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Electrical profiles in the midgut of two larvae of lepidoptera

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Fisiologia. — *Electrical profiles in the midgut of two larvae of lepidoptera* (*). Nota di GIANLUIGI MONTICELLI e BARBARA GIORDANA, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — L'intestino medio delle larve dei Lepidotteri non assorbe Na, ma svolge un ruolo omeostatico nella escrezione di K, tramite una pompa che estrude questo catione nel lume intestinale. Nel presente lavoro sono stati analizzati i profili elettrici degli enterociti delle larve di Philosamia cynthia e Bombyx mori, misurando il potenziale cellulare della membrana rivolta verso l'emolinfa (V_s) e verso il lume (V_m). Si è osservato che i valori di V_s possono essere suddivisi in due intervalli (0–15 e 16–70 *m*V), che corrispondono ai due tipi di cellule costitutive del tessuto, le cellule colonnari e quelle a coppa. Il valore di V_s misurato nelle cellule colonnari di B. mori non è diverso dal potenziale di equilibrio del K calcolato, mentre più complessa sembra essere la genesi del potenziale della membrana controluminale in P. cynthia.

INTRODUCTION

The knowledge of transmembrane electrical potential differences across luminal and basolateral membranes as well as intracellular ion activities in epithelial cells is necessary to calculate ion electrochemical gradients across the single barriers, in order to investigate the nature of ion transport. The electric profile of the widely distributed sodium-transporting epithelia are at present well characterized, while less data are available on typical potassium transporting tissue such as the midgut of Lepidopteran larvae. The midgut epithelium, which consists of a single layer of two kinds of epithelial cells—columnar and goblet cells—whose morphology has been studied in detail [1–3], bears a transepithelial electrical potential difference of 100–150 mV, lumen positive with respect to haemolymph side, that is almost entirely accounted for by a Na independent electrogenic K^+ pump. Midgut intracellular potential profiles reported up to date have been recorded only in two different larvae of Lepidoptera, i.e. Hyalophora cecropia and Manduca sexta [4–6].

In the present paper we intend to add a contribution to the knowledge of midgut potential profiles from impalements performed on midgut cells of Philosamia cynthia and Bombyx mori larvae.

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(**) Nella seduta del 25 novembre 1982.

Materials and methods

Larvae in the fifth instar of Philosamia cynthia or of Bombyx mori have been used. The larvae were reared in the laboratory and fed on Ailanthus glandulosa and Morus alba leaves respectively. The midgut was dissected from the larvae as a cylinder, and the peritrophic membrane with the enclosed intestinal contents as well as the malpighian tubules were accurately removed. The midgut was then mounted as a cylinder between two sections of glass tubing, with a gap of about 0.5 cm, and separated two compartments: a large volume of saline bathed the haemolymph side of the tissue, while the lumen side was flushed with the solution by means of a peristaltic pump. The composition of the standard saline was (mmol/l) 1.7 NaCl, 21 KCl, 25 $KHCO_3$, 44 $MgSO_4$, 9 $CaCl_2$, 110 sucrose for B. mori and 4 NaCl, 25 $KHCO_3$, 37 $MgSO_4$, 9 $CaCl_2$, 156 sucrose for P. cynthia. Salines were kept in equilibrium with a 95 % O_2–5 % CO_2 gas mixture, pH 7.4. The experiments were performed at room temperature (20–22 °C). In some experiments luminal and haemolymph potassium was substituted by equal amounts of sodium. In other experiments, L–alanine to a final concentration of 10 mmol/l was added to the saline. Transepithelial electrical potential recordings were obtained by means of a fixed Ag–AgCl electrode in the lumen side of the midgut and a reference electrode in the haemolymph side.

Intracellular recordings were obtained using glass microelectrodes filled with 3 M KCl and having an electrical restistance of 10–20 MΩ. The electrical circuit was of the conventional type. The midguts were always impaled from the haemolymph side, and potential profiles were measured between the microelectrode and the haemolymph side reference electrode.

Results and discussion

The impalements were always performed by advancing the microelectrode towards the tissue from the haemolymph side. Fig. 1 shows typical potential profiles recorded in B. mori (A, B) and P. cynthia (C) midgut isolated as cylinders and perfused with standard saline. Impalements performed on B. mori midguts mounted as a sac (not reported in this paper) gave the same profiles. In A, as well as in C, the progressive advancement of the microelectrode from the haemolymph to the intracellular compartment and across the mucosal border is shown. In C the transepithelial electrical potential difference (PD) was also recorded by means of a fixed electrode (it must be noted that the scale bars of the two recordings are different).

These potential profiles are in agreement with those recorded for H. cecropia and M. sexta [4–6] and referred to as high potential difference (HPD) profiles. As a matter of fact, two kinds of cells are present in lepidopteran midgut, i.e. columnar and goblet cells (see Akai, 1969, for B. mori [2]), and Blankemeyer

and Harvey [5] have given evidence that HPD profiles, with basal steps between —16 mV and —46 mV, should be considered typical of columnar cells while low potential difference (LPD) profiles, with basal steps on the order of —1 mV to —15 mV, appear typical of goblet cells.

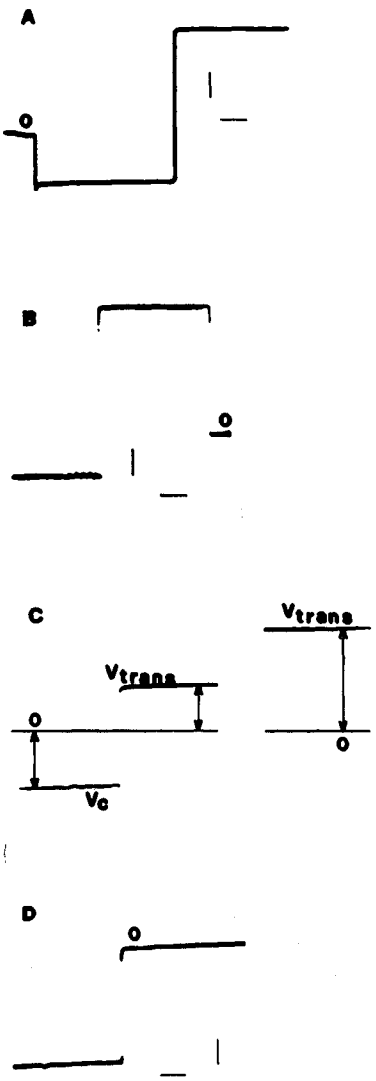

Fig. 1. – Electrical potential profiles obtained as a microelectrode is moved from the haemolymph side through a midgut isolated from B. mori (A, B) or P. cynthia (C, D). A, B, C: both luminal and haemolymph sides were bathed with standard saline. D: electrical profile recorded in the absence of K ions on both sides. C shows the contemporary traces of the transepithelial electrical potential difference, recorded by means of a fixed electrode placed in the lumen side, and of the potential profile recorded by means of a penetrating microelectrode. A reference electrode was put in the haemolymph side. Ordinate: A, B, C, D 20 mV; in C the ordinate of the transepithelial PD, recorded separately, is 10 mV. Abscissa: A 2 sec; B, C 1 sec; D 200 m sec.

The frequency distribution of all the impalements performed in standard conditions are reported in Fig. 2 (B. mori) and Fig. 3 (P. cynthia). Considering these distributions as representative of a single population, the mean value ($\pm$ S.E.) of the basolateral potential (basal step) is 28.5 ± 1.6 mV ($n = 86$) and 30.9 ± 0.9 mV ($n = 103$) respectively, cell negative with respect to haemolymph. It should be noted that when the midgut of the first larva, i.e., P. cynthia (Fig. 3) was studied, all basal step recordings inferior to —5 mV were considered arti-

facts and not registered and only in later experiments (Fig. 2, B. mori), on the basis of the cited Authors, were all recordings taken into account. Therefore, only the distribution reported in Fig. 2 can legitimately be considered as representative of two populations corresponding to columnar and goblet cells. In this case, the mean value for columnar basal steps (HPD profiles) is 34.1 $\pm$ 1.2 mV (69 impalements). This value is not different from the K^+ equilibrium

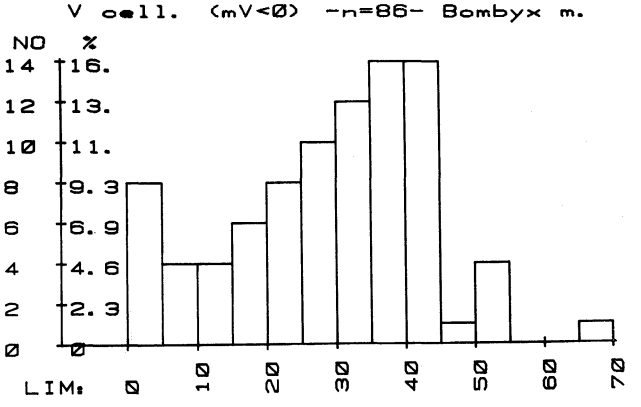

Fig. 2. – Frequency distribution of 86 impalements from 4 different preparations. Epithelial cells of B. mori midgut.

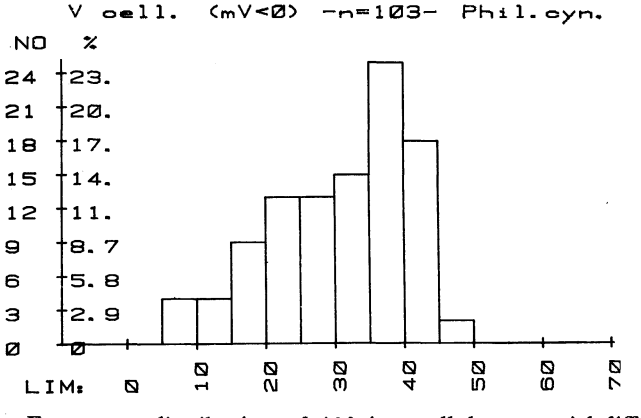

Fig. 3. – Frequency distribution of 103 intracellular potential differences from 5 different preparations. Epithelial cells of P. cynthia midgut.

potential (32.2 mV) calculated from the Nernst equation, taking into account an intracellular K^+ activity of 128 mequiv/l cell water, estimated from intracellular concentrations [7], assuming an activity coefficient of 0.8 [8]. Conversely, the experimental mean value found for P. cynthia, 31.9 $\pm$ 0.8 mV. (95 impalements; basal steps inferior to —15 mV have been excluded) is far from the calculated K^+ equilibrium potential (49.9 mV); therefore some other ion must be implied in the genesis of this potential.

Fig. 4 reports intracellular recordings (each point an impalement) as well as transepithelial PD before and after the removal of K ions from perfusing solutions in P. cynthia. As already observed [9], the transepithelial PD (upper trace) rapidly declines in the absence of haemolymph K ions, and the polarity

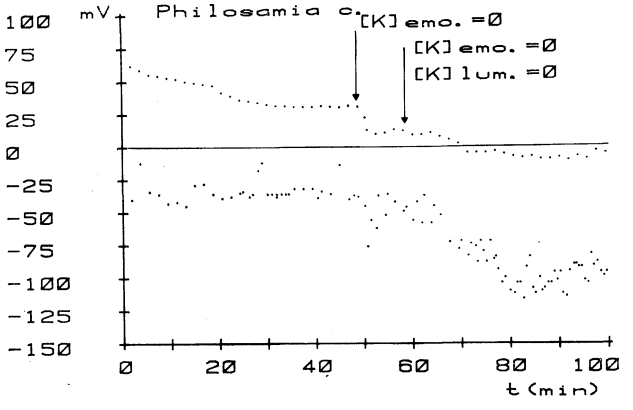

Fig. 4. – Effect of K ion removal on the transepithelial (upper trace) and cellular (lower trace) electrical potentials in P. cynthia midgut.

reverses, giving rise to an appreciable lumen negative potential. At the same time, a marked increase of basolateral membrane potential (lower trace) is recorded (see also Fig. 1 D), as demonstrated by the frequency distribution of 59 impalements reported in Fig. 5. The hyperpolarization of the membrane

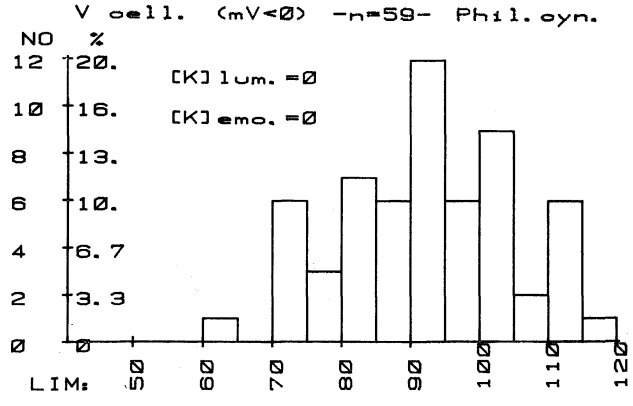

Fig. 5. – Frequency distribution of 59 intracellular recordings in P. cynthia midgut cells when K ions are removed from both sides.

can be explained by the diffusion potential caused by K^+ diffusion from the cell. Besides, as it has also been reported for H. cecropia and M. sexta [4, 6], a ten-fold decrease in external K^+ concentration does not cause a 58 mV increase of the basal step.

The experiment reported in Fig. 6 shows the effect of L-alanine 10 mM added to the haemolymph compartment, on the transepithelial PD (upper trace) and on basal potential of B. mori (lower trace; no discrimination between HPD and LPD profiles has been made). It has already been shown [10] that L-alanine, expecially from the haemolymph side of the midgut, causes a relevant increase of the transepithelial PD that counteracts the typical potential decay profile of

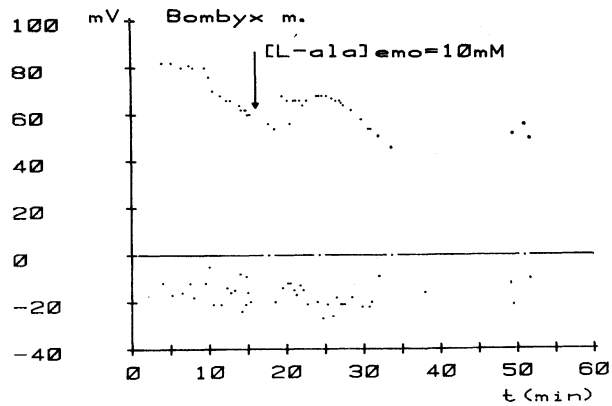

Fig. 6. – Effect of L-alanine on the transepithelial electrical potential difference (upper trace) and on cellular potential (lower trace) in B. mori midgut.

Lepidopteran midguts in vitro [11, 12]. Because metabolic products of L-alanine metabolization, such as pyruvic acid, also mimic the amino acid effect, the suggestion has been made that L-alanine, which is metabolized by midgut cells [13] could provide energy supply for the K^+ pump. Fig. 6 shows that the enhancement of the transepithelial PD is not due to a depolarization of the basolateral transmembrane potential, thus giving support to the hypothesis that the effect of the amino acid is exerted on the K^+ pump located on the luminal membrane [5].

References

[1] E. Anderson and W. R. Harvey (1966) – *Active transport by the Cecropia midgut – II. Fine structure of the midgut epithelium.* « J. Cell. Biol. », *31*, 107–134.

[2] H. Akai (1969) – *Ultrastructural localization of phosphatases in the midgut of the silkworm Bombyx Mori.* « J. Insect Physiol. », *15*, 1623–1628.

[3] M. Cioffi (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.

[4] J. L. Wood, P. S. Farrand and W. R. Harvey (1969) – *Active transport of potassium by the cecropia midgut – IV. Microelectrode potential profile.* « J. Exp. Biol. », *50*, 169–178.

[5] J. T. Blankemeyer and W. R. Harvey (1978) – *Identification of active cell in potassium transporting epithelium.* « J. Exp. Biol. », *77*, 1–13.

[6] D. F. Moffett and R. Hudson (1980) – *Cell membrane potential, internal* K^+ *activity and external* K^+ *in the* K^+*–transporting midgut of the tabacco Hornworm.* « J. Gen. Physiol. », *76*, 13a.

[7] B. GIORDANA and V. F. SACCHI (1977) – *Cellular ionic concentrations in the midgut of two larvae of Lepidoptera* in vivo *and* in vitro. « Comp. Biochem. Physiol. », *59*, 17–20.

[8] K. ZERAHN (1975) – *Potassium exchange between bathing solution and midgut of Hyalophora cecropia and time delay for potassium flux through the midgut* « J. exp. Biol. », *63*, 295–300.

[9] B. GIORDANA and F. SACCHI (1977) – *Some ionic and electrical parameters of the intestinal epithelium in three mature larvae of Lepidoptera.* « Comp. Biochem. Physiol. », *56A*, 95–99.

[10] B. GIORDANA and V. F. SACCHI (1978) – *Glycine and L-alanine influence on transepithelial electrical potential difference in the midgut of Bombyx mori larva* in vitro. « Comp. Biochem. Physiol. », *61*, 605–609.

[11] T. W. SCHULTZ and A. M. JUNGREIS (1977) – *Origin of the short circuit decay profile and maintenance of the cation transport capacity of the larval lepidopteran midgut* in vitro *and* in vivo. « Tissue and Cell », *9*, 255–272.

[12] B. GIORDANA and V. F. SACCHI (1980) – *The transepithelial electrical potential decay across the isolated midgut of two larvae of Lepidoptera* (Bombyx mori and Philosamia cynthia). « Comp. Biochem. Physiol. », *66*, 533–536.

[13] V. F. SACCHI and B. GIORDANA (1980) – *Absorption of glycine, L-alanine and L-phenylalanine in the midgut of the larvae of Bombyx mori.* « Experientia », *36*, 659–660.