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ROLE OF MIDGUT ELECTROGENIC K^+ PUMP POTENTIAL DIFFERENCE IN REGULATING LUMEN K^+ AND pH IN LARVAL LEPIDOPTERA

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Summary

Larvae of *Manduca sexta* were fed on tobacco leaves or synthetic diets with differing potassium content. For all diets tested, midgut lumen K^+ activity coefficients were low, and midgut lumen K^+ activities, K^+ concentrations and pH values were as closely regulated as in blood. The transmidgut electrochemical potential difference for potassium based on activities was lower than had previously been estimated from concentrations, but was dominated by the electrical gradient established by the electrogenic K^+ ATPase. Midgut K^+ activities and pH profiles were similar, as would be suggested by a model in which the generation of high pH was linked to electrogenic K^+ transport.

Introduction

Although the potassium pump of lepidopteran midgut is functionally well characterized (Harvey *et al.* 1983), its role remains obscure (Dow, 1986). Traditionally, it is argued that the high potassium level in foliage (around 250 mmol l^{-1}) is reflected by a high potassium level in the midgut lumen (around 280 mmol l^{-1}), which is much higher than that in the blood (32 mmol l^{-1}) (Jungreis *et al.* 1973; Harvey *et al.* 1975; Giordana & Sacchi, 1977). This transmidgut potassium concentration difference, combined with a large electrical potential difference (PD) (around 150 mV), is thought to promote a rapid flux of potassium from lumen to blood. This passive potassium movement is thought to be countered by active transport from blood to lumen, *via* the specialized goblet cells. This simple pump–leak interaction accounts in large part for the constancy of blood and midgut cellular K^+ concentrations. Moreover, the K^+ pump provides the energy for amino acid absorption (Nedergaard, 1977) *via* amino-acid–K^+ symports (Giordana *et al.* 1982). Thus amino acid uptake *via* midgut columnar cells is dependent on the electrical PD across the columnar cell apical membrane.

However, the K^+ pump–leak steady state alone does not account for the close

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regulation of K^+ in the midgut contents reported here. Under a wide range of dietary K^+ loadings, both the concentration and the activity of potassium along the midgut lumen are uniform and constant. The <10-fold K^+ activity difference (lumen > blood) is not the largest ion gradient across the midgut. The lumen is normally more alkaline than the blood side, by at least 3 pH units (Dow, 1984), corresponding to a >1000-fold H^+ activity difference, which is much more impressive. It has been argued that the energy for this pH difference is supplied by the electrical PD from the K^+ pump (Dow, 1984). The constant lumen and blood K^+ activities would ensure a constant electrical PD which in turn would ensure a constant lumen pH. These observations support the suggestion by Dow (1984) that the primary role of the K^+ pump is to establish the electrical PD, rather than to excrete K^+. The PD is then used for K^+ regulation, amino acid and nutrient uptake, and pH regulation.

Materials and methods

Larvae of *Manduca sexta* were obtained from Carolina Biological Supply, and raised on a standard synthetic diet, after Riddiford (1968), consisting mainly of wheatgerm, casein, vitamins and mineral salts in an agar gel. These were termed 'normal diet' larvae. Other larvae were raised from the second instar on tobacco plants ('leaf diet'). In some feeding experiments, larvae were switched to 'high K^+ diet' or 'very high K^+ diet', at the end of the fourth instar. High K^+ diet comprised normal diet plus K_2SO_4 to a final potassium concentration of 82 mmol l^{-1}; very high K^+ diet contained K_2SO_4 to a final potassium concentration of 132 mmol l^{-1}. No effort was made to control for differences in diet osmolality under these conditions, but larval feeding and growth patterns were not observed to differ among treatments.

After 3 days' feeding, in which they attained a mass of around 5 g, larvae were anaesthetized by chilling, and samples of diet, fresh faecal pellets, blood and midgut contents (subdivided into three regions) were taken according to the protocol described in Dow (1984).

pH

Sample pH was measured with a MI-410 microcombination pH probe (Microelectrodes, Inc.) against pH standards (Arthur H. Thomas, Ltd).

Potassium activities

Potassium activities were measured with a commercial valinomycin-based K^+ electrode (Orion Research model 931900), simultaneously with sample pH, and using the pH electrode reference barrel for reference measurement. Potassium activities were calculated from standards of the same approximate pH as the sample, to eliminate any pH-sensitivity of the electrode. Potassium standards were run before and after every reading.

Potassium concentrations

Potassium concentrations were measured by flame photometry against KCl standards, after 1:500 dilution. Sodium values were also measured, and sodium interference in potassium measurements compensated for, in the few cases where sodium levels were detectable. Potassium activity coefficients (γ_{K^+}) were calculated for each sample as potassium activity/potassium concentration.

Dry mass fractions

Samples were weighed immediately upon collection (where necessary extrapolating to zero time to correct for water loss between larva and balance), then reweighed after drying to constant mass over silica gel in a desiccator. Dry mass fractions were calculated as dry mass/fresh mass.

Results

Potassium concentrations, activities and activity coefficients, along with sample pH and dry mass fractions, for larvae fed on synthetic diet, are shown in Table 1. Potassium concentrations are in close agreement with previously published values (Harvey *et al.* 1975; Giordana & Sacchi, 1977; Dow *et al.* 1984), as are pH values (Dow, 1984) and dry mass fractions of diet, gut contents and faeces (Reynolds *et al.* 1985). Both the concentration and activity of potassium rise in the midgut, compared with either diet or faeces (which resemble each other in potassium levels).

Similar data for larvae raised on tobacco are presented in Table 2. Although tobacco has twice the potassium content of normal synthetic diet, the blood potassium concentration and activity are very close to those for diet-reared larvae. Significantly, the resemblance extends to both midgut potassium concentration and activity, implying that midgut luminal [K^+] might be under regulation as tightly as blood [K^+]. To investigate this possibility, similar data were obtained for larvae fed on synthetic diet with elevated K^+ levels. A comparison of potassium activities for normal, high K^+, very high K^+ and leaf diets is shown in Fig. 1. Again, both blood and midgut potassium activities are constrained to lie within very narrow limits, despite a very wide range of diet (and faeces) potassium levels.

Profiles of transepithelial chemical potential differences ($\Delta\mu$) for potassium ions and protons are shown in Fig. 2. These are expressed in mV, and the values for diet and faeces (relative to blood) are included, although they do not necessarily reflect transepithelial chemical differences across the foregut or rectum.

The K^+ activity coefficient in midgut lumen is typically 0·4. This value is remarkably low compared with those of 0·8 for an aqueous 200 mmol l^{-1} solution of KCl or 0·6–0·7 for intracellular K^+ (Moffett *et al.* 1982). This low activity coefficient implies that potassium, the dominant cation in midgut fluid, is not all in free solution. A possible explanation for this may lie in the effect of high pH on proteins. The lumen pH of 10 is far above the isoelectric point of virtually all proteins which accordingly will be polyanionic and bind cations (Edsall & Wyman,

Table 1. *Potassium levels, pH and dry mass fractions of blood, synthetic diet and gut contents of fifth-instar larvae of* Manduca sexta *fed on normal diet*

	Blood	Diet	Midgut contents			Faeces
			Anterior	Middle	Posterior	
Potassium						
Concentration ($mmol\,l^{-1}$)	32 ± 1	74 ± 3	190 ± 15	204 ± 6	211 ± 11	77 ± 11
Activity ($mmol\,l^{-1}$)	17 ± 1	23 ± 3	80 ± 0·2	83 ± 1	84 ± 1	44 ± 5
Activity coefficient	0·54 ± 0·03	0·31 ± 0·05	0·44 ± 0·02	0·41 ± 0·02	0·40 ± 0·02	0·46 ± 0·05
pH	6·6 ± 0·1	5·1 ± 0·03	10·4 ± 0·1	10·8 ± 0·2	10·0 ± 0·2	4·9 ± 0·1
Dry mass fraction	0·06 ± 0·003	0·23 ± 0·01	0·10 ± 0·01	0·09 ± 0·005	0·10 ± 0·007	0·24 ± 0·01

Data are mean ± S.E.M. ($N = 6$).

Table 2. *Potassium levels, pH and dry mass fractions of blood, diet, and gut contents of fifth-instar larvae of* Manduca sexta *fed on tobacco plants*

	Blood	Diet	Midgut contents			Faeces
			Anterior	Middle	Posterior	
Potassium						
Concentration ($mmol\,l^{-1}$)	37 ± 2	137 ± 28	181 ± 5	180 ± 4	185 ± 6	128 ± 13
Activity ($mmol\,l^{-1}$)	25 ± 2	122 ± 13	62 ± 5	62 ± 4	50 ± 4	101 ± 13
Activity coefficient	0·68 ± 0·06	1·0 ± 0·05	0·33 ± 0·03	0·34 ± 0·02	0·27 ± 0·02	0·98 ± 0·15
pH	6·5 ± 0·06	5·7 ± 0·08	10·2 ± 0·2	10·5 ± 0·3	9·6 ± 0·2	5·4 ± 0·4
Dry mass fraction	0·047 ± 0·002	0·09 ± 0·005	0·04 ± 0·003	0·04 ± 0·004	0·04 ± 0·002	0·09 ± 0·001

Data are mean ± S.E.M. ($N = 8$).

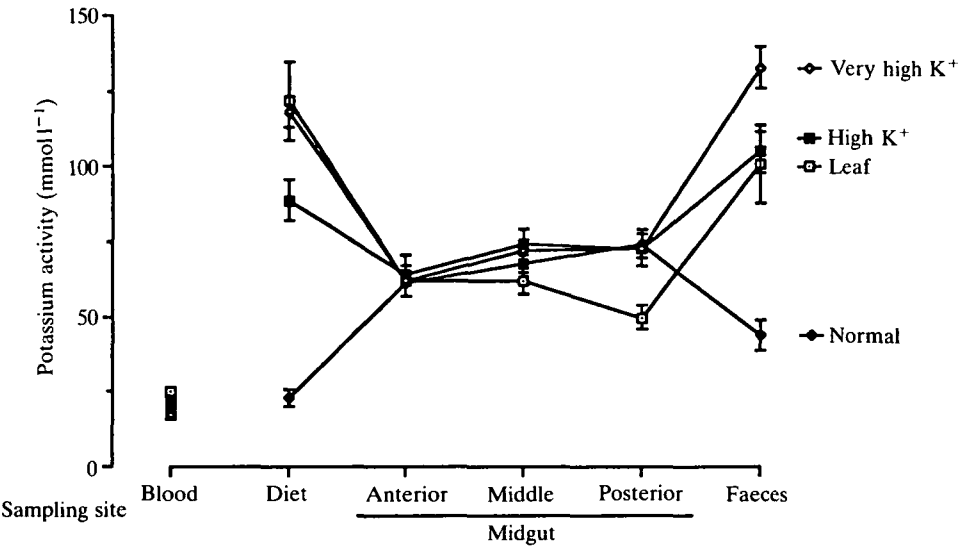

Fig. 1. Profiles of potassium activities in food, faeces and gut contents of *Manduca* larvae as a function of diet. Data are shown as mean ± S.E.M. of at least six determinations, except where error bars are smaller than the symbols used. The diets are as described in the text.

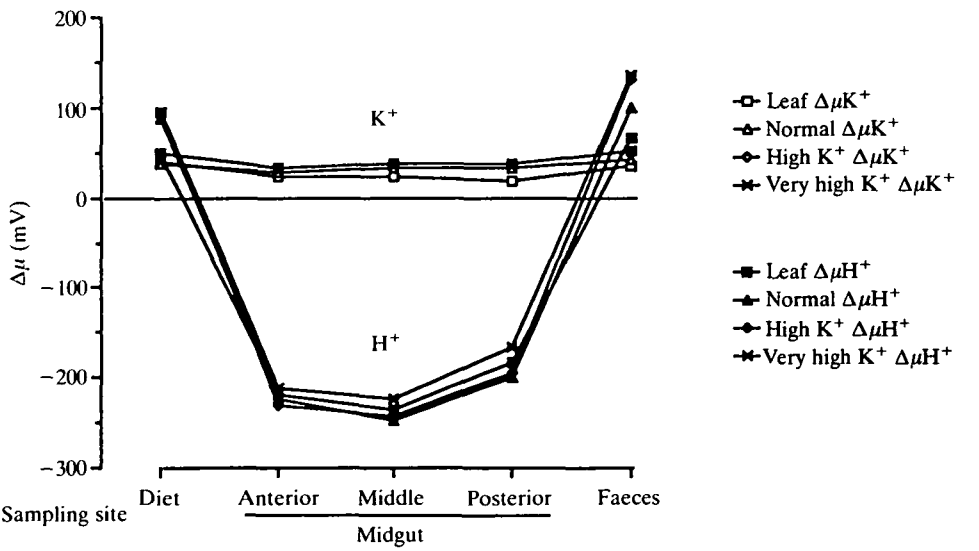

Fig. 2. Comparison of transmidgut electrochemical potential differences ($\Delta\mu$) for K^+ and H^+ and as a function of sampling site for larvae raised on various natural and synthetic diets, as described in the text. Error bars are omitted for clarity. Chemical potential differences are calculated in mV as $59\log A_L/A_B$, where A_L and A_B are the activities in lumen and blood, respectively.

1958). Such interactions are believed to be relatively tight, and so would exclude bound ions from solution (Robertson, 1920). Since calcium and magnesium are insoluble at this high pH, and sodium is virtually absent, gut proteins may act as K^+ adsorption matrices in the alkaline environment of the caterpillar midgut. Some evidence for this was obtained in titration experiments on gut contents: when acidified to low pH, the potassium activity coefficient (γ_{K^+}) rose markedly from $0{\cdot}3 \pm 0{\cdot}02$ ($N = 6$) at pH 10 to $0{\cdot}63 \pm 0{\cdot}03$ ($N = 4$) at pH 4. However, this is unlikely to be a complete explanation: the faeces of diet-reared (but not tobacco-reared) larvae also show a low γ_{K^+}, despite a low pH. It is possible that the gel-like nature of the synthetic diet produces anomalies in solution chemistry.

Discussion

It is clear from the data presented here that under a wide range of dietary K^+ loadings, midgut potassium levels (measured either as activity or concentration) are as tightly regulated as those in the blood. Under some conditions, the midgut potassium level is higher that that in either food or faeces; a midgut pump–leak steady state alone could not account for this result. Clearly the Malpighian tubules and rectal cryptonephridial system must be involved in the regulation of lumen K^+ levels as well as the blood K^+ level, under some circumstances actually conserving K^+. The constant pH in the lumen and blood also appears to be dependent on the K^+ pump. We will attempt to incorporate this new information into a model for the interconnected K^+ and H^+ homeostasis in lepidopteran larvae.

In the case of potassium, the chemical and electrical driving forces across the midgut act in the same direction. The chemical PD driving potassium from lumen to blood, as calculated from activities, is lower than the value estimated from concentrations. Taking the data from Table 2 for posterior midguts of *Manduca* larvae fed on tobacco leaves, the chemical PD at 25°C, with blood as reference, based on activities is +18 mV whereas that based on concentrations is +41 mV. However, when the transepithelial potential (TEP) is taken into account, these differences become relatively insignificant. Assuming the transmidgut electrical PD *in vivo* to be +155 mV (lumen positive) (Harvey *et al.* 1986), the corresponding electrochemical PDs would be +173 mV based on activities and +196 mV based on concentrations. These data emphasize that the electrical PD generated by the K^+ pump dominates the driving force for potassium from lumen to blood side.

With the forces driving K^+ in mind, a preliminary explanation of the regulation of lumen K^+ can be attempted. When dietary potassium is high the chemical PD driving K^+ from lumen to blood would increase; potassium would be driven to the blood faster than it is pumped to the lumen. The resulting increase in blood $[K^+]$ would favour potassium excretion by the Malpighian tubules (Irvine, 1969), until blood levels returned to normal. When dietary potassium is low the chemical component of the driving force would decrease; potassium would be driven from lumen to blood more slowly than it was pumped from blood to lumen. The resulting decrease in blood $[K^+]$ would favour K^+ reabsorption by the cryptoneph-

ridial system, until lumen potassium returned to normal. The electrical component of the potassium driving force would be relatively insensitive to these fluctuations, because the pump PD reaches maximum values at very low transport rates (Harvey & Zerahn, 1972). In his conclusion to his analysis of the cryptonephric condition in lepidopteran larvae, Ramsay (1976) anticipated this midgut–Malpighian tubule–rectal complex cooperativity. 'Selection pressure on lepidopteran larvae living on leaves is for rapid growth; this is achieved by a high throughput of food and by a digestive system which involves massive internal fluxes of ions; the Malpighian tubules have been pressed into the direct service of the rectal epithelium to provide a powerful and quickly acting mechanism to compensate for any maladjustment of these fluxes...'.

In the case of H^+ the chemical and electrical driving forces across the midgut are opposed. Again taking the values from Table 2 for posterior midgut, the chemical PD is −183 mV, driving protons towards the lumen. Of course, these values are still higher in the anterior and middle midgut sections, as reported previously (Dow, 1984). The assumed transepithelial electrical PD of +155 mV driving protons towards the blood is comparable with that required for protons to be in electrochemical equilibrium, at least across the posterior midgut. The closeness of this agreement must await simultaneous determination of PD and pH gradients in the midgut *in vivo*. However, if the pH gradient is established by the polarization of the goblet apical membrane, as argued by Dow (1984), then the midgut pH would be significantly higher than predicted by the gross TEP, as the goblet cavity is significantly positive, compared with the apical lumen, in both posterior (Moffett & Koch, 1988) and middle (Dow, 1986; Dow & Peacock, 1989) midgut. The lumen K^+ and H^+ profiles resemble each other quite closely, as would be predicted from a model linking the generation of high pH to electrogenic K^+ transport. The constancy of lumen K^+ activity may help maintain the lumen pH at a level optimal for growing larvae.

These pH profiles, obtained under varying dietary [K^+] in a single species, complement those previously obtained for four different species (Dow, 1984). It is interesting to note their consistency, the slight decline in pH in posterior midgut, and the much more drastic decline presumably achieved by the hindgut. The columnar cells of posterior midgut contain carbonic anhydrase (Ridgway & Moffett, 1986), implying that they may serve a role in the neutralization of the gut contents. However, the major site for acidification of the gut contents may be the hindgut, and possibly the cryptonephridial system, which may serve a role in ionic homeostasis (Ramsay, 1976).

Summary of midgut K^+, H^+ homeostasis

Active electrogenic K^+ transport from blood to midgut lumen gives rise to a large electrical PD (lumen positive). The electrical PD along with a small chemical PD drives K^+ from lumen to blood. The resulting K^+ pump–leak steady state regulates blood and midgut cell K^+ levels. The blood level may be fine-tuned by the Malpighian tubules and cryptonephridial system if blood K^+ cannot be held

constant by the pump–leak steady state. The electrochemical PD-driven K^+ leak is used for nutrient absorption. The electrical PD regulates lumen pH. Lumen $[K^+]$ is influenced by the midgut K^+ pump–leak steady state and regulated by the cryptonephridial system in response to changes in blood $[K^+]$. The regulation of luminal $[K^+]$ would aid processes dependent on the transmidgut K^+ gradient.

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