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Source: Zoological Science, 19(6) : 629-632
Published By: Zoological Society of Japan
URL: https://doi.org/10.2108/zsj.19.629
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 (\(\psi\)) being less than the extracellular Na+ \(\psi\) and the intracellular K+ \(\psi\) being more than the extracellular K+ \(\psi\). 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 (\(\psi\)) being less than the extracellular Na+ \(\psi\) while, conversely the intracellular K+ far exceeds the extracellular K+ \(\psi\) (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 (\(\Psi_m\)), hyperpolarization of the transepithelial potential difference (\(\Psi_{tm}\)), increase in intracellular Na+ activity (a\(_{Na}\)) 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 Mariner 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. 42KCl 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_{m}) 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}, 7H_{2}O, 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 Ψ_{s} 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_{m} - Ψ_{s}) 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 Ψ_{m} 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 Nemstian characteristics to further test its validity and, therefore, the validity of the biological measurement (Gerencser, 1983). The intracellular K⁺ activity (a_k) was calculated using Equation 1 where Ψ is the potential of the K⁺ electrode in the cell.

\[ a_k = a_i^{\Psi} e^{\frac{\Psi}{RT} \ln \frac{a_i}{a_k}} \]

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).

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.

![Fig. 1.](https://bioone.org/journals/zoological-science/26-sep-2019/terms-of-use)

**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.

| 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. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Before AIB addition (Control) | Ψ_{m}(mV) | Ψ_{s}(mV) | Ψ_{m}(mV) | a_k(MM) | Ψ_{m}^o (joules/equiv.) | Ψ_{s}^o (joules/ equiv.) | n |
| −65.8±2.1 (46) | +64.9±2.2 (46) | −9.0±0.2 (46) | 350±22 (55) | −2700±50 (55) | +2950±50 (55) | 20 |
| After AIB addition | −60.0±1.9 (25) | +57.1±2.1 (25) | −2.9±0.3 (25) | 358±21 (22) | −2800±25 (22) | +3185±75 (22) | 10 |
| After AIB + Ba²⁺ addition | −59.8±2.0 (22) | +55.3±1.8 (21) | −3.6±0.5 (20) | 410±15 (16) | −3000±55 (16) | +3465±70 (16) | 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_{s} is relative to the mucosal solution. Polarity of calculated Ψ_{m} is relative to cytoplasm. a_k was calculated by means of Eq. 1. Ψ_{m} and Ψ_{s} were calculated by means of Eq. 2. (−) Ψ_{m}^o represents uphill energy gradient from mucosal solution to cytosol. (+) for Ψ_{s} (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.

<table>
<thead>
<tr>
<th></th>
<th>Ψᵐ(mV)</th>
<th>Ψˢ(mV)</th>
<th>Ψᵐˢ(mV)</th>
<th>aₖ(mM)</th>
<th>Ψᵐ⁻(joules/eqv.)</th>
<th>Ψˢ⁻(joules/eqv.)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before AIB addition</td>
<td>–65.8±2.1(26)</td>
<td>+64.9±2.2(26)</td>
<td>–0.9±0.2(26)</td>
<td>350±22(35)</td>
<td>–2700±50(35)</td>
<td>+2950±50(35)</td>
<td>20</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After AIB addition</td>
<td>–59.6±1.8(18)</td>
<td>+56.3±1.1(18)</td>
<td>–3.1±0.1(18)</td>
<td>352±31(18)</td>
<td>–2875(18)</td>
<td>+3185±75(22)</td>
<td>10</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>N.S.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>After Ouabain addition</td>
<td>–64.8±2.5(20)</td>
<td>+64.6±2.7(15)</td>
<td>0.2±0.1(15)</td>
<td>270±18(10)</td>
<td>–1650±100(10)</td>
<td>+1690±150(10)</td>
<td>10</td>
</tr>
<tr>
<td>N.S.</td>
<td>N.S.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

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 Ψᵐ and Ψˢ are relative to the mucosal solution. Polarity of calculated Ψˢ is relative to cytoplasm. aₖ was calculated by means of Eq. 1. Ψᵐ⁻ and Ψˢ⁻ were calculated by means of Eq. 2. (–) Ψᵐ⁻ represents uphill energy gradient from mucosal solution to cytosol. (+) for Ψˢ⁻ 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 ⁴²K⁺ taken up into the epithelium

<table>
<thead>
<tr>
<th></th>
<th>cpm</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1236±126</td>
<td>18</td>
</tr>
<tr>
<td>After addition of AIB to M</td>
<td>2168±205</td>
<td>7</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After addition of AIB to S</td>
<td>1138±98</td>
<td>6</td>
</tr>
<tr>
<td>N.S.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After addition of AIB to M and ouabain to S</td>
<td>736±109</td>
<td>6</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

RESULTS

As demonstrated in the present study (Table 1), mucosally-applied 80 mM AIB significantly depolarized Ψᵐ, and significantly hyperpolarized Ψᵐˢ. Therefore the calculated change in Ψˢ exceeded the empirically determined change in Ψᵐ. Also seen in Table 1 are the findings that mucosally-applied AIB did not significantly alter aₖ from control but the Δ μ⁺ across the BLM exceeded the Δ μ⁻ across the mucosal membrane. Additionally, mucosally-applied AIB significantly raised aₖ above control when 5 mM Ba²⁺ 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 aₖ significantly decreased as compared to control as did the μ⁻ for intracellular K⁺ across both the luminal and basolateral membranes. Table 3 demonstrates that the foregut epithelial cells are stimulated to take up serosal ⁴²K⁺ in the presence of mucosal 80 mM AIB. Table 3 also shows that serosal 1 mM ouabain abolished the AIB-driven uptake of ⁴²K⁺ into the foregut epithelial cells.

DISCUSSION

Mucosally-applied AIB caused a depolarization of Ψᵐ, a hyperpolarization of Ψᵐˢ and no significant change in aₖ 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⁺-couple at the apical membrane (Gerencser, 1981b) this would depolarize the Ψᵐ 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, et al., 1996) there would be a decrease in the negativity of Ψˢ by both the additive increased electrogenic Na⁺/K⁺- and Cl⁻-pump activities; and, the linkage of Ψᵐ to Ψˢ through a low resistance extracellular shunt (Gerencser and Loughlin, 1983) which would lead to a greater serosally-negative Ψᵐˢ (Schultz, 1977) as shown in Table 1. When AIB is present in the mucosal bathing solution the μ⁻ against which the Na⁺/K⁺ pump is moving Na⁺ out of the cell across the BLM decreases significantly relative to μ⁻ across the BLM in the absence of AIB, as is shown in Table 1. Conversely, the μ⁻ for K⁺ entry into the cells across the BLM via the Na⁺/K⁺-ATPase decreases significantly relative to μ⁻ across the BLM in the absence of AIB.
pump increases because of the depolarization of $\Psi_s$ 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 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 breakdown needs within these cells as previously suggested (Gerencser, 1996).

ACKNOWLEDGEMENTS

I would like to acknowledge the excellent technical assistance of F. Robbins. This investigation was supported by the Epplle Foundation for Research, Inc.

REFERENCES


(Received January 23, 2002 / Accepted March 18, 2002)