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Energetics of Potassium Ion Transport in *Aplysia* Gut

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ABSTRACT—Basolateral membranes of *Aplysia californica* foregut epithelia contain an ATP-dependent Na^+/K^+ transporter (Na^+/K^+ pump or Na^+/K^+ -ATPase). This Na^+/K^+ pump accounts for both the intracellular Na^+ electrochemical potential ($\bar{\mu}$) being less than the extracelluar Na^+ $\bar{\mu}$ and the intracellular K^+ $\bar{\mu}$ being more than the extracellular K^+ $\bar{\mu}$. Also, K^+ channel activity resides in both luminal and basolateral membranes of the *Aplysia* foregut epithelial cells. Increased activity of the Na^+/K^+ pump, coupled to luminal and basolateral membrane depolarization altered the K^+ transport energetics across the basolateral membrane to a greater extent than the alteration in K^+ transport energetics across the luminal membrane. These results suggest that K^+ transport, either into or out of the *Aplysia* foregut epithelial cells, is rate-limiting at the basolateral membrane.

Key words: electrochemical, K^+ transport, Na^+/K^+- ATPase

INTRODUCTION

Active Na^+ absorption by the *Aplysia californica* (seahare) foregut is mediated by a basolaterally-localized Na^+/K^+-stimulated ATPase (Gerencser and Lee, 1985a). This Na^+/K^+ pump accounts for the intracellular Na^+ electrochemical potential ($\bar{\mu}$) being less than the extracellular Na^+ $\bar{\mu}$ while, conversely the intracellular K^+ far exceeds the extracellular K^+ $\bar{\mu}$ (Gerencser, 1983; 1984). Na^+ transport across the apical membrane into the cytosol of these cells is mediated by, at least, two different mechanisms (Gerencser, 1985). One of these mechanisms is a Na^+ channel (Gerencser, 1981a) while the other mechanism is a compilation of symport processes (Gerencser, 1978; 1981b; Gerencser and Levin, 2000). Aminoisobutyric acid (AIB), a nonmetabolizable amino acid, is actively accumulated by the *Aplysia* foregut in the presence of Na^+, and AIB also stimulates the absorptive flux of Na^+; both of these events being mediated by a common symporter (Gerencser, 1981b). The cotransport of AIB and Na^+ across the apical membrane of the *Aplysia* foregut absorptive cell initiates a series of events: 1) depolarization of the mucosal membrane potential difference (Ψ_m), hyperpolarization of the transepithelial potential difference (Ψ_{ms}), increase in intracellular Na^+ activity (a^i_{Na}) and an increase in Na^+ absorption (Gerencser, 1981b; Gerencser, 1996; Gerencser *et al*., 1999.) All of these events intersect with the Na^+/K^+ pump located in the basolateral membrane (BLM) of these cells. The increase in intracellular Na^+ stimulated by AIB has a profound effect on the Na^+/K^+ -ATPase activity and consequently, on the energetic profiles of Na^+ and K^+ across both the apical or mucosal membrane and the BLM of these *Aplysia* gut epithelial cells. There are K^+ channels also present in both the mucosal and basolateral membranes of these *Aplysia* foregut absorptive cells (Gerencser *et al*., 1999) and activity by these channels can also alter the energetic profiles of Na^+ and K^+. In view of the luminal membrane coupling of Na^+ and AIB, the present study was undertaken to determine whether this event altered the energy gradient of K^+ across the mucosal and/or BLM where the Na^+/K^+ pump transports Na^+ uphill out of the cell and transports K^+ uphill into the cell (Gerencser and Lee, 1985; Gerencser, 1996).

MATERIALS AND METHODS

Mollusc and Chemicals

Adult seahares (*Aplysia californica*) were obtained from Marinus Inc. (Westchester, CA) and were maintained at 25°C in circulating filtered sea water. Adult *Aplysia* (600–1000 g) were used in these experiments. Aminoisobutyric acid, barium chloride and ouabain were purchased from Sigma Chemical. All other reagent-grade chemicals were purchased from Fisher Scientific. ^{42}KCl was purchased from Amersham.

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Experimental Procedures and Incubation Medium

The animals were sacrificed and their posterior foreguts were removed, slit longitudinally, rinsed and then positioned between two halves of a Lucite chamber described previously (Gerencser, 1983) which allowed measurement of transepithelial electrical potential (Ψ_{ms}) and, simultaneously, the introduction of microelectrodes into the surface epithelial cells. The chamber exposed the tissue to an oxygenated seawater medium. The formula for the seawater medium was (in mM): NaCl, 462.0; $MgSO_4 \cdot 7H_2O$, 2.4; KCl, 10.0; $KHCO_3$, 2.4; $MgCl_2$, 9.8; $CaCl_2$, 11.4. The total osmolality of the bathing medium was 1000 mosmol/l and the final pH was 7.8. Microelectrodes for measurements of mucosal membrane potential (Ψ_m) and $\bar{\mu}_k$ were constructed and utilized as previously described (Gerencser, 1983; Gerencser, 1993). Briefly, the experimental protocol was as follows: after the excised tissue as a sheet was placed between the two halves of a lucite chamber which allowed measurement of transepithelial potential difference (Ψ_{ms}) and, simultaneously, the introduction of microelectrodes into the cells lining the gut villi to obtain an independent estimate of Ψ_m. Then K^+-selective microelectrodes were passed into the villus epithelial cells to measure the intracellular $\bar{\mu}_K$ as seen in Fig. 1 which is a typical recording obtained with a K^+ -specific microelectrode. In Fig. 1, the K^+ specific microelectrode was advanced via micromanipulation across the mucosal membrane of the *Aplysia* foregut epithelial cell. Upon attainment of a steady-state potential difference, the electrode was left in place (intracellularly) for at least one minute. The microelectrode was then withdrawn from the cell. If the K^+ potential difference of the microelectrode did not return to a value within 1.0 mV of its original value prior to cellular penetration then the data was considered invalid. If the electrode potential returned to a value within 1.0 mV of its original value after its withdrawal from the cell, then it was retested for its original Nernstian characteristics to further test its validity and, therefore, the validity of the biological measurement (Gerencser, 1983). The intracellular K^+ activity (a^i_k) was calculated using Equation 1 where Ψ^i is the potential of the K^+ electrode in the cell.

$$a^i_k = a^{"}_k / e^{2.303\,[\Psi i - \Psi m) - \Psi"]/S} \quad (1)$$

and a^i_k is the activity of K^+, $\Psi^{"}$ the potential of the K^+ electrode in 500 mM KCl, and Ψ_m is the mean mucosal membrane potential. S is the slope of the electrode response and is defined as described previously (Gerencser, 1983). In Table 1, $\bar{\mu}_K$ is expressed as reversible work, in joules, required to transfer one equivalent of K^+ across the mucosal or basolateral (serosal) membrane of a foregut villus epithelial cell and is calculated from Equation 2:

$$\bar{\mu}_K = RT \ln a^i_K / a^o_K + z\Psi F \quad (2)$$

where R, T, z and F have their usual physicochemical meanings and Ψ can either be Ψ_m or Ψ_s (basolateral or serosal membrane potential). Ψ_s was calculated as previously described (Gerencser, 1983; Schultz, 1977).

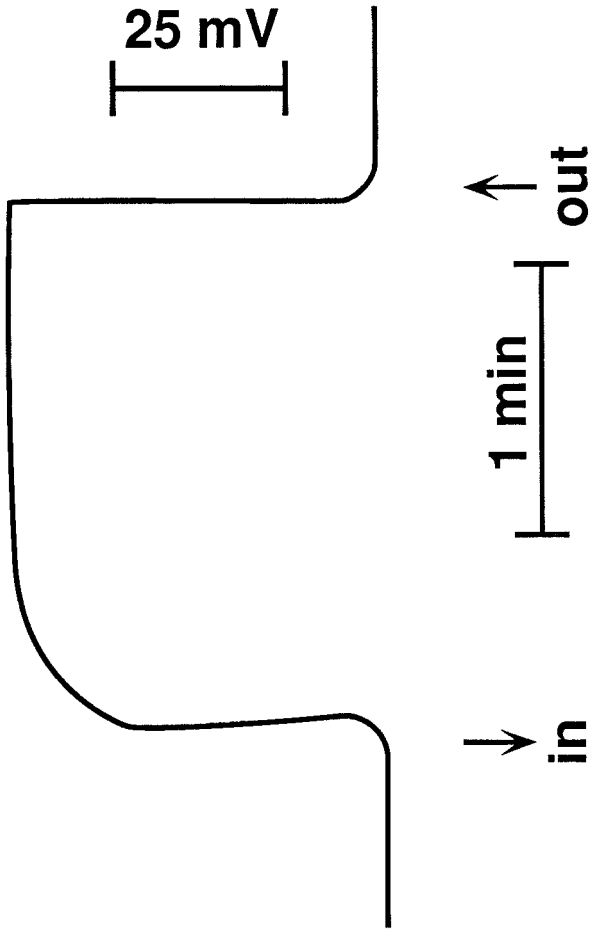

Fig. 1. Recording of an acceptable impalement with a K-selective microelectrode in an *Aplysia* gut epithelial cell. ↑ and ↓ indicate time of apical impalement and withdrawal of the microelectrode, respectively.

The application of radiotracer $^{42}K^+$, rinsing and scraping of the epithelium, dissolution of the epithelium and counting of $^{42}K^+$ were as described previously (Goldinger *et al.*, 1983; Gerencser, 1984). Briefly, after mucosal application of AIB and serosal application of $^{42}K^+$ to the isolated *Aplysia* foregut, the enterocytes were scraped

Table 1. Potential profiles, intracellular K^+ activities and transmembrane K^+ electrochemical potential differences in *Aplysia californica* foregut bathed in NaCl seawater medium in the presence and absence of mucosal aminoisobutyric acid.

	Ψ_m(mV)	Ψ_s(mV)	Ψ_{ms}(mV)	a^i_K(mM)	$\bar{\mu}^m_K$ (joules/equiv.)	$\bar{\mu}^s_K$(joules/equiv.)	n
Before AIB addition (Control)	–65.8±2.1 (46)	+64.9±2.2 (46)	–0.9±0.2 (46)	350±22 (55)	–2700±50 (55)	+2950±50 (55)	20
After AIB addition	–60.0±1.9 (25) P<0.05	+57.1±2.1 (25) P<0.05	–2.9±0.3 (25) P<0.05	358±21 (22) N.S.	–2800±25 (22) P<0.05	+3185±75 (22) P<0.05	10
After AIB + Ba^{2+} addition	–58.9±2.0 (22) N.S.	+55.3±1.8 (21) N.S.	–3.6±0.5 (20) P<0.05	410±15 (16) P<0.05	–3000±55 (16) P<0.05	+3465±70 (16) P<0.05	10

Values are means (S.E. N.S. is non-significant. Numbers in parentheses are number of observations; n is the number of animals. Polarity of Ψ_m and Ψ_{ms} are relative to the mucosal solution. Polarity of calculated Ψ_s is relative to cytoplasm. a^i_K was calculated by means of Eq. 1. $\bar{\mu}^m_K$ and $\bar{\mu}^s_K$ were calculated by means of Eq. 2. (–) $\bar{\mu}^m_K$ for represents uphill energy gradient from mucosal solution to cytosol. (+) for $\bar{\mu}^s_K$ (represents downhill energy gradient from cytosol to serosal solution.

Table 2. Potential profiles, intracellular K^+ activities and transmembrane K^+ electrochemical potential differences in *Aplysia californica* foregut bathed in NaCl seawater medium in the presence and absence of mucosal aminoisobutyric acid.

	Ψ_m(mV)	Ψ_s(mV)	Ψ_{ms}(mV)	a^i_K(mM)	$\bar{\mu}^m_K$ (joules/equiv.)	$\bar{\mu}^s_K$ (joules/equiv.)	n
Before AIB addition (Control)	–65.8±2.1 (26)	+64.9±2.2 (26)	–0.9±0.2 (26)	350±22 (35)	–2700±50 (35)	+2950±50 (35)	20
After AIB addition	–59.6±1.8 (18) P<0.05	+56.3±1.1 (18) P<0.05	–3.1±0.1 (18) P<0.05	352±31(18) N.S.	–2875 (18) P<0.05	+3185±75 (22) P<0.05	10
After Ouabain addition	–64.8±2.5 (20) N.S.	+64.6±2.7 (15) N.S.	0.2±0.1 (15) P<0.05	270±18 (10) P<0.05	–1650±100 (10) P<0.05	+1690±150 (10) P<0.05	10

Values are means (S.E. N.S. represents non-significance. Numbers in parentheses are number of observations; n is the number of animals. Polarity of Ψ_m and Ψ_{ms} are relative to the mucosal solution. Polarity of calculated Ψ_s is relative to cytoplasm. a^i_K was calculated by means of Eq. 1. $\bar{\mu}^m_K$ and $\bar{\mu}^s_K$ were calculated by means of Eq. 2. (–) $\bar{\mu}^m_K$ for represents uphill energy gradient from mucosal solution to cytosol. (+) for $\bar{\mu}^s_K$ represents downhill energy gradient from cytosol to serosal solution.

Table 3. Uptake of K^+ into *Aplysia* foregut epithelial cells as measured by the radioactivity of $^{42}K^+$ taken up into the epithelium

	cpm	N
Control	1236±126	18
After addition of AIB to M	2168±205 P<0.05	7
After addition of AIB to S	1138±98 N.S.	6
After addition of AIB to M and ouabain to S	736±109 P<0.05	6

Values are means±S.E. n is the number of animals. M. represents the mucosal aspect of the tissue while S represents the serosal aspect of the tissue.

from the tissue and emptied into nitric acid for dissolution. The dissolved tissue was then counted for gamma radiation in a gamma counter (Packer Prias). The data obtained were analyzed statistically by Student's t-test utilizing P<0.05 as the statistically significant quantitative criterion.

RESULTS

As demonstrated in the present study (Table 1), mucosally-applied 80 mM AIB significantly depolarized Ψ_m and significantly hyperpolarized Ψ_{ms}. Therefore the calculated change in Ψ_s exceeded the empirically determined change in Ψ_m. Also seen in Table 1 are the findings that mucosally-applied AIB did not significantly alter a^i_k from control but the $\Delta\,\bar{\mu}_k$ across the BLM exceeded the $\Delta\,\bar{\mu}_k$ across the mucosal membrane. Additionally, mucosally-applied AIB significantly raised a^i_k above control when 5 mM Ba^{2+} was placed in both the mucosal and serosal bathing solutions. As shown in Table 2, when 1 mM ouabain was added to the serosal medium, the a^i_k significantly decreased as compared to control as did the $\bar{\mu}$ for intracellular K^+ across both the luminal and basolateral membranes. Table 3 demonstrates that the foregut epithelial cells are stimulated to take up serodal $^{42}K^+$ in the presence of mucosal 80 mM AIB. Table 3 also shows that serosal 1 mM ouabain abolished the AIB-driven uptake of $^{42}K^+$ into the foregut epithelial cells.

DISCUSSION

Mucosally-applied AIB caused a depolarization of Ψ_m, a hyperpolarization of Ψ_{ms} and no significant change in a^i_K in the foregut cells of Aplysia californica (Table 1). These observations can be explained as follows: If an actively transported amino acid such as AIB is Na^+-coupled at the apical membrane (Gerencser, 1981b) this would depolarize the Ψ_m because of the electrogenic or rheogenic nature of the mechanism (Schultz, 1977). In other words, AIB would stimulate Na^+ transport across the mucosal membrane into the cytosol of the Aplysia foregut absorptive cell (Gerencser, 1981b; Gerencser, *et al*., 1999). The basolaterally-located electrogenic Na^+/K^+ pump or Na^+/K^+-ATPase [Gerencser and Lee, '85] would then accommodate the increased thermodynamic activity of Na^+ (Na^+ transport pool) by increasing its rate of work much as a variable output device which was described by Michaelis-Menten kinetics in an isolated Na^+ pump system (Gerencser and Lee, 1985; Gerencser, *et al*., 1999). In addition, this was previously demonstrated by an increase in the unidirectional mucosal-to-serosal Na^+ flux after mucosal AIB addition to the voltage-clamped, isolated *Aplysia* foregut (Gerencser, 1981b). The increased Na^+ flux, and concomitant increased intracellular Na^+ activity, would then stimulate an extracellular serosal to cytosol K^+ flux (Table 3) which is mediated by a ouabain-sensitive Na^+/K^+-ATPase as is also shown in Table 3. Since it has been shown that the major portion of the SCC before and after AIB addition to the mucosal solution is a Cl^- current (Gerencser, 1981b) which is generated by a Cl^- pump (Gerencser, 1996) there would be a decrease in the negativity of Ψ_s by both the additive increased electrogenic Na^+/K^+- and Cl^--pump activities; and, the linkage of Ψ_m to Ψ_s through a low resistance extracellular shunt (Gerencser and Loughlin, 1983) which would lead to a greater serosally-negative Ψ_{ms} (Schultz, 1977) as shown in Table 1. When AIB is present in the mucosal bathing solution the $\bar{\mu}$ against which the Na^+/K^+ pump is moving Na^+ out of the cell across the BLM decreases significantly relative to $\bar{\mu}$ across the BLM in the absence of AIB, as is shown in Table 1. Conversely, the $\bar{\mu}$ for K^+ entry into the cells across the BLM via the Na^+/K^+

pump increases because of the depolarization of Ψ_s despite there being no change in a^i_K (Table 1). The reason a^i_K does not change is because Na^+/K^+ pump activity (K^+ influx) is in a steady state with K^+ channel efflux activity which are present in both the apical and BLM's and this steady-state phenomenon is demonstrated in Table 1 where it was shown that Ba^{2+} added to both mucosal and serosal compartments, causes an increase in a^i_k. Ba^{2+} is a known inhibitor of K^+ channel activity (Van Driessche, *et al*., 1988) and, therefore, slows the exit of K^+ from the foregut cells which would increase a^i_K, as shown in Table 1. Serosal ouabain decreases the a^i_k (Table 2) suggesting that the Na^+/K^+ -ATPase is the mechanism responsible for maintaining a^i_k above electrochemical equilibrium since ouabain is a specific inhibitor of Na^+/K^+ -ATPase activity (Skou, 1965). In summary, it appears that the greatest energetic change for K^+ transport in the *Aplysia* foregut absorptive cells, in the presence of a Na^+ -coupled compound that can change Na^+/K^+ -ATPase activity such as AIB, occurred at the BLM (Table 1). Therefore, logically the BLM appears to be the rate limiting step for K^+ transport in *Aplysia* foregut epithelial cells whether the transport is extracellular to intracellular or vice versa.

The energetics for K^+ transport in *Aplysia* gut far exceeds those values found for K^+ transport in vertebrate epithelia (Gerencser, 1985) and most other invertebrate epithelia (Gerencser, 1985; 1996; Gerencser *et al*., 1999). This can be accounted for by the exceptionally steep gradients for K^+ directed from the extracellular to intracellular compartments (Table 1, Gerencser, 1983). Therefore, it would be expected that most of the transport work or energy expenditure by the *Aplysia* gut cells would be via Na^+/K^+-ATPase activity as previously alluded to (Gerencser, 1996). This ion-transport work by the Na^+/K^+ pump would set up secondary gradients of ions needed for nutritional uptake and growth and even catabolic or breakdown needs within these cells as previously suggested (Gerencser, 1996).

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