SHORT COMMUNICATION

INHIBITION OF A CHLORIDE PUMP BY ACETAZOLAMIDE IN THE INTESTINE OF APLYSIA CALIFORNICA

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The isolated intestine of Aplysia californica, bathed in a substrate-free NaCl seawater bathing medium, generates a spontaneous transepithelial potential difference such that the serosal surface is negative relative to the mucosal surface (Gerencser, 1978a). The short-circuit current (Isc) is accounted for by active absorptive mechanisms for both Na⁺ and Cl⁻, the Cl⁻ transport mechanism being more vigorous than that for Na⁺ (Gerencser, 1978a). However, Cl⁻ transport appeared to be independent of Na⁺ transport, for the Isc measured in an Na⁺-free seawater bathing medium was shown to be identical to a net active absorptive flux of Cl⁻ (Gerencser, 1984a). It was hypothesized that active Cl⁻ absorption in Aplysia enterocytes was mediated by a primary active transport process, because it had been demonstrated that the intracellular Cl⁻ electrochemical potential was less than that measured in the extracellular medium (Gerencser & White, 1980), even in the absence of extracellular Na⁺ (Gerencser, 1983). Lending strength to this hypothesis, Gerencser & Lee (1983, 1985a) demonstrated the existence of a Cl⁻-stimulated ATPase activity in Aplysia enterocyte plasma membranes, suggesting a cause-and-effect relationship between ATPase activity and Cl⁻ transport. The ATPase activity stimulated by Cl⁻ was strongly inhibited by acetazolamide. In addition, Gerencser (1984b) and Gerencser & Lee (1985b) have demonstrated an ATP-dependent Cl⁻ uptake in Aplysia inside-out enterocyte plasma membrane vesicles (EPMV). Therefore, the present study was undertaken to assess the effect of acetazolamide on the ATP-driven Cl⁻ uptake mechanism in EPMV.

Seahares (Aplysia californica) were obtained from Marins Inc. (Westchester, CA) and were maintained at 25°C in circulating filtered seawater. Adult Aplysia (600–1000 g) were used in these experiments. The plasma membrane vesicles were prepared from Aplysia intestinal enterocytes by homogenization and differential and discontinuous sucrose density-gradient centrifugation techniques as described previously (Gerencser & Lee, 1985a). Vesicle transport experiments were also performed as previously described (Gerencser & Lee, 1985b).

The transmembrane electrical potential (Δψ) was estimated from the distribution of the lipophilic cation triphenylmethylphosphonium (TPMP⁺) between the

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extra- and intravesicular space by ultrafiltration as described above and by a double-
labelling method as described by Lee & Pritchard (1983). Non-specific binding
of TPMP⁺ to the vesicular membranes (Goldinger, Duffey & Hong, 1983) was
assessed by using non-ionic media in the membrane preparative, reaction mixture
and ultrafiltration stages of the TPMP⁺ electrical potential difference assay. When
converted into a ΔΨ, 31.1 mV was then subtracted from the total to give the ATP-
dependent ΔΨ.

As demonstrated in the present study (Table 1), the addition of ATP, in the
presence of Mg²⁺, to EPMV of Aplysia elicited a rapid Cl⁻ uptake significantly above
that of control. This difference in Cl⁻ uptake is the ATP-dependent portion of the
total Cl⁻ uptake into the EPMV and it is inhibited 50.3 ± 6.1% by 1 mmol l⁻¹
acetazolamide. Similarly, in the same preparation of EPMV, the ΔΨ was inhibited by
acetazolamide 90.6 ± 2.1%, which is similar quantitatively to the effect of
1 mmol l⁻¹ acetazolamide on Cl⁻-stimulated ATPase activity in the same preparation
(Gerencser & Lee, 1985a). The above values are means ± s.e. for 9–12 different
experiments (36–42 animals).

The present finding (Table 1) that acetazolamide inhibited the ATP-dependent
Cl⁻ uptake and intravesicular negative potential (ΔΨ) in Aplysia EPMV is
consistent with the following previous findings: (1) acetazolamide inhibition of active
Cl⁻ absorption and Iₑ in in vitro Aplysia intestine (Gerencser, 1984a) and
(2) acetazolamide inhibition of Cl⁻-stimulated ATPase activity in Aplysia EPMV
(Gerencser & Lee, 1985a). Although acetazolamide, at low concentrations, has been
shown to be a specific inhibitor of carbonic anhydrase (Maren, 1977), it has also been
demonstrated to be a good Cl⁻ transport inhibitor (White, 1980). Thus the data
further strengthen the idea that the Cl⁻-stimulated ATPase, which is inhibited by
acetazolamide, may be involved in Cl⁻ transport across the Aplysia intestine.

Table 1. Effect of acetazolamide on ATP-dependent Cl⁻ transport and ΔΨ

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Cl⁻ transport (nmol mg⁻¹ protein)</th>
<th>Inhibition (%)</th>
<th>ΔΨ (mV)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None, +ATP</td>
<td>106.7 ± 10.3</td>
<td>—</td>
<td>34.9 ± 2.5</td>
<td>—</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>78.2 ± 6.5</td>
<td>50.3 ± 16.1</td>
<td>3.3 ± 0.6</td>
<td>90.6 ± 2.1</td>
</tr>
<tr>
<td>None, −ATP</td>
<td>50.0 ± 9.2</td>
<td>—</td>
<td>31.1 ± 5.3*</td>
<td>—</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>48.9 ± 2.3</td>
<td>2.2 ± 6.4</td>
<td>32.8 ± 6.2*</td>
<td>−5.4 ± 5.8</td>
</tr>
</tbody>
</table>

Acetazolamide (1 mmol l⁻¹) was preincubated with membrane vesicles in the reaction mixture (50 μl containing 10 mmol l⁻¹ Tris/Hepes, pH 7.8, 250 mmol l⁻¹ sucrose, 3 mmol l⁻¹ MgSO₄ and 25 mmol l⁻¹ choline chloride) for 10 min at 25°C. ATP-independent Cl⁻ uptake at 15 s was determined to be 50.0 nmol mg⁻¹ protein. This value was used in the final computation of 'Inhibition (%)'.

Non-specific bound TPMP⁺ to membrane vesicles was accounted for in the final computation of ΔΨ in the upper part of the table.

ΔΨ refers to non-specific vesicle-bound triphenylmethylphosphonium (TPMP⁺) and the effect of acetazolamide on this component (lower part of the table, ΔΨ column).

Values are means ± s.e. for 9–12 different experiments (36–42 animals).
Additionally, the finding that ATP, in the presence of Cl\textsuperscript{-}, can stimulate \( \Delta \psi \) (increase in intravesicular negativity), as seen in Table 1, also suggests that the mechanism responsible for this phenomenon is electrogenic.

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REFERENCES