectively (Fig. 7). The tricarboxylic acid citrate stimulated the PD by 145% (Fig. 7). Fig. 8 demonstrates stimulation of the PD by the medium-chain fatty acid octanoate (121%) and the short-chain fatty acids hexanoate (166%) and acetate (69%).

Addition of organic anions to just one side of the tissue stimulated the PD (Table 3). All substrates, except citrate, were equally effective at stimulating the

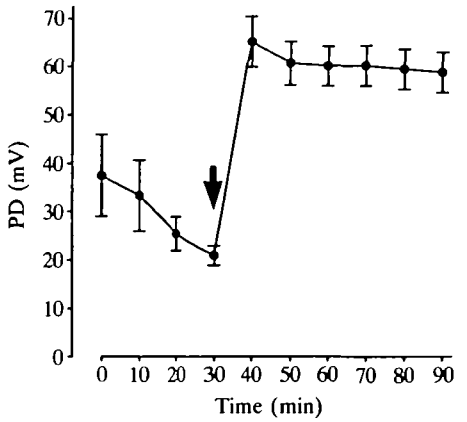

Fig. 3. The effects on PD ($\bar{x} \pm$ S.E.) of adding control saline (Table 1) to both sides of substrate-depleted midguts. The vertical arrow indicates addition of control saline 1·5–3 h after dissection ($N = 5$).

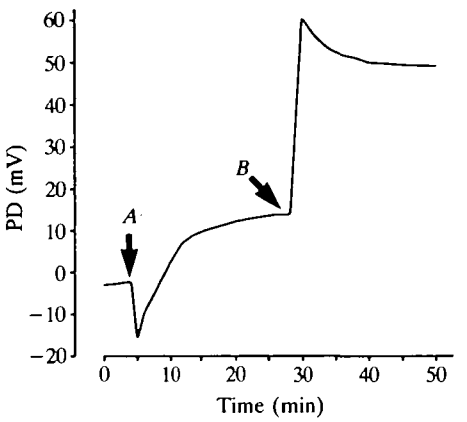

Fig. 4. Typical trace of PD with time of an individual substrate- and potassium-depleted midgut (saline C; Table 1) after addition of substrates and KCl. *A* indicates the bilateral addition of K^+-free control saline (D, Table 1) which contains physiological levels of amino acids and sugars, but no potassium. *B* indicates the bilateral addition of KCl to a final concentration of 28·9 mmol l^{-1}. Mean values for replicate experiments are given in the text.

PD from either the haemolymph or lumen side of the tissue. Citrate was a more effective stimulant when added to the haemolymph side of the tissue.

Fig. 9A illustrates the 26% decline in PD when 10 mmol l^{-1} aminooxyacetate, an inhibitor of transaminases, was added to substrate-free saline or substrate-free saline plus 10 mmol l^{-1} sucrose. This inhibitor caused a dramatic decline (102%) in the PD of midguts bathed in 10 mmol l^{-1} alanine. In contrast, the PD across midguts bathed in 10 mmol l^{-1} glutamate showed only a 26% decline when

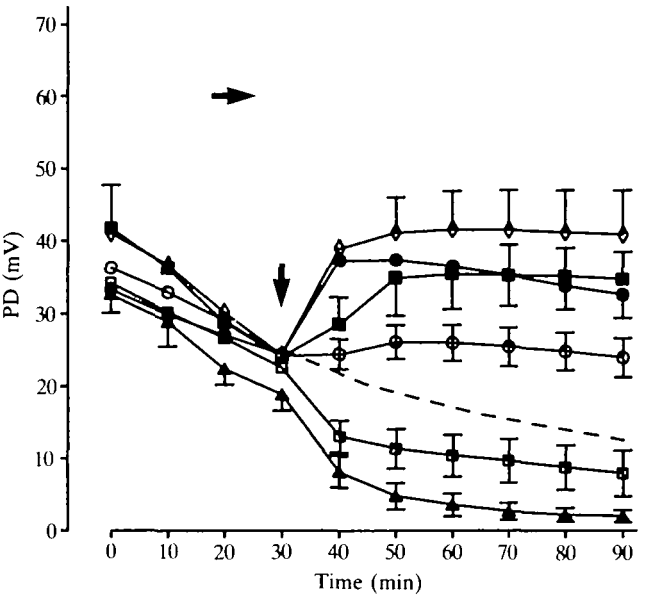

Fig. 5. The effects on PD ($\bar{x} \pm$ S.E.) of adding individual amino acids to both sides of substrate-depleted midguts. The vertical arrow indicates the addition of 10 mmol l^{-1} amino acid and the horizontal arrow indicates the elevated level of PD achieved by adding control saline to substrate-depleted midguts (taken from Fig. 3). The dashed line represents the time course of PD of substrate-depleted midguts when no exogenous substrates are added (taken from Fig. 3). Amino acids were added 1·5–3 h after dissection: ◇, glutamate ($N = 6$); ■, alanine ($N = 4$); ●, glutamine ($N = 6$); ○, aspartate ($N = 5$); □, glycine ($N = 4$); ▲, proline ($N = 5$).

Table 3. *Effect of unilateral substrate additions on the potential difference across* Manduca sexta *midgut*

Substrate	% Increase in PD	
	Luminal addition	Haemolymph addition
Succinate	116·6 ± 23·6 (4)	121·4 ± 13·8 (4)
Malate	71·8 ± 12·5 (4)	89·4 ± 8·6 (4)
Acetate	101·0 ± 17·6 (4)	92·8 ± 11·1 (5)
Citrate	48·1 ± 14·2 (4)	129·7 ± 25·0 (4)*
Glutamate	122·7 ± 13·0 (3)	75·3 ± 21·7 (4)
NaCl	2·9 ± 4·4 (3)	1·3 ± 1·3 (3)

Values are expressed as $\bar{x} \pm$ S.E. with the value in parentheses indicating the number of midguts.

All substrates are 10 mmol l^{-1}; NaCl is 20 mmol l^{-1}.

* Denotes a significant difference between luminal and haemolymph additions.

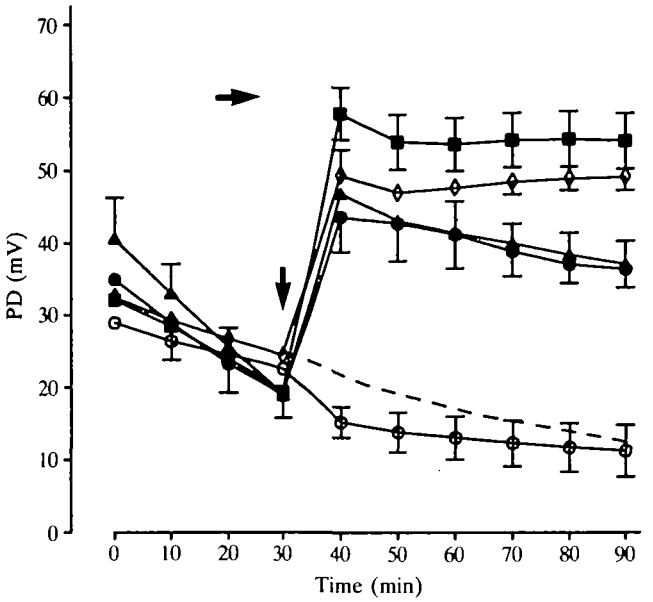

Fig. 6. The effects on PD ($\bar{x} \pm$ S.E.) of adding individual sugars to both sides of substrate-depleted midguts. Addition of 10 mmol l^{-1} sugars 1·5–3 h after dissection: ■, fructose ($N = 6$); ◇, sucrose ($N = 6$); ▲, glucose ($N = 6$); ●, trehalose ($N = 6$); ○, 3-methyl-*O*-glucose ($N = 3$). Arrows and dashed line are as described in Fig. 5.

exposed to aminooxyacetate (data not shown in Fig. 9A). The addition of 10 mmol l^{-1} 2-deoxy-D-glucose, an inhibitor of glycolysis, caused no significant reductions in PD when the tissue was bathed in substrate-free saline or substrate-free saline plus 10 mmol l^{-1} glutamate. In contrast, a 30 % reduction in PD was observed when 2-deoxy-D-glucose was added to midguts bathed with substrate-free saline plus 10 mmol l^{-1} sucrose (Fig. 9B). Mercaptopropionic acid, an inhibitor of mitochondrial fatty acid oxidation (Sabbagh *et al.* 1985), caused a drop in PD in the presence (21 %) and absence (29 %) of 10 mmol l^{-1} sucrose (Fig. 9C). The addition of all three inhibitors to midguts bathed in substrate-free saline produced a 58 % decline in PD (Fig. 9D).

Discussion

One of the problems in studying solute transport across the larval midgut of *Manduca sexta* is the decline in PD and short-circuit current during the course of *in vitro* experiments. Three reasons have been proposed to explain the decay in these transport parameters: (1) loss of goblet cell matrix material (Schultz & Jungreis, 1977), (2) loss of haemolymph-borne stimulants (Wolfersberger & Giangiacomo, 1983), or (3) an inadequate saline used in *in vitro* experiments (Schultz & Jungreis, 1977; Thomas & May, 1984). The first explanation (Schultz & Jungreis, 1977) has been challenged by Cioffi (1980). The addition of haemolymph extracts causes a

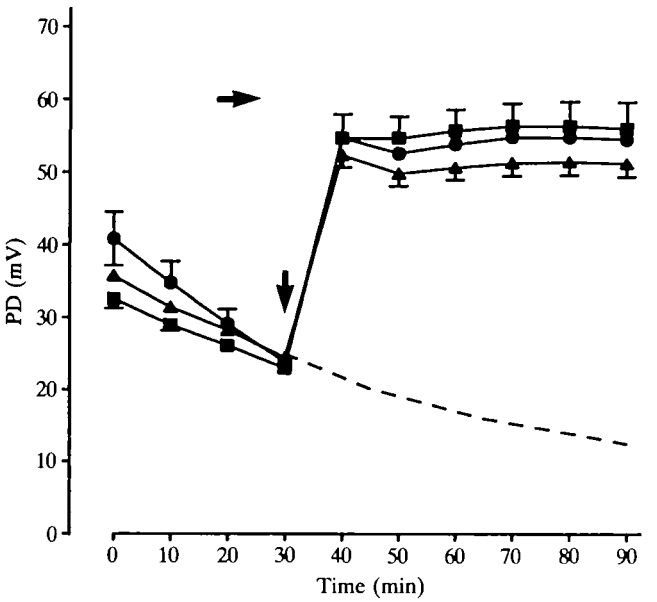

Fig. 7. The effects on PD ($\bar{x} \pm$ S.E.) of adding individual dicarboxylic or tricarboxylic acids to both sides of substrate-depleted midguts. Addition of 10 mmol l^{-1} organic acids 1·5–3 h after dissection: ■, citrate ($N = 6$); ●, succinate ($N = 5$); ▲, malate ($N = 6$). Arrows and dashed line are as described in Fig. 5.

modest stimulation of short-circuit current, but this treatment does not result in sustained high levels of active transport (Wolfersberger & Giangiacomo, 1983). It appears that the third explanation, saline content, is the most consequential. The results in this study show that maintenance of PD across tobacco hornworm midgut *in vitro* requires the presence of exogenous substrates. In the absence of these substrates the PD falls to zero within a few hours. The saline which has been used previously to study *in vitro* ion transport across tobacco hornworm midgut (32K saline; see Cioffi & Harvey, 1981) maintains the PD better than the substrate-free saline. The midgut tissue contains invertase (Koch & Moffett, 1987), and therefore oxidation of the sucrose present in the 32K saline probably supplies some energy for electrogenic processes. However, when bathed in this saline, there is a continuous decline in PD as has been previously observed by other workers (Moffett, 1979; Dow *et al.* 1984). Bathing the midgut in a saline which more closely mimics the inorganic and organic composition of the haemolymph minimizes the decay in PD and results in sustained high levels for several hours.

Oxidation of endogenous long-chain fatty acids may sustain active transport in the absence of substrates. Cioffi (1979) observed lipid droplets in electron micrographs of tobacco hornworm midgut and the present study demonstrated depression of PD by an inhibitor of fatty acid metabolism, 3-mercaptopropionic acid. However, this inhibitor also depressed the PD of midguts exposed to 10 mmol l^{-1} sucrose. This inhibitor is specific to fatty acid oxidation in isolated

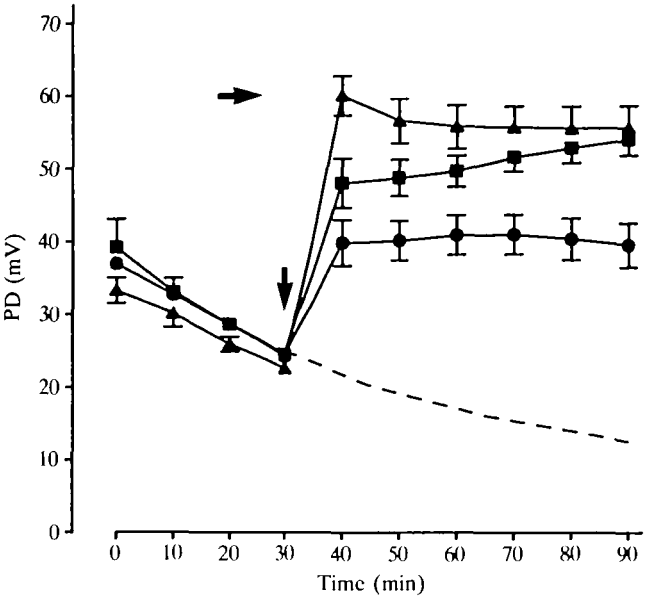

Fig. 8. The effects on PD ($\bar{x} \pm$ S.E.) of adding fatty acids to both sides of substrate-depleted midguts. Addition of fatty acids 1·5–3 h after dissection ($N = 6$): ▲, hexanoate (0·5 mmol l^{-1}); ■, octanoate (0·5 mmol l^{-1}); ●, acetate (10 mmol l^{-1}). Arrows and dashed line are as described in Fig. 5.

midgut mitochondria (M. E. Chamberlin & K. A. Jones, unpublished observations) and therefore this inhibitor's depression of the PD of midguts bathed with 10 mmol l^{-1} sucrose indicates that lipids are still oxidized under these conditions. Endogenous amino acids appear to be metabolized when no exogenous substrates are available, since aminooxyacetate inhibited the PD of midguts bathed in substrate-free saline. Endogenous glucose or glycogen does not appear to be an important energy source, since 2-deoxy-D-glucose did not inhibit the PD of midguts in substrate-free saline. The addition of all three inhibitors did not produce a total abolition of PD, presumably because not all metabolic pathways are blocked. However, it should be noted that their effects were additive since their summed individual inhibitions (60% decrease in PD) nearly equals the percentage inhibition (58%) observed when the inhibitors were added simultaneously.

The addition of a control saline to substrate-depleted midguts stimulates the PD. Some of this stimulation can be seen in the absence of exogenous potassium. No systematic ion-substitution studies were performed to determine which ion or ions are responsible for this response. However, since the K^+-ATPase is relatively non-specific with regard to its monovalent cation requirement (Wieczorek *et al.* 1986), it seems likely that sodium may be transported under these circumstances. When potassium is present, the increase in PD is fully manifested, indicating that

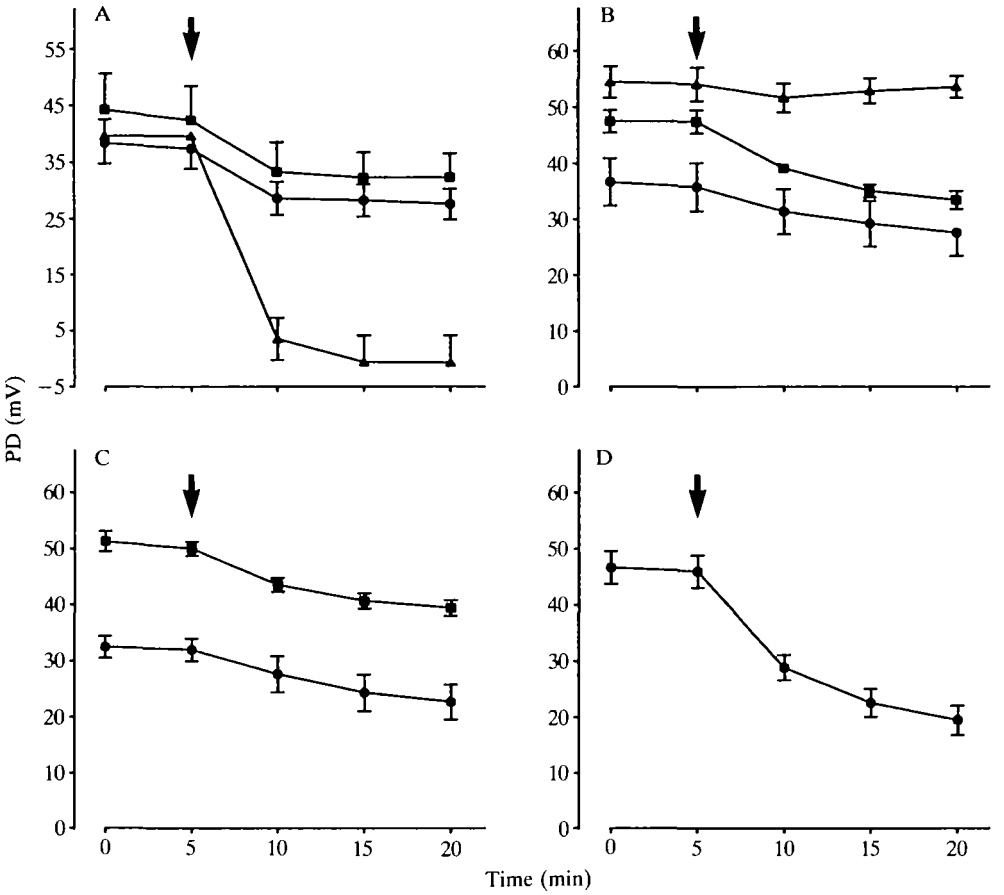

Fig. 9. The effects of inhibitors ($\bar{x} \pm$ S.E.) on the PD of midguts. (A) Bilateral addition of 10 mmol l^{-1} aminooxyacetate to midguts exposed to 10 mmol l^{-1} sucrose (■, $N = 3$), 10 mmol l^{-1} alanine (▲, $N = 3$) or no exogenous substrates (●, $N = 4$). (B) Bilateral addition of 10 mmol l^{-1} 2-deoxy-D-glucose to midguts exposed to 10 mmol l^{-1} sucrose (■, $N = 3$), 10 mmol l^{-1} glutamate (▲, $N = 3$) or no exogenous substrates (●, $N = 6$). (C) Bilateral addition of 0·5 mmol l^{-1} mercaptopropionic acid to midguts exposed to 10 mmol l^{-1} sucrose (■, $N = 3$) or no exogenous substrates (●, $N = 5$). (D) Bilateral addition of 10 mmol l^{-1} aminooxyacetate, 10 mmol l^{-1} 2-deoxy-D-glucose and 0·5 mmol l^{-1} mercaptopropionic acid to midguts bathed with substrate-free saline ($N = 6$). Inhibitors were added at the times shown by the arrows.

potassium transport is responsible for a large percentage of the responses detailed in this study.

The stimulation of PD upon the addition of substrates to midguts in potassium-free saline was preceded by a transient decrease in PD. Histidine has a net positive charge at the pH used in these studies and uptake of this amino acid could account for the transient decrease in PD. However, this decrease would be consistent with uptake of a cation, probably sodium, with a neutral substrate. This transient decline in PD upon substrate addition was also observed when potassium was

present in the bathing medium (data not shown). Measurements of intracellular potassium and sodium levels indicate that there is an electrochemical gradient favouring the entry of both these cations across the apical membrane (see Moffett & Koch, 1988; Moffett *et al.* 1982; Dow *et al.* 1984). Therefore, the influx of organic solutes may be coupled to either sodium or potassium influx. In support of this view, Hanozet *et al.* (1984) showed that uptake of amino acids by *Philosamia cynthia* midgut vesicles can be energized by a sodium gradient as well as by a potassium gradient. However, potassium-coupled transport may be most important in the *in vivo* situation since the luminal concentration of potassium far exceeds that of sodium (Dow *et al.* 1984). More studies will be needed to elucidate the organic transport mechanisms in *Manduca sexta* midgut.

Several substrates were able to stimulate the PD. This was not an osmotic effect since the addition of the nonmetabolizable sugar, 3-methyl-*O*-glucose, did not stimulate the PD. All tested metabolizable sugars stimulated the PD. The midgut tissue contains the disaccharidases invertase (Koch & Moffett, 1987) and trehalase (Dahlman, 1970), which can hydrolyse sucrose and trehalose to their monosaccharide components. The midgut tissue can metabolize these monosaccharides completely since it has high levels of glycolytic enzymes and the mitochondria oxidize pyruvate (Chamberlin, 1987). As shown in this study, oxidation of exogenous sucrose can be partially inhibited by 2-deoxy-D-glucose, an inhibitor of glycolysis. The stimulation of *Manduca sexta* midgut PD by sugars conflicts with the results obtained in similar experiments on *Bombyx mori* midgut (Parenti *et al.* 1985). Although the midgut of *Bombyx mori* has higher activities of glycolytic enzymes than that of *Manduca sexta* (Chamberlin, 1987), Parenti *et al.* (1985) found no stimulation of *Bombyx mori* midgut PD upon addition of glucose. However, it should be noted that these workers had sucrose present in the saline at all times. The midgut of *Bombyx mori* contains invertase (Ito & Tanaka, 1959), and therefore oxidation of glucose could be masked when sucrose is present.

Addition of several amino acids stimulated the PD. Mitochondria isolated from the tobacco hornworm midgut have a limited capacity for amino acid oxidation. However, homogenates of whole posterior midgut tissue contain high levels of glutamate–oxaloacetate transaminase, glutamate–pyruvate transaminase and glutamate dehydrogenase (Chamberlin, 1987). Apparently, the oxidation of alanine occurs *via* glutamate–pyruvate transaminase since its stimulatory effect could be blocked by aminooxyacetate. In contrast, stimulation of PD by glutamate was unaffected by this inhibitor, indicating that glutamate oxidation occurs predominantly *via* glutamate dehydrogenase. Oxidation *via* glutamate dehydrogenase, a mitochondrial enzyme, would be consistent with the observation that glutamate was the only tested amino acid oxidized by isolated mitochondria (Chamberlin, 1987).

Although glutamate was an effective stimulant when added from the haemolymph or luminal side of the tissue *in vitro*, haemolymph levels of this amino acid are very low and therefore this amino acid may not normally enter the cells from this side *in vivo*. Two amino acids, proline and glycine, which are found at

relatively high concentrations in the haemolymph, inhibited the PD. It has been shown that proline is not oxidized by midgut mitochondria (Chamberlin, 1987). The results presented in this study suggest that glycine also is not metabolized by tobacco hornworm midgut. After addition of these two amino acids there was a fall in the PD. This is consistent with uptake of these amino acids with a cation as discussed above.

The larval haemolymph of *Manduca sexta* contains the carboxylic acids, succinate, malate and citrate. All three of these substrates stimulated the PD. These results agree with previous observations made by other investigators. Thomas & May (1984) observed that the PD and short-circuit current of *Manduca sexta* midgut could be sustained at high levels *in vitro* when citrate was in the bathing medium. Mandel *et al.* (1980*a*) showed a stimulation of short-circuit current upon the bilateral addition of succinate. If this stimulation were due to net absorption of these anions, then stimulation should only occur when these substrates are added to the luminal side of the tissue. This was not observed. Malate and succinate stimulated the PD equally well from either the luminal or haemolymph side of the tissue and citrate was more effective when added to the haemolymph side. Therefore, it appears that the stimulation of PD cannot be due solely to anion absorption, but must reflect oxidation of these carboxylic acids. This is supported by the observation that malate, succinate and citrate are all oxidized by isolated midgut mitochondria (Chamberlin, 1987).

In this study the midgut was exposed to short- and medium-chain fatty acids. Long-chain fatty acids were not tested because of their insolubility in saline. Hexanoate and octanoate greatly stimulated the PD across posterior midgut. These fatty acids are oxidized by isolated mitochondria (Chamberlin, 1987). In contrast, acetate, which is poorly oxidized by isolated midgut mitochondria, elicited a smaller stimulation of PD.

In summary, it appears that the larval midgut of *Manduca sexta* requires the presence of exogenous substrates to sustain the PD. The midgut epithelium can transport and oxidize a wide variety of solutes, including large organic anions, to support active transport processes which generate this PD. Although the results presented in this study were obtained under open-circuit conditions, preliminary evidence indicates that short-circuit current is also stimulated by metabolic substrates (M. E. Chamberlin, unpublished observations). It is not known whether both the goblet cells and the columnar cells transport metabolic substrates, or what proportion of organic solutes are absorbed intact so that they may be used by other tissues in the rapidly growing larva. Details of these transport mechanisms await further experimentation.

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