Acid/base transport across the midgut of the tobacco hornworm, *Manduca sexta*

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Received 15 June 1998; accepted 24 August 1998

Abstract

Larval Lepidoptera generate a large pH gradient across the midgut epithelium. The in vitro rate of luminal alkalinization (\(J_{\text{OH}^-}\)) and hemolymph acidification (\(J_{\text{H}^+}\)) under nominally CO\(_2\)-free conditions was measured in the three morphologically distinct regions of the tobacco hornworm midgut. Under open-circuit conditions, the highest \(J_{\text{OH}^-}\) and \(J_{\text{H}^+}\) was observed in the anterior section and the lowest was in the middle section. In all three sections the \(J_{\text{H}^+}\) was equal to \(J_{\text{OH}^-}\) indicating transepithelial movement of acid or base equivalents. Furthermore, the rate at which the midgut transported acid or base was the same under open- and short-circuit conditions, indicating that acid/base transport is an active process. Although the inhibitors, acetazolamide and ethoxyzolamide, inhibited the activity of carbonic anhydrase in tissue homogenates, they had no effect on \(J_{\text{OH}^-}\), \(J_{\text{H}^+}\), or transepithelial potential. Therefore, under the nominally CO\(_2\)-free conditions of this study, it is unlikely that hydration of CO\(_2\) and the formation of HCO\(_3^-\) is involved in luminal alkalinization. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: pH; *Manduca*; Midgut; Transport; Carbonic anhydrase

1. Introduction

Larval Lepidoptera generate and maintain a large pH gradient across their midgut epithelia, with the lumen being several pH units more alkaline than the hemolymph (Dow, 1984). The pH of the midgut lumen does vary among species, but typically ranges from pH 8 to 11 (Berenbaum for review, 1980). In a study of one lepidopteran species, the tobacco hornworm (*Manduca sexta*), Dow (1984) demonstrated that the pH of the anterior midgut contents rises significantly above that of the ingested food and the pH continues to rise until it reaches a value of 11.3 in the middle midgut region. As the food progresses through the posterior region, the pH drops, but remains alkaline. In contrast to the highly alkaline midgut, the pH of the hemolymph remains constant at 6.7. Dow (1984) concluded from these results that the transepithelial pH gradient is generated by the anterior and middle midgut regions, but that the posterior region is incapable of generating an alkaline secretion. In vitro studies, however, later showed that the posterior midgut does engage in luminal alkalinization (Chamberlin, 1990b).

The generation of a high luminal pH could be achieved by passive or active transport. It was initially proposed that active K\(^+\) secretion energized passive base secretion into the lumen (Dow, 1984; Dow and Harvey, 1988). Moffett and Cummings (1994), however, presented convincing evidence to support the hypothesis that acid equivalents are actively transported across the midgut epithelium. They made simultaneous measurements of the in situ transepithelial potential difference (PD) and the transepithelial H\(^+\) gradient across tobacco hornworm midgut. Their data showed that protons are not in electrochemical equilibrium across the midgut and, therefore, in order for this tissue to generate the observed pH gradient it must actively transport acid equivalents from the lumen to hemolymph (or base equivalents to the lumen). Only one study, however, has measured the rate of luminal alkalinization (\(J_{\text{OH}^-}\)) in lepidopteran midgut. Chamberlin (1990b) demonstrated that
under short-circuit conditions, when no transepithelial pH or electrical gradient is present, luminal alkalinization is maintained. Although these in vitro results appear to support the hypothesis that luminal alkalinization is an active process, passive movement of a base from the epithelial cells into the lumen (assuming cell pH > 6.7) could not be discounted because Chamberlin (1990b) did not measure the rate of hemolymph acidification \( J_{\text{OH}^-} \) along with \( J_{\text{H}^+} \). If \( J_{\text{OH}^-} \) equals \( J_{\text{H}^+} \), then luminal alkalinization is due to transepithelial transport of acid or base equivalents and not simply leakage of acid or base equivalents across the membranes. The present study examines whether these fluxes are equal in all three sections of the tobacco hornworm midgut.

Another aspect of midgut acid/base transport that has not been addressed is the nature of the base equivalent that is secreted. It has been postulated that bicarbonate is generated from metabolic CO\(_2\) inside the goblet cell of the midgut and secreted into the midgut lumen where it then dissociates into carbonate and a proton (Dow, 1984). The proton is then transported by the goblet cell to the hemolymph. In order for the production of \( \text{HCO}_3^-/\text{CO}_3^{2-} \) to occur rapidly, the enzyme carbonic anhydrase (CA), responsible for catalyzing the hydration of CO\(_2\), must be present in epithelial cells of the midgut. Histochemical studies have confirmed the presence of CA in the goblet cells of the anterior and middle regions of the tobacco hornworm midgut and in the columnar cells of the posterior midgut (Ridgway and Moffet, 1986). Although acetazolamide (ACZ) inhibits midgut CA activity (Jungreis et al., 1981), the effect of CA inhibitors on acid/base transport by lepidopteran midgut has not been examined. The present study explores if inhibition of CA alters \( J_{\text{OH}^-} \) and \( J_{\text{H}^+} \), in the anterior and posterior regions of the midgut.

2. Materials and methods

2.1. Insects

Feeding fifth-instar \( M. \text{ sexta} \) larvae weighing between 4.0 and 5.0 g were used in all experiments. The larvae were raised from eggs obtained from a colony in the Department of Biological Sciences at Ohio University. Larvae were maintained at 25°C, on a 16 hr:8 hr L:D cycle and fed commercial diet (no. 9783, Bioserv, Frenchtown, NJ, USA) ad libitum.

2.2. Experimental chambers

The chambers in this study were a miniaturized and modified version of a design previously described by Chamberlin (1990b). These chambers were designed to minimize physical damage to the tissue and maximize oxygenation and circulation past both sides of the tissue.

The apparatus consists of two half chambers and, unlike the chambers used by Chamberlin (1990b), these chambers were designed so that an equal area of tissue faced each half chamber. One half has an opening that is surrounded by a ring of 8 pins, on which to secure the midgut tissue. The other half of the chamber has openings into which these pins are inserted. The area of the opening across which the midgut is exposed is 0.164 cm\(^2\) and each half chamber has a volume of 3 ml.

2.3. Electrophysiological and acid/base flux measurements

Larvae were decapitated and a dorsal incision was made to expose the midgut. The midgut was cleaned of Malpighian tubules and trachea and the midgut opened so that the morphologically distinct regions (anterior, middle, or posterior; see Cioffi, 1979) could be identified. The appropriate midgut section was dissected out and impaled on the pins, hemolymph side down, on the chamber. The two half chambers were then joined together and clamped in a vise. Saline (Table 1), which had been bubbled with 100% O\(_2\) for 2 hours to reduce the CO\(_2\) tension to negligible levels (Thomson et al., 1991), was added to each side of the chamber.

The PD was measured using calomel electrodes connected to KCl agar bridges (3 M KCl, 4% agar), which were inserted into the saline on either side of the midgut. Short-circuit current was passed with agar bridges that connected the chamber to silver/silver chloride electrodes. Both PD and \( I_{\text{sc}} \) were monitored using an epithelial voltage-clamp (World Precision Instruments, DVC 1000) and recorded using a computer data acquisition program, Datacan V (Sable Systems Inc., version 1.0).

Five minutes after mounting the midgut in the...
chamber, the midgut was bilaterally perfused with pre-oxygenated saline at a constant rate (2.5 ml min\(^{-1}\)) using a peristaltic pump. After 10 minutes of perfusion, the tissue was either accepted or rejected according to its electrophysiological characteristics. Minimal acceptable values were 200 µA and 70 mV for the anterior section, 50 µA and 50 mV for the middle section, and 100 µA and 80 mV for the posterior section. These values are similar to \(I_c\) and PD achieved in previous studies (Chamberlin, 1990a,b).

If the tissue was deemed acceptable, then the \(J_{\text{OH}^-}\) or \(J_{\text{H}^+}\) was measured under either open-circuit or short-circuit conditions. It should be noted that continuous titration could not be maintained under short-circuit conditions due to interference between the pH meter and voltage clamp. Therefore, to be consistent, both open- and short-circuit experiments were carried out in the same manner. Furthermore, because only one titrating system was available, it was not possible to determine \(J_{\text{OH}^-}\) or \(J_{\text{H}^+}\) simultaneously. Therefore, these fluxes were sequentially measured using the two different protocols, termed Protocol 1 and Protocol 2. Both protocols yield a total of two measurements of \(J_{\text{OH}^-}\) and \(J_{\text{H}^+}\) from the same tissue. The only difference between the two protocols is the order of measurements. Protocol 1 began with measurement of \(J_{\text{OH}^-}\), followed by measurement of \(J_{\text{H}^+}\), and the measurements are repeated in that order. To quantify \(J_{\text{OH}^-}\), perfusion was stopped on the lumen side and \(I_c\) or PD recorded for 15 min while perfusion continued on the hemolymph side. A pH electrode was then inserted into the lumen side of the chamber and the \(J_{\text{OH}^-}\) determined by monitoring the amount of acid (0.1 M or 0.2 M HCl) required to return the saline to pH 6.7 by using a automatic titrating system. (Radiometer PHM84 pH meter, ABU 80 autoburette and a TTT 80 titrator).

Bilateral perfusion was then resumed for another 10 min, after which the perfusion was stopped on the hemolymph side for 15 min and saline titrated back to pH 6.7 with 0.1 M NaOH. Protocol 2 was identical to Protocol 1 except that \(J_{\text{H}^+}\) was determined first. During the 15 min in which the perfusion was stopped, the pH changed 0.01 to 0.2 units, depending on the midgut section. Titrations back to pH 6.7 generally took about 1 min. Physical damage to the epithelium was determined at the conclusion of each experiment by observing whether amaranth leaked across the tissue when it was added to the lumen side of the chamber. The \(J_{\text{OH}^-}\) or \(J_{\text{H}^+}\) was determined for each section of the midgut under open-circuit conditions using both Protocols 1 and 2.

Because Protocol 1 and 2 yielded the same \(J_{\text{OH}^-}\) and \(J_{\text{H}^+}\) under open-circuit conditions and the anterior and posterior sections had the highest \(J_{\text{OH}^-}\) and \(J_{\text{H}^+}\) (see Results), measurements of \(J_{\text{OH}^-}\) and \(J_{\text{H}^+}\) under short-circuit conditions were done only on the posterior and anterior sections using Protocol 2.

Studies regarding the effects of CA inhibitors on acid/base transport were restricted to just the anterior and posterior sections and conducted under open-circuit conditions using a modification of Protocol 2. After the first determinations of \(J_{\text{H}^+}\) and \(J_{\text{OH}^-}\), the saline was switched to one containing either ACZ (10\(^{-3}\) M) or ethoxzyolamide (ETZ; 10\(^{-5}\) M) and the second measurements of \(J_{\text{H}^+}\) and \(J_{\text{OH}^-}\) were undertaken. Because of this experimental protocol, the tissue was exposed to the CA inhibitors 25 min before the determination of \(J_{\text{H}^+}\), and 50 min before the determination of \(J_{\text{OH}^-}\). Because ACZ and ETZ did not affect \(J_{\text{OH}^-}\) or \(J_{\text{H}^+}\) under our experimental conditions (see Results), studies were conducted to confirm that the midgut from our population of tobacco hornworms had a CA that was sensitive to ACZ and ETZ. The activity of CA in the posterior section of tobacco hornworm midgut was measured using a modified method of Bruns et al. (1986). Briefly, this assay involves monitoring the time it takes for phenol red to turn from red (alkaline conditions) to yellow (acid conditions) when CO\(_2\)-bubbled H\(_2\)O is added to a poorly buffered solution. For each enzyme preparation three posterior sections were dissected from tobacco hornworm larvae, rinsed in buffer, blotted dry, and homogenized in 1 ml cold buffer (12.5 mM imidazole, pH 8.2). All reactions were carried out in shell vials immersed in ice slush. In the control assay, reagents were added to a vial in the following order and then stirred: 920 µl 12.5 mM imidazole buffer (pH 8.2), 25 µl 0.1% phenol red, 5 µl DMSO (inhibitor vehicle), 100 µl tissue homogenate. While stirring, 1 ml CO\(_2\)-bubbled water (ice-cold water bubbled with 100% CO\(_2\), for one hour) was added and the time it took for the solution to turn from red to yellow was timed with a stop watch and recorded. The effect of inhibitors on CA activity was assessed by adding all reagents in same order as in the control assay, except that 5 µl of inhibitor dissolved in DMSO was added (final concentrations equal 10\(^{-3}\) M and 10\(^{-5}\) M for ACZ and ETZ, respectively).

\[ \text{2.4. Statistics} \]

Statistical calculations were carried out using Statistica, (Version 5, Tulsa, OK). Either a 1-way analysis of variance (ANOVA) or multi-way ANOVA with repeated measures were conducted. A post-hoc test (Tukey’s Test) was applied, when appropriate, to determine differences among the means. A \(P\) value of \(\leq 0.05\) was considered statistically significant.

\[ \text{3. Results} \]

The PD of the middle and anterior regions stabilized 45 minutes after mounting the midguts in the Ussing chamber whereas the PD of the anterior midgut stabi-
ized after 60 minutes (Fig. 1). A comparison of the flux measurements determined using Protocol 1 versus Protocol 2 revealed no significant difference (repeated measures ANOVA) between the first and second reading of either \( J_{OH^-} \) or \( J_{H^+} \) in any of the midgut sections (Fig. 2). Therefore, the values reported in Table 2 are the mean of \( J_{OH^-} \) and \( J_{H^+} \) observed in Protocols 1 and 2, and readings 1 and 2. A 2-way ANOVA revealed that \( J_{OH^-} \) was not significantly different from the \( J_{H^+} \) in all three sections of the midgut when examined under open-circuit conditions (Table 2). Nevertheless, the mean \( J_{OH^-} \) and \( J_{H^+} \) observed in each section of the midgut was significantly different from the other two sections (Table 2). The anterior section had the highest \( J_{OH^-} \) and \( J_{H^+} \), whereas the middle section had the lowest.

All midgut regions reached a stable \( I_{sc} \) within 15 min (Fig. 3). Reading 1 and reading 2 of \( J_{OH^-} \) and \( J_{H^+} \) were not significantly different (repeated measures ANOVA) from each other in both the anterior and posterior sections of the midgut (Fig. 4). Furthermore, \( J_{OH^-} \) was not significantly different from hemolymph \( J_{H^+} \) in either the anterior or the posterior section under the short-circuit conditions (Table 2). A comparison (4-way ANOVA) between the \( J_{OH^-} \) and \( J_{H^+} \) observed under short-circuit and open-circuit revealed that \( J_{OH^-} \) and \( J_{H^+} \) measured under short-circuit conditions were not different from those measured under open-circuit conditions (Table 2).

Exposure to ACZ did not affect the PD across both anterior and posterior sections (Fig. 5). Furthermore, a 3-way repeated measures ANOVA revealed that \( J_{OH^-} \) and \( J_{H^+} \) in both the posterior or anterior midgut sections were unaffected by the presence of ACZ (Fig. 6). Similar results were observed with ETZ (data not shown). In spite of the absence of effects on \( J_{OH^-} \) and \( J_{H^+} \), the CA activity assay confirmed that ACZ and ETZ both significantly inhibited CA in the midgut tissue (Table 3).

### 4. Discussion

Although larval lepidoptera generate and maintain a huge pH gradient across the midgut epithelium, few studies have attempted to examine acid/base transport across this epithelium in vitro. The present study is the first to demonstrate that the rate of \( J_{OH^-} \) is equal to \( J_{H^+} \) in the midgut of a larval lepidopteran insect. Therefore, the observed luminal alkalinization takes place by trans-epithelial transport and not a passive leak of base equivalents from the epithelial cells. The results of the present study also indicate that the anterior section transports acid and base equivalents at the highest rate and this result is similar to that described by Chamberlin (1990b). Although Dow (1984) demonstrated that the lumen of the middle midgut section has the highest pH in vivo, the results of the present study do not necessarily contradict his findings. If the luminal contents of the anterior section are rapidly alkalinized and pushed into the middle section as the animal continues to feed, this may result in the highest pH being observed in lumen of middle midgut in vivo.

The present study also indicates that the posterior midgut secretes base equivalents, yet Dow (1984) showed that the pH dropped in the posterior midgut section in vivo. The disparity between the results of the present study and that of Dow (1984) could be explained by the luminal environment that is present in vivo. As the midgut contents are pushed posteriorly, extracellular protein digestion may increase the concentration of free amino acids in the lumen and this may drive the pH downward. Therefore the \( J_{OH^-} \) of the posterior midgut may not be sufficient to maintain a high pH. It is also possible that reflux of the acidic fluid from the Malpighian tubules or rectum (Moffett, 1994) into the midgut may also contribute to the lowering the posterior midgut pH in vivo, although this hypothesis has not been tested.

The later explanation seems plausible since Moffett (1994) showed that when the posterior midgut is separated from the hindgut by ligating the whole body at the midgut/hindgut junction, the posterior midgut lumen was very alkaline (10.5±0.63).

Under the conditions of the present study it does not appear that \( HCO_3^- \) and/or \( CO_3^{2-} \) is the base that is secreted, because inhibition of CA had no effect on acid/base flux under open-circuit conditions. Using an in vitro assay, it was verified that the enzyme present in tobacco hornworm midgut was sensitive to the CA inhibitors, ACZ and ETZ. This result confirms earlier studies (Johnston and Jungreis, 1979; Johnston and Jungreis, 1981; Jungreis et al., 1981) that demonstrated the
inhibition of tobacco hornworm midgut CA tissue by ACZ. Two possible explanations could account for the failure of ACZ and ETZ to inhibit $J_{\text{OH}^-}$ and $J_{\text{H}^+}$. First, the inhibitors may not be able to physically access CA within the tissue. This seems unlikely because sulfonamides such as ACZ and ETZ are fairly lipophilic and are effective in many other epithelia (Maren, 1967). The second, and more likely, explanation is that under the conditions of the study in which the saline was bubbled with 100% O$_2$, CA could not mediate luminal alkalinization as metabolic CO$_2$ may be removed from the system before it can be hydrated to form HCO$_3^-$. Insects use a CO$_2$/HCO$_3^-$ hemolymph buffering system in vivo (Harrison et al., 1995) and therefore, it is quite likely that under conditions where CO$_2$ tensions are closer to physiological levels, the production of HCO$_3^-$ via CA may play an important role in luminal alkalinization. Investigation of the role of extracellular CO$_2$ in the formation of base equivalents is beyond the scope of the present study, but this aspect of acid/base transport by the midgut warrants future investigation.

The non-bicarbonate $J_{\text{OH}^-}$ measured in the present study cannot account for the rate of luminal alkalinization estimated to occur in vivo. Artificial diet has pH of 5.3 and this rises to 11.0 in the anterior midgut lumen (Dow, 1984). The fifth instar tobacco hornworm consumes 0.214 g of food hr$^{-1}$ (Reynolds et al., 1985) and the buffering capacity of artificial caterpillar diet is 32
Table 2
Mean $J_{OH^-}$ and $J_{H^+}$ in the three midgut sections under open- and short-circuit conditions.

<table>
<thead>
<tr>
<th>Midgut section</th>
<th>$J_{OH^-}$ (µequiv cm$^{-2}$ hr$^{-1}$)</th>
<th>$J_{H^+}$ (µequiv cm$^{-2}$ hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-Circuit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>5.82±1.19</td>
<td>4.88±1.0</td>
</tr>
<tr>
<td>Middle</td>
<td>0.59±0.12</td>
<td>0.71±0.14</td>
</tr>
<tr>
<td>Posterior</td>
<td>1.89±0.28</td>
<td>2.14±0.44</td>
</tr>
<tr>
<td>Short-Circuit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>4.97±0.43</td>
<td>3.99±0.41</td>
</tr>
<tr>
<td>Posterior</td>
<td>1.99±0.20</td>
<td>1.94±0.18</td>
</tr>
</tbody>
</table>

Values are means±SE; $N$ = 12 (open-circuit) $N$ = 6–8 (short-circuit).

Fig. 3. Change in $I_{sc}$ over time in isolated midgut tissue. The circled numbers (1–4) indicate the time when a titration occurred during the course of the experiment. Values are means±SE, $N$ = 8 for the anterior section and $N$ = 6 for the posterior section.

μequiv pH$^{-1}$ g$^{-1}$ (Dow, 1984). Therefore, the approximate in vivo rate at which the anterior section secretes base equivalents is 39.0 µequiv hr$^{-1}$. The midgut comprises 41.3% of the total body weight and the tissue represents only 10% of the weight of the filled midgut (Reynolds et al., 1985). Using these conversion factors, a caterpillar that weighs 4.2 g (average weight of the caterpillars used in the present study) has a total midgut tissue weight of 0.174 g and the anterior section would represent approximately one third of this weight or 0.058 g. This tissue weight is equivalent to 0.85 cm$^2$ using a conversion factor reported by Cioffi and Harvey (1981). Therefore, the $J_{OH^-}$ required to shift the pH of the diet to that of the anterior midgut lumen is 45.9 µequiv cm$^{-2}$ hr$^{-1}$. This estimated in vivo rate is 8 times higher than that measured in vitro in the present study (see Table 2).

The disparity between in vivo and in vitro $J_{OH^-}$ may be explained by CO$_2$-dependent base secretion. It is also possible that hormonal, neural, or nutritional factors may lead to a higher $J_{OH^-}$ in vivo.

The present study demonstrated that the midgut is capable of luminal alkalinization and hemolymph acidification under short-circuit conditions. Therefore, these acid/base equivalents are transported via active processes. Furthermore, the magnitude of $J_{OH^-}$ and $J_{H^+}$ were the same under open- and short-circuit conditions, indicating that the presence of a large PD did not augment acid/base transport. Collectively these findings confirm the conclusions of the study by Moffett and Cummings (1994) in which these workers demonstrated that the pH gradient across the midgut epithelium in vivo was one unit higher than predicted by the Nernst equ-
Fig. 5. Effect of ACZ on midgut PD. The tissue was exposed bilaterally to ACZ at the time indicated by the arrow. The circled numbers (1–4) indicate when a titration occurred during the course of each experiment. Values are means±SE, N=6.

ation, and therefore, must result from an active process. It should be emphasized that luminal alkalinization may be brought about by the absorption of H⁺ or another acid equivalent. Because $J_{\text{OH}^-}$ and $J_{\text{H}^+}$ are equal under the conditions of the present study, we can refer to acid/base transport as transepithelial acid absorption. Net movement of H⁺ from lumen to hemolymph under short-circuit conditions was shown in this study, and, therefore, transepithelial acid absorption is electrogenic. Active H⁺ absorption may help explain the discrepancy between $I_{\text{sc}}$ and net ionic flux observed by Chamberlin (1990a). Chamberlin (1990a) observed that net K⁺ secretion exceeded the $I_{\text{sc}}$. This was in contrast to previous studies which showed net K⁺ secretion equivalent to the $I_{\text{sc}}$ when a simple KCl-based saline was used (Cioffi and Harvey, 1981). The disparity between net K⁺ flux (35.8 μequiv cm⁻² hr⁻¹) and $I_{\text{sc}}$ that Chamberlin (1990a) observed was not balanced by active secretion of Cl⁻ or absorption of Na⁺. A net absorption of H⁺ would account for a further 8.9±0.7 μequiv cm⁻² hr⁻¹ (Chamberlin, 1990b) or 5.0±0.4 μequiv cm⁻² hr⁻¹ (the present study), reducing the discrepancy observed between $I_{\text{sc}}$ and measured ion fluxes.

Based on the pH (6.7) of the saline used in the present study and the posterior midgut’s intracellular pH (7.2), apical membrane potential (−90 mV), and basolateral membrane potential (−35mV) measured by Moffett and Koch (1992), it appears that proton transport across the apical membrane (lumen to cell) would be passive. One way protons could enter the midgut cells is via the

2H⁺/K⁺ exchanger on the apical membrane of the goblet cell (Azuma et al., 1995).

In contrast to the apical membrane, proton transport across the basolateral membrane must be active (1.48 kcal mol⁻¹; calculated from data in Moffett and Koch, 1992). The presence of a basolateral proton ATPase could mediate proton absorption because the energy released by the hydrolysis of ATP (approx. −13.6 kcal mol⁻¹; Nicholls and Ferguson, 1992) would be sufficient to drive the proton transport. Nevertheless, no basolateral proton ATPase has been identified in *M. sexta*

Fig. 6. The effect of ACZ on the $J_{\text{OH}^-}$ and $J_{\text{H}^+}$ in the anterior and posterior midgut sections under open-circuit conditions. Values are presented as means±SE, N=6.

Table 3
The effect of ACZ and ETZ on CA activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reaction time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.2±2.3</td>
</tr>
<tr>
<td>ACZ</td>
<td>67.2±3.1b</td>
</tr>
<tr>
<td>ETZ</td>
<td>69.1±2.4b</td>
</tr>
</tbody>
</table>

a Values are means±SE, N=6
b Significantly different from control.
midgut. It is also possible that a K⁺/H⁺ exchanger could be involved in transporting protons across the basolateral membrane, since the driving force for movement of K⁺ from the hemolymph into the cell is thermodynamically favorable (−0.154 kcal mol⁻¹; calculated from data in Moffett and Koch, 1992). Given that the energy required to transport one proton out of the cell is greater than the energy released by the transport of one K⁺ into the cell, the K⁺/H⁺ exchanger would have to exchange more than one K⁺ per proton.

Acknowledgements

We wish to thank Simona Aizicovici for her technical assistance. This work was supported by a National Science Foundation grant (IBN-9407313) to MEC.

References


Cioffi, M., 1979. The morphology and fine structure of the larval midgut of a moth (Manduca sexta) in relation to active ion transport. Tissue and Cell 11, 467–479.


