Enhanced Net K⁺ Uptake Capacity of NaCl-Adapted Cells

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ABSTRACT

Maintenance of intracellular K⁺ concentrations that are not growth-limiting, in an environment of high Na⁺, is characteristic of NaCI-adapted cells of the glycophyte, tobacco (Nicotiana tabacum/gossii). These cells exhibited a substantially greater uptake of ⁴²Rb⁺ (i.e. an indicator of K⁺) relative to unadapted cells. Potassium uptake into NaCl-adapted cells was 1.5-fold greater than unadapted cells at 0 NaCl and 3.5-fold greater when cells were exposed to 160 millimolar NaCl. The difference in net K⁺ uptake between unadapted and NaCl-adapted cells was due primarily to higher rates of entry rather than to reduced K⁺ leakage. Presumably, enhanced K⁺ uptake into adapted cells is a result of electrophoretic flux, and a component of uptake may be linked to vanadate-sensitive H⁺ extrusion.

Intracellular ion uptake is an integral component of osmotic adjustment necessary for adaptation to salt (3, 7, 10). However, because of the deleterious effects of high ion concentrations on cytosolic physiology and biochemistry (7, 10, 28), it is essential that ion levels in this compartment be stringently controlled. This is apparently accomplished by precise coordination and regulation of intracellular ion accumulation, vacuolar ion compartmentation, and cell expansion, while “compatible” organic solutes serve as solutes in the cytosol (7, 10, 20, 21, 28). This coordination facilitates the utilization of ions for osmotic adjustment to balance the water status of the cell and to generate sufficient turgor for growth while maintaining satisfactory cytosolic ion levels compatible with cell metabolism.

The inhibitory effects of Na⁺ may be mediated through interactions with other cations, specifically K⁺ and Ca⁺². High external concentrations of Na⁺ affect intracellular K⁺ accumulation to the extent that K⁺ deficiency is considered to be one of the detrimental effects of exposure to NaCl (12, 15, 16). Extracellular Na⁺ perturbs K⁺ uptake, presumably by competing for sites through which transmembrane influx of both K⁺ and Na⁺ occur (6, 12, 16). Increased plasma membrane permeability or “leakiness,” elicited by high NaCl concentrations, results in the inability of cells to maintain adequate intracellular K⁺ levels (16). Loss of membrane integrity due to Na⁺ displacement of Ca⁺² from the plasma membrane can be reversed by increased Ca⁺² (5).

Proton extrusion across the plasma membrane is stimulated by a reduction in the external water potential mediated by any number of osmotic solutes (17, 18, 26, 29). A substantial component of increased H⁺ pumping was vanadate-sensitive and it has been inferred that the plasma membrane H⁺-ATPase has a central function in the response to alterations in the osmotic or ionic environments (2, 17, 18, 26). Intracellular sugar uptake has been linked to this enhanced H⁺ pumping capacity (17).

The data presented here indicate that NaCl adaptation has resulted in enhanced capacity for K⁺ (⁴²Rb⁺) uptake. This capacity is maintained at external NaCl concentrations that substantially inhibit K⁺ uptake in unadapted cells. Increased K⁺ uptake into NaCl adapted cells may be linked to the enhanced H⁺ pumping capacity of these cells and also to greater capacity of K⁺ uptake in the presence of vanadate.

MATERIALS AND METHODS

Cell Suspensions

Tobacco (Nicotiana tabacum/gossii) cell cultures and procedures for the maintenance and growth of the cells were as described previously (27). NaCl-adapted cells were isolated from unadapted cells and maintained for 4 years in medium containing 300 mM NaCl. Prior to use, the NaCl-adapted cells were transferred to medium without NaCl by incubating the cells for 24-h periods, sequentially, into media where the NaCl concentration was reduced by 50 mM increments (26). Both unadapted and adapted cells were grown for 3 d in fresh medium without NaCl before use in experiments. Adapted cells transferred to medium without NaCl exhibited equivalent growth when reincubated into medium with 300 mM NaCl as cells that were maintained in medium with 300 mM NaCl (26, 27).

Cells were separated from medium by filtration through Miracloth and then rinsed with a solution (150 mg fresh weight of cells to 1 mL of rinse solution) containing 5 mM CaSO₄ (pH 6.0) and resuspended in that solution for 5 min. The cells were then resuspended from the medium and subsequently preincubated in the CaSO₄ solution (similar cell to volume ratio as above) for 2 h to allow recovery from shock resulting from filtration (25, 26). Aliquots of the cell suspensions were then transferred to separate vessels for experimentation. Concentrated solutions of salts were added to cell suspensions to achieve the desired ion concentrations.
Measurement of H⁺ Secretion

Net H⁺ secretion from cells was determined by back titration (26). Because unadapted and NaCl-adapted cells were morphometrically similar and cell viabilities did not vary substantially (data not shown), the number of viable cells in an equivalent fresh weight was similar for the two cell lines.

86Rb⁺ Uptake Determination

86Rb⁺ (86RbCl carrier free; Amersham) was added to a final concentration of 0.0037, 0.0074, and 0.037 MBq/mL in uptake solutions containing 5 mm CaSO₄ plus 10, 20, or 100 mm KCl, respectively. After incubation, the cells were harvested by vacuum filtration and rinsed three times with an ice-cold solution (0.9 g fresh weight of cells to 10 mL of rinse solution) containing 5 mm CaSO₄, 1 mM KCl, and mannitol. Mannitol was added to the rinse solution to make it isosmotic with the uptake solution to reduce osmotic shock (1).

To determine 86Rb⁺ leakage from cells, a portion of the cells was collected by filtration 2 h after incubation with 86Rb⁺. The cells were rinsed with an ice-cold solution (0.45 g fresh weight of cells to 20 mL of rinse solution) containing 5 mm CaSO₄, 1 mM KCl, and mannitol (keeping this solution isosmotic with the uptake solution). 86Rb⁺ leakage from the cells was monitored at the time intervals indicated after the cells were transferred into solution without 86Rb⁺.

Cell Viability and Osmotic Potentials

Viability was based on the percent of cells that included neutral red (13). Cell osmotic potentials were measured by determining incipient plasmolysis in NaCl solutions graded at 0.05 molal increments (11).

| Table I. Proton Secretion from Unadapted and NaCl (300 mm)-Adapted Tobacco Cells Treated with 10 or 100 mm KCl in the Presence or Absence of 10 μM Fusicoccin or 0.1 mm Sodium Vanadate after 1 h
|------------------------------------------|------------------------------------------|------------------------------------------|
| Unadapted Cells | NaCl-Adapted Cells | NaCl Adapted/Unadapted
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Increase (+) or decrease (−)</td>
</tr>
<tr>
<td>86Rb⁺ solution</td>
<td>247 ± 28</td>
<td>—</td>
</tr>
<tr>
<td>+ FC</td>
<td>438 ± 52</td>
<td>+191</td>
</tr>
<tr>
<td>+ Van</td>
<td>0</td>
<td>−247</td>
</tr>
</tbody>
</table>

Results

H⁺ Secretion and K⁺(86Rb⁺) Uptake into Unadapted and NaCl-Adapted Cells

KCl stimulated net H⁺ secretion from both unadapted and NaCl-adapted cells with a greater acidification of the external medium occurring in the presence of 100 mm KCl (Table I). Net H⁺ extrusion was greater for NaCl-adapted cells, a difference that was magnified by treatment with fusicoccin (Table I). Enhanced H⁺ secretion may be due to a more sensitive response to turgor reduction (17, 19). Acidification that occurred in response to 10 mm KCl was completely inhibited by vanadate (Table I). Similar results were obtained at external KCl concentrations of 20 or 40 mm (data not shown). However, a component of H⁺ extrusion elicited by 100 mm KCl was not inhibited by vanadate (Table I). These results are indicative that at 10 mm KCl, H⁺ efflux is due primarily to an E₁,E₂-type H⁺-translocating plasma membrane ATPase (4, 8, 22–24, 26).

The increased H⁺ secretion elicited by 100 mm KCl relative to 10 mm KCl, in both unadapted and NaCl-adapted cells (203 and 260 nmol H⁺ [g fresh weight]⁻¹ h⁻¹, respectively), was equivalent to that which was not inhibited by vanadate at 100 mm KCl (185 and 250 nmol H⁺ [g fresh weight]⁻¹ h⁻¹). This implies that H⁺ extrusion that is vanadate-insensitive at 100 mm KCl could be due to a cation/H⁺ antiport operating in the K⁺ inward H⁺ outward direction (18). Differences in KCl-stimulated acidification of the external medium between unadapted and NaCl-adapted cells were not attributable to substantial disparities in membrane surface area to volume ratios of the cells.

Using 86Rb⁺ as an indicator of K⁺ (14), NaCl-adapted cells had a greater capacity for K⁺ uptake than unadapted cells, and at least a portion of K⁺ uptake was correlated with H⁺ secretion (compare vanadate-inhibitable H⁺ secretion and K⁺

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uptake in Tables I and II. Fusicoccin did not greatly enhance K+ uptake into unadapted cells but resulted in greater K+ uptake into NaCl-adapted cells, particularly in the presence of 10 mM KCl (Table II). These data suggest that the greater pumping capacity of the plasma membrane H+ -ATPase in NaCl-adapted cells may contribute to enhanced K+ uptake exhibited by these cells. At 10 mM KCl, uptake of K+ that occurs in the absence of detectable H+ secretion could be attributable to the electrophoretic flux of K+ through channels driven by the residual membrane potential difference (19, 26). Enhanced K+ uptake at 100 mM KCl relative to 10 mM KCl in the absence of H+ pumping is greater in NaCl-adapted cells and infers that adapted cells have enhanced K+ permeability via either channels or a cation/H+ antiport.

### Intracellular Uptake and Loss of K+\(^{86}\text{Rb}^+\) in Response to NaCl

Net K+ uptake was greater for NaCl-adapted than for unadapted cells regardless of the external Na+ concentration (Figs. 1 and 2). K+ uptake by unadapted cells was depressed by external NaCl above 80 mM (Fig. 2). No inhibition could be observed in the case of NaCl-adapted cells. An external concentration of 20 mM KCl was used in these experiments to simulate the K+ concentration of the nutrient medium in which the cells were maintained. The endogenous K+ content did not differ greatly between the two cell lines (26). The intracellular osmotic potentials of cells used in these experiments were comparable (−7.5 and −8.2 bars, respectively, for

### Table II. K+\(^{86}\text{Rb}^+\) Uptake by Unadapted and NaCl (300 mM)-Adapted Tobacco Cells Treated with 10 or 100 mM KCl in the Presence or Absence of 20 μM Fusicoccin or 0.1 mM Sodium Vanadate after 1 h

<table>
<thead>
<tr>
<th></th>
<th>Unadapted Cells</th>
<th>NaCl-Adapted Cells</th>
<th>NaCl Adapted:Unadapted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (\mu\text{mol K+ (g fresh wt)}^{-1})</td>
<td>Increase (+) or decrease (−)%</td>
<td>Total (\mu\text{mol K+ (g fresh wt)}^{-1})</td>
</tr>
<tr>
<td>10 mM KCl</td>
<td>337 ± 82</td>
<td>—</td>
<td>528 ± 82</td>
</tr>
<tr>
<td>+FC(^c)</td>
<td>387 ± 23</td>
<td>+50 +15</td>
<td>990 ± 97</td>
</tr>
<tr>
<td>+Van</td>
<td>152 ± 22</td>
<td>−185 −55</td>
<td>212 ± 26</td>
</tr>
<tr>
<td>100 mM KCl</td>
<td>1320 ± 106</td>
<td>—</td>
<td>2046 ± 184</td>
</tr>
<tr>
<td>+FC(^c)</td>
<td>1502 ± 220</td>
<td>+182 +14</td>
<td>2650 ± 180</td>
</tr>
<tr>
<td>+Van</td>
<td>990 ± 69</td>
<td>−330 −25</td>
<td>1432 ± 299</td>
</tr>
</tbody>
</table>

* Difference = sodium vanadate or fusicoccin minus KCl only. *% Increase (+) or decrease (−) = Van or FC − KCl only \(\times 100\%\). \(^c\) FC = fusicoccin; Van = sodium vanadate.
unadapted and NaCl-adapted cells). Over the course of the experiment, cell viability was about 80% and did not change substantially with the exception that there was about a 12% reduction in viability of unadapted cells in 160 mM NaCl after 4 h.

The ability of adapted and unadapted cells to maintain K+ uptake in the face of increasing external concentrations of NaCl was examined. Moreover, since differences in net K+ uptake might be due either to enhanced K+ entry or reduced loss to the medium, both entry and loss were followed (Figs. 1 and 3). To accomplish this, cells of each line were incubated for 2 h in 20 mM K+ (86Rb+) medium, in the absence or presence of increasing NaCl concentrations. A portion of the cells was then transferred to uptake medium lacking 86Rb+ (see “Materials and Methods”). Release of 86Rb+ to the external solution was followed over the next 2 h (see Fig. 1). In parallel, 86Rb+ uptake was followed in those cells remaining in the uptake media. The higher net K+ uptake into NaCl-adapted cells was due apparently to enhanced entry rather than to appreciable differences in K+ leakage at comparable NaCl concentrations (Fig. 3).

**DISCUSSION**

Evidence has been presented indicating that adaptation of glycophyte cells to NaCl results in increased capacity for K+ uptake, and this enhanced capacity is even greater under saline conditions. The principal effect of such an adaptation is to regulate intracellular K+ concentrations in environments where the uptake of K+ is under competition from other cations (12, 16). Presumably, this is an adaptive mechanism that contributes to the ability of NaCl-adapted glycophyte cells to maintain intracellular levels of K+ adequate for survival and growth in saline environments (27).

K+ uptake and H+ secretion have been shown to be influenced in some systems by turgor pressure (17, 18), however, the turgor pressures of NaCl-adapted cells were similar to those of unadapted cells. This situation would not be expected to stimulate K+ uptake. Apparently, the response of cells to turgor must be a reaction to transitory turgor reduction. K+ uptake may also be regulated by feedback from the internal K+ content (9) but in the present case internal K+ content did not differ substantially between the cell lines (26).

K+ uptake appears to be linked to H+ pumping by the plasma membrane H+-ATPase. Such linkage could be mediated by a common transport mechanism (e.g., cation/H+ exchange) or perhaps K+ entry could be passive through specific channels driven by the electrochemical potential gradient produced by the electrogenic H+-ATPase. The enhanced capacity of the plasma membrane H+-ATPase in NaCl-adapted cells (Table I) may be linked to the greater K+ uptake observed in these cells.

In the presence of vanadate, when net H+ pumping was inhibited (Table I, 10 mM KCl), K+ entry into the cells continued (Table II), presumably because the residual negative membrane potential difference drives the electrophoretic flux of K+. Moreover, passive 86Rb+ entry would continue until isotopic equilibrium has been achieved. H+ extrusion also continued in the presence of vanadate but only at high (100 mM) K+ levels. It has been suggested that this H+ secretion may be the result of the reverse action of a cation/H+ antiporter driven by the K+ electrochemical potential (18, 26).

The data presented here indicate that salt adaptation of glycophyte cells involves mechanisms that appear to enhance the capacity for K+ uptake. K+ uptake into NaCl-adapted cells was 1.5-fold greater than into unadapted cells in the absence of NaCl and increased to 3.5-fold at 160 mM NaCl. K+ uptake into NaCl-adapted cells was substantially unaffected by high external NaCl concentrations. Mechanistically, our data suggest that enhanced K+ uptake can be linked to increased H+ pumping capacity of the plasma membrane H+-ATPase. Furthermore, increased K+ selectivity of the uptake mechanism(s) seems also to occur as a function of salt adaptation.
ACKNOWLEDGMENT

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LITERATURE CITED