The Physiological Relevance of Na⁺-Coupled K⁺-Transport

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Plant roots utilize at least two distinct pathways with high and low affinities to accumulate K⁺. The system for high-affinity K⁺ uptake, which takes place against the electrochemical K⁺ gradient, requires direct energization. Energization of K⁺ uptake via Na⁺ coupling has been observed in algae and was recently proposed as a mechanism for K⁺ uptake in wheat (Triticum aestivum L.). To investigate whether Na⁺ coupling has general physiological relevance in energizing K⁺ transport, we screened a number of species, including Arabidopsis thaliana L. Heynh. ecotype Columbia, wheat, and barley (Hordeum vulgare L.), for the presence of Na⁺-coupled K⁺ uptake. Rb⁺-flux analysis and electrophysiological K⁺-transport assays were performed in the presence and absence of Na⁺ and provided evidence for a coupling between K⁺ and Na⁺ transport in several aquatic species. However, all investigated terrestrial species were able to sustain growth and K⁺ uptake in the absence of Na⁺. Furthermore, the addition of Na⁺ was either without effect or inhibited K⁺ absorption. The latter characteristic was independent of growth conditions with respect to Na⁺ status and pH. Our results suggest that in terrestrial species Na⁺-coupled K⁺ transport has no or limited physiological relevance, whereas in certain aquatic angiosperms and algae this type of secondary transport energization plays a significant role.

The level of K⁺ encountered by both aquatic and terrestrial plant roots ranges from low micromolar to supramillimolar levels. Sufficient accumulation of K⁺ in these widely varying conditions is of vital importance for both cellular and whole plant homeostasis (Maathuis and Sanders, 1996). At millimolar external K⁺ concentrations uptake is mediated via a low-affinity mechanism constituting inward-rectifying ion channels and driven entirely by the membrane potential. However, in many soils and aquatic environments the available K⁺ is limited to micromolar levels, thus rendering adequate K⁺ accumulation dependent on energization in addition to that of the membrane potential (Maathuis and Sanders, 1993). The nature of the energizing principle for K⁺ uptake from micromolar external K⁺ concentrations has been extensively debated (for a review, see Maathuis and Sanders, 1996). Coupling to the inwardly directed H⁺ and Na⁺ gradients has been described as a means for energizing high-affinity K⁺ accumulation.

High-affinity K⁺ uptake into K⁺-starved internodal cells of charophyte algae was shown to be Na⁺-dependent. Further studies merited the conclusion that uptake of K⁺ into these cells was mediated by a K⁺:Na⁺ symport with a 1:1 stoichiometry (Smith and Walker, 1989).

The status of Na⁺-coupled K⁺ transport in terrestrial species is unclear, although early studies by Rains and Epstein (1967) showed that Na⁺ inhibits high-affinity K⁺ uptake (measured with Rb⁺) in barley (Hordeum vulgare L.). In Arabidopsis thaliana L. Heynh. ecotype Columbia the presence of micromolar Na⁺ inhibited K⁺-induced membrane depolarizations, which is strong evidence against Na⁺ coupling (Maathuis and Sanders, 1994). Furthermore, analysis of current-voltage relationships in A. thaliana root protoplasts indicated that in this species high-affinity K⁺ uptake was associated with transmembrane proton transport. Subsequent analysis of the reversal potentials identified a K⁺:H⁺ symport with a stoichiometric coupling coefficient of 1 as the most likely mechanism (Maathuis and Sanders, 1994).

Current-voltage data obtained from oocytes expressing the wheat (Triticum aestivum L.) K⁺ transporter HKT1 initially led to a similar conclusion regarding high-affinity K⁺ uptake in roots of wheat (Schachtman and Schroeder, 1994). However, ensuing studies with HKT1 expressed in yeast and oocytes demonstrated a significant stimulation of Rb⁺ uptake and inward K⁺ currents by micromolar levels of external Na⁺ (Rubio et al., 1995). Moreover, it was demonstrated that HKT1 mediated both K⁺ and Na⁺ influxes, functioning as a high-affinity K⁺-uptake mechanism at micromolar external Na⁺ and as a low-affinity Na⁺-influx pathway at millimolar external Na⁺.

Although no data are available on HKT1 function in planta, the observations obtained from HKT1 expressed in oocytes and yeast cells suggest a possible role for Na⁺-driven high-affinity K⁺ transport and the occurrence of Na⁺ coupling as a general mechanism for energization of solute transport in plants (Walker and Sanders, 1991). We tested this hypothesis in five different species, including the wheat cultivar that was the source for the HKT1 cDNA (Atlas 66).

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We also varied the pH and Na⁺ level in the growth conditions to optimize the activation of Na⁺-coupled K⁺ transport. However, our results failed to provide any evidence for Na⁺-coupled K⁺ transport in the terrestrial species that we surveyed, and they indicate that the occurrence of this mechanism may be of significance in certain aquatic species only.

**MATERIALS AND METHODS**

**Plant Growth**

Seeds of *Arabidopsis thaliana* L. Heynh. ecotype Columbia were germinated in soil and grown on Murashige-Skoog medium (Murashige and Skoog, 1962) with the [K⁺] reduced to 50 μM as described previously (Maathuis and Sanders, 1995).

Wheat (*Triticum aestivum* L., cv Maris Dove, cv Karchia, cv Hereward, and cv Atlas 66) and barley (*Hordeum vulgare* L., cv Igris) seeds were germinated between tissue paper moistened with tap water. After germination, plants to be used for radiometric experiments were transferred to 1-L plastic containers and grown for 5 to 7 d on 1 mM CaCl₂ buffered to pH 6.0 (5 mM Mes/bis-Tris-propane), pH 7.5 (5 mM Mes/Tris), or pH 9.0 (5 mM Tris/3-(cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid). Additionally, plants were grown at pH 6.0 with Na⁺ (1 mM, added as NaCl). Wheat cultivars used for electrophysiological experiments were grown on 2 mM zwitterionic buffer (Mes, Hepes, or 5 mM Mes/Tris, or 5 mM Tris/3-(cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid). Additionally, plants were grown at pH 6.0 with Na⁺ (1 mM, added as NaCl). Wheat cultivars used for electrophysiological experiments were grown on 2 mM zwitterionic buffer (Mes, Hepes, or 3-[cyclohexylamino]-1-propane-sulfonic acid) brought to pH (6.0, 7.0, or 9.0) with Ca(OH)₂. Growth medium pH was monitored daily and adjusted if necessary.

Growth chamber conditions were 25/20°C day/night temperatures, 16-h daylength, 80% RH, and 35 W m⁻² light intensity.

Maize (*Zea mays* L., cv Fronica) seeds were germinated between moistened filter paper and dark-grown for 5 d on 0.5 mM CaSO₄ prior to use.

*Riccia fluitans* L. cells were grown on 1% Murashige-Skoog medium, pH 6.7, under diffuse light at 21°C. Prior to experimental use, plants were washed in a medium containing 0.1 mM CaCl₂ and 2 mM Hepes/Ca(OH)₂ at pH 7.3, and kept under diffuse light until used.

*Egeria densa* L. seeds were germinated and grown in artificial pond water for several weeks under natural light conditions at room temperature.

**Rb⁺ Uptake**

The ⁸⁶Rb⁺ uptake protocol was performed as described previously (Maathuis and Sanders, 1995). Intact plants were acclimatized to the uptake solution for 1.5 h prior to each uptake experiment, which was started by transferring plants into 20-mL vials containing (in mM): 0.05 RbCl, 1.0 CaCl₂, 0.5 MgCl₂, 1 Mes/Tris, pH 6.0, and 0.2 to 0.4 μCi/mL ⁸⁶Rb⁺. After a 10-min uptake period, plants were transferred to an ice-cold solution of similar composition but with 1 mM nonradioactive RbCl. This 10-min wash step was included to remove any ⁸⁶Rb⁺ from the apoplast and was repeated for another 10 min in fresh, ice-cold wash solution. Subsequently, roots were excised and weighed into scintillation vials containing 3 mL of scintillation liquid (Ultima Gold XR, Packard, Groningen, The Netherlands). Radioactivity in the tissue was liberated through 2 h of exposure to the scintillation liquid, after which samples were analyzed on a scintillation counter (Minibeta 1212, LKB, Bromma, Sweden). Initial experiments demonstrated that ⁸⁶Rb⁺ uptake is linear for at least 20 min. Results are of duplicate experiments using three plants per treatment.

**Membrane Potential Measurements**

Whole *A. thaliana* plants (3–5 weeks old) were mounted in a Perspex chamber and continuous perfusion of the standard solution (0.5 mM CaCl₂, 0.5 mM MgCl₂, 2 mM Mes/Tris, pH 6.0, 5 mM Tris/Mes, pH 8.0, or 5 mM bis-Tris-propane/Mes, pH 9.8) was maintained around the roots (approximately 10 mL min⁻¹). Both K⁺ and Na⁺ were added as Cl⁻ salts. The membrane potential was recorded by impaling epidermal and cortical cells with single-barrel electrodes.

Thallus cells of *R. fluitans* were mounted in a Perspex chamber and impaled with single-barrel electrodes. The standard bathing solution consisted of 0.1 mM CaCl₂ and 2 mM Hepes/Ca(OH)₂, pH 7.3.

Roots were detached from 5- to 10-d-old *T. aestivum* and *E. densa* plants and mounted in a Perspex chamber perfused with the standard solution containing 0.5 mM CaCl₂, 0.5 mM MgCl₂, and 5 mM Tris/Mes, pH 8.0, or 5 mM bis-Tris-propane/Mes, pH 9.8, or with a medium containing 0.125 mM CaCl₂ and 1 mM zwitterionic buffer brought to pH 4.4 or 6.1 with Ca(OH)₂, or, in some experiments, 0.5 mM Na₂SO₄. K⁺ was added as KCl. The membrane potential was recorded from epidermal and cortical cells by inserting single-barrel microelectrodes.

Electrodes were pulled on a vertical puller and filled with 200 mM KCl. The electrode output was zeroed in 100 mM KCl to minimize junction potentials and connected via Ag/AgCl half-cells to an electrometer (model 7050, World Precision Instruments, New Haven, CT).

**Root Surface Potential Measurements**

Detached roots of *T. aestivum* and *Z. mays* were mounted in a Perspex chamber consisting of two solution wells of approximately 0.3-mL volume, as described previously (Walker, 1994). Each well was connected to an Ag/AgCl half-cell and was electrically separated by the silicon-grease-embedded root. In one well, a high-solution flow rate was maintained (approximately 1 mL s⁻¹), enabling a rapid change. Solutions contained 0.125 mM CaCl₂ and 1 mM zwitterionic buffer brought to pH 6.1 with Ca(OH)₂. K⁺ was added as K₂SO₄; Na⁺ was added as NaCl.

In all growth and experimental procedures utmost care was taken to avoid Na⁺ contaminations. All manipulations were carried out in plastic and high-purity water was obtained from a purifying system (Purite, Oxon, UK). Na⁺...
contamination in all solutions was less than 1.0 μM, as determined by atomic absorption spectrometry.

RESULTS

Rb⁺-Uptake Experiments

Radiolabeled Rb⁺ is a reliable K⁺ analog in short-term experiments (Huang et al., 1992) and allowed us to follow intact plant Rb⁺ uptake as a function of the external level of Na⁺. Unidirectional influx in three different species (A. thaliana, T. aestivum, and H. vulgare) was determined by exposing roots to ⁸⁶Rb⁺ for a 10-min period. In all experiments the external Rb⁺ concentration was kept at 50 μM to keep it well within the range of high-affinity uptake (K₉₅ 40 μM in A. thaliana [Maathuis and Sanders, 1994], 25 μM in barley [Epstein et al., 1963]) and to exclude any channel-mediated uptake (K₉₅ 5-30 mM in A. thaliana [Maathuis and Sanders, 1995]). Furthermore, all species were grown in K⁺-starvation conditions to maximize activation of high-affinity uptake.

Table I shows Rb⁺ influxes for plants grown at different pHs and in the absence or presence of Na⁺. Clearly, for all three species grown at a pH of 6.0 in the absence of Na⁺, high-affinity uptake proceeds without the addition of Na⁺ to the uptake buffer.

The effect of Na⁺ addition to the uptake buffer was tested over a range of Na⁺ concentrations. In A. thaliana, the presence of 500 μM Na⁺ was without any effect or was slightly inhibitory on the measured fluxes. In wheat and barley, Na⁺ concentrations up to 150 μM were similarly without effect, but 500 μM Na⁺ led to a significant decrease in uptake rates (up to 20%).

Alkaline growth conditions might be expected to limit the contribution of K⁺:H⁺-symport-mediated K⁺ influx and could therefore provide a favorable circumstance for derepression of Na⁺-coupled K⁺ transport. However, the Rb⁺-uptake pattern observed in plants grown at pH 7.5 (wheat and barley only) was similar to that for plants grown at pH 6.0. Again, uptake rates were significant in the absence of Na⁺ and were not in any way augmented in its presence.

Growth at pH 9.0 had a detrimental effect on plant yield, which was up to 50% lower compared with growth at pH 6.0. The generally poorer plant condition was reflected in a reduction of Rb⁺-uptake rates. In wheat (cv Maris Dove) Rb⁺ uptake was slightly enhanced in the presence of 20 to 50 μM Na⁺. However, the increase was modest (approximately 17% in the presence of 20 μM Na⁺) and was reversed at higher (0.5 mM) Na⁺ concentrations.

Most plants are exposed to some level of Na⁺ in field situations and artificial growth conditions. However, in a laboratory environment, plant growth can proceed in the virtual absence of Na⁺ (<1 μM). It is possible that in these conditions the expression/activation of Na⁺-coupled transport is suppressed because transport requires induction by Na⁺. In general, the presence of 1 mM Na⁺ in the growth medium stimulates growth rates at either pH 6.0 or 9.0 (data not shown) and reduces the Rb⁺ influx in all species (Table I). However, in all three species the presence of Na⁺ during growth failed to generate any form of Na⁺ dependence on the observed Rb⁺ influx (Table I).

Electrophysiological Experiments

In many plant cells, especially those derived from K⁺-starved tissues, an increase in the external K⁺ concentra-

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth Medium pH/±Na⁺</th>
<th>Na⁺ Concentration in Uptake Buffer (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A. thaliana</td>
<td>6.0/-</td>
<td>1.81 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>6.0/+</td>
<td>1.45 ± 0.09</td>
</tr>
<tr>
<td>T. aestivum</td>
<td>6.0/-</td>
<td>2.04 ± 0.06</td>
</tr>
<tr>
<td>(cv Maris Dove)</td>
<td>6.0/+</td>
<td>1.64 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>7.5/-</td>
<td>2.56 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>9.0/-</td>
<td>1.38 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>9.0/+</td>
<td>1.71 ± 0.22</td>
</tr>
<tr>
<td>T. aestivum</td>
<td>6.0/-</td>
<td>1.92 ± 0.15</td>
</tr>
<tr>
<td>(cv Hereward)</td>
<td>6.0/+</td>
<td>1.50 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>7.5/-</td>
<td>1.73 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>9.0/-</td>
<td>2.61 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>9.0/+</td>
<td>1.59 ± 0.02</td>
</tr>
<tr>
<td>H. vulgare</td>
<td>6.0/-</td>
<td>1.58 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>6.0/+</td>
<td>0.92 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>7.5/-</td>
<td>1.31 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>9.0/-</td>
<td>0.96 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>9.0/+</td>
<td>0.61 ± 0.12</td>
</tr>
</tbody>
</table>

* n.d., Not determined.
tion from effectively zero to micromolar levels generates a membrane depolarization that originates from an increased inward current through K⁺ transporters. Hence, measurement of such depolarizations provides a relatively simple and quick assay for the presence of high-affinity K⁺-transport activity. By directly monitoring the membrane voltage, microelectrodes and surface electrodes can assay K⁺ influx and its dependence on Na⁺ with a large time resolution. Furthermore, intracellular electrodes can be used to access different cell types in the root tissue. The possibility of coupling of the K⁺ flux to Na⁺ can be assessed by recording the separate depolarization generated by K⁺ or Na⁺ alone and subsequently the depolarization in the presence of both substrates. A synergistic response in the presence of both ions strongly indicates coupling between the two fluxes. This relatively simple test for flux coupling is feasible on a single root cell level with impaling microelectrodes or, alternatively, by detecting the surface potential difference in entire parts of the root using surface electrodes (Due, 1993). However, quantitative analysis of such data has to be undertaken with caution, since the magnitude of the depolarizations not only depends on the currents carried by the K⁺ transporter but also on the background membrane conductance.

Figure 1 shows traces of K⁺-influx-generated membrane depolarization in a K⁺-starved A. thaliana cortical root cell. Addition of 10 or 100 μM K⁺ to the bathing medium produced a membrane depolarization of 6 ± 4 mV (n = 8) and 23 ± 9 mV (n = 11), respectively (pH in assay medium, 6.0). Addition of Na⁺ (10 or 100 μM) evoked a smaller depolarization than the equivalent Κ⁺ concentration, indicating the presence of a high-affinity K⁺-influx system that discriminates against Na⁺. The extent of the depolarization was shown to depend on the external [K⁺] in a saturating manner, with a maximum depolarization of approximately 30 mV when external [K⁺] reached 300 μM and an apparent Kₘ of 30 μM (Maathuis and Sanders, 1994). It is significant that the membrane depolarizations not only proceeded in the absence of Na⁺ but also failed to show any increase in the presence of Na⁺ (Fig. 1).

A more extensive investigation of K⁺-induced membrane depolarizations was performed in three wheat cultivars. Figure 2 depicts a representative trace of root surface potentials in the wheat cv Karchia grown at pH 9.0. Clearly, the addition of 1 mM Na⁺ has only a marginal effect on the root surface potential difference and in no way augments the K⁺-dependent depolarization. A statistical analysis of measurements in cv Atlas 66, cv Karchia, and cv Hereward is summarized in Table II. As expected for a high-affinity K⁺ transporter, growth in the presence of K⁺ severely repressed the Vₘₐₓ. Furthermore, for all cultivars grown at pH 6.0 in the absence of Na⁺, Vₘₐₓ (defined as the maximum depolarization) was significantly reduced in the presence of 1 mM Na⁺. Additionally, growth in the presence of 1 mM Na⁺ did not change this characteristic. Even growth at pH 9.0 failed to induce any detectable Na⁺ effect on K⁺-induced depolarizations. Na⁺ inhibited the extent of the K⁺-induced depolarization in each case, the effect being approximately 30%.

In situ hybridization suggests that the high-affinity K⁺ transporter HKT1 is expressed mainly in cortical root cells (Schachtman and Schroeder, 1994). Therefore an underestimation of the contribution of HKT1-mediated fluxes could arise in surface potential difference measurements. To test this, we impaled cortical root cells of wheat with...
Table II. The effect of $K^+$ and $Na^+$ on the membrane surface potential difference in wheat roots

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Growth Condition</th>
<th>$V_{max}$</th>
<th>Effect of $Na^+$ on $V_{max}$</th>
<th>$K_m$</th>
<th>Effect of $Na^+$ on $K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlas 66</td>
<td>pH 6.0</td>
<td>27.5 ± 3.9 (4)</td>
<td>-9.6 ± 1.0 (4)</td>
<td>23.3 ± 1.1 (4)</td>
<td>-5.6 ± 2.6 (4)</td>
</tr>
<tr>
<td>Atlas 66</td>
<td>pH 6.0 + $Na^+$</td>
<td>41.3 ± 4.8 (5)</td>
<td>-12.2 ± 1.1 (5)</td>
<td>37.9 ± 8.4 (5)</td>
<td>-4.6 ± 3.2 (5)</td>
</tr>
<tr>
<td>Atlas 66</td>
<td>pH 6.0 + $Na^+$ + $K^+$</td>
<td>4.6 ± 0.4 (4)</td>
<td>-1.4 ± 0.6 (5)</td>
<td>46.5 ± 20.1 (4)</td>
<td>-0.1 ± 11.2 (4)</td>
</tr>
<tr>
<td>Hereward</td>
<td>pH 6.0 + $Na^+$</td>
<td>30.1 ± 6.3 (5)</td>
<td>-4.5 ± 1.6 (5)</td>
<td>25.9 ± 5.4 (5)</td>
<td>-5.3 ± 3.3 (5)</td>
</tr>
<tr>
<td>Karchia</td>
<td>pH 6.0 + $Na^+$</td>
<td>45.4 ± 3.6 (10)</td>
<td>-11.0 ± 1.5 (10)</td>
<td>11.5 ± 1.3 (10)</td>
<td>-0.5 ± 0.7 (10)</td>
</tr>
<tr>
<td>Karchia</td>
<td>pH 9.0</td>
<td>34.4 ± 4.7 (4)</td>
<td>-9.0 ± 3.4 (4)</td>
<td>17.9 ± 2.9 (5)</td>
<td>-1.3 ± 1.6 (5)</td>
</tr>
</tbody>
</table>

Microelectrodes, as described for A. thaliana. As recorded with surface electrodes, additions of 1 to 100 $\mu$M $K^+$ caused substantial membrane depolarization, clearly demonstrating that high-affinity $K^+$ uptake does proceed in cortical cells (Fig. 3); we detected no evidence for coupling of this $K^+$ influx to $Na^+$, measured at either pH 4.4 or 6.1.

However, at these pH values, $K^+$ uptake dominated by $H^+$-coupled transport may obscure other mechanisms, whereas a high external pH, which would considerably reduce the proton-motive force, might enhance the ability to observe any coupling of $K^+$ transport to $Na^+$. To investigate this possibility we did a number of intracellular recordings in cortical cells of A. thaliana and wheat with the pH of the root-bathing solution increased to either 8.0 or 9.8 (Fig. 4). For Arabidopsis, the addition of 50 $\mu$M $K^+$ depolarized the membrane by $23 ± 5$ mV ($n = 4$) in the absence of $Na^+$ and by $24 ± 4$ mV ($n = 4$) in the presence of $Na^+$ at an assay pH of 9.8. For wheat these values were $41 ± 9$ ($n = 8$) and $44 ± 11$ ($n = 8$), respectively. Similarly, with an external pH of 8.0, the $K^+$-induced depolarizations were comparable in the presence and absence of $Na^+$ (data not shown).

Maize (Z. mays) exhibits $C_4$ metabolism and many such species require $Na^+$ as a micronutrient. Potential differences at the root surface of maize responded to the addition of micromolar $K^+$, as was also observed in wheat roots, but, again, the detected signals showed no $Na^+$ dependence (results not shown).

The liverwort Riccia can sustain growth in both terrestrial and aquatic environments. Although essentially a freshwater plant, it is exposed to low millimolar external $Na^+$ levels in most conditions. In $K^+$-starved cells mem-

Figure 3. Original trace showing an intracellular membrane potential recording in a wheat (cv Atlas 66) cortical root cell. Upper trace, Membrane depolarizations induced by 10 or 100 $\mu$M $K^+$ in the presence and absence of $Na^+$ determined at pH 4.4. Lower trace, Same as upper trace but measured at pH 6.1. Closed symbols represent $K^+$ addition; open symbols represent washout. The bar at the top indicates the presence of 1 mM $Na^+$ in the perfusion medium. Plants were grown at pH 9.0 in the presence of $Na^+$.

Figure 4. Original traces showing intracellular membrane potential recordings in cortical root cells of wheat cv Hereward (A) and Arabidopsis (B). Both traces show membrane depolarizations induced by 50 $\mu$M $K^+$ in the presence and absence of 0.5 mM $Na^+$ determined at pH 9.8. Closed symbols represent $K^+$ addition; open symbols represent washout. The bar at the top indicates the presence of 0.5 mM $Na^+$ in the perfusion medium. Plants were grown at pH 9.0.
brane potentials reach values as high as −300 mV in the absence of K⁺. By gradually increasing the ambient K⁺ up to 1 mM, the membrane depolarizes to values of approximately −200 mV in a saturable manner, with an apparent Kₘ of 25 ± 2 μM and a maximum depolarization of 79 ± 2 mV, indicative of high-affinity K⁺ transport. Figure 5 shows a representative example of the membrane potential response after exposure to 40 μM K⁺ and then to 40 μM K⁺ plus 300 μM Na⁺. The addition of Na⁺ fails to affect the membrane potential in the absence of K⁺. Furthermore, the extent of the K⁺-induced depolarization in the presence of Na⁺ (44 ± 4 mV, n = 3) is not significantly different from that with K⁺ alone (45 ± 6 mV, n = 8).

Figure 6 shows an original trace of the membrane potential in an Egeria leaf cell. The top trace shows exposure to different K⁺ levels in the presence of 0.4 mM Na⁺, leading to depolarizations with an apparent Kₘ for K⁺ of approximately 8 μM. The bottom trace shows a similar exposure to K⁺, but without Na⁺, recorded in the same cell. Exposure to 20 or 50 μM evoked small K⁺-induced depolarizations in the absence of Na⁺. The latter indicates the presence of a Na⁺-independent K⁺ pathway, possibly through K⁺ channels. Depolarizations in the presence of Na⁺ always exceeded those for K⁺ alone, in spite of the depolarizing effect of Na⁺ itself (which tends to decrease the driving force for K⁺). Clearly, high-affinity K⁺ uptake in Egeria leaves is largely Na⁺-dependent.

A similar Na⁺ dependence was recorded in root cells of Egeria (results not shown) and of Elodea (Walker, 1994), with an apparent Kₘ of 13 μM for K⁺ and of 160 μM for Na⁺. Furthermore, in leaves of Vallisneria and Egeria Na⁺ influx was stimulated up to 3-fold by micromolar external K⁺ (Walker, 1994).

**DISCUSSION**

The Na⁺ content of soils and freshwater in most temperate regions averages 0.1 to 1 mM, which is similar to that of K⁺ (Marschner, 1995). However, in many arid and coastal regions, Na⁺ levels reach as high as 200 mM, which can considerably impair plant growth. In spite of this Na⁺ abundance, studies have shown that Na⁺ is not an essential growth requirement for most plant species, and even in halophytic species, in which the presence of Na⁺ significantly enhances growth, Na⁺ behaves as a micronutrient rather than a macronutrient (Marschner, 1995). To counteract the toxic effects of high Na⁺ levels, most plants have developed a high degree of discrimination against Na⁺ uptake and also maintain a high cytoplasmic K⁺/Na⁺ ratio (Flowers et al., 1977).

In contrast to plant cells, Na⁺ is essential for animal cells to survive. This strict Na⁺ dependence is reflected by a transport economy that is dominantly Na⁺-coupled and relies on the action of primary Na⁺/K⁺-exchanging pumps. In plant cells, however, uphill transport of solutes and nutrients is generally coupled to the proton gradient, which is maintained by H⁺-extruding primary pumps at the plasma membrane and tonoplast.

However, dominance of H⁺ coupling in plant transport is not complete. In K⁺-starved halophytes such as Chara australis and Nitella translucens, high-affinity K⁺ uptake strongly depends on the presence of external Na⁺ (Smith and Walker, 1989; Walker and Sanders, 1991). Also, Na⁺ coupling is not restricted to K⁺ uptake but is implicated in the transport of urea, amino acids, and sugars (Walker et al., 1993; Walker, 1994). In higher plants, evidence for Na⁺-coupled K⁺ transport was found in Egeria (Fig. 6) and Vallisneria leaves and in Elodea and Egeria roots (results not shown). Preliminary results also indicate that NO₃⁻ transport in Zostera marina is Na⁺-dependent (results not shown).

Na⁺-coupled transport in higher terrestrial plants is a largely unexplored phenomenon. HKT1, the gene coding for a high-affinity K⁺ transporter in wheat, restored yeast growth and K⁺ uptake (Schachtman and Schroeder, 1994) with a proposed stoichiometry of approximately two K⁺ ions transported per ion of Na⁺ (Rubio et al., 1995). Onoshi et al. (1990) reported that in C₄ plants, pyruvate transport into the chloroplast is coupled to either an H⁺ gradient (maize and related species) or to a Na⁺ gradient (all other tested species) with a 1:1 stoichiometry.

These results could suggest a wider physiological role for Na⁺-coupled transport in terrestrial plant species. Nev-
Nevertheless, data presented in this study show that Arabidopsis, H. vulgare, and T. aestivum are capable of surviving and growing in the effective absence of Na⁺ (<1.0 μM as determined by atomic absorption spectrometry). This demonstrates that any potential Na⁺-coupled transport mechanism is not of vital importance in the growth regimes that we applied. Also, two independent techniques provide evidence that high-affinity K⁺ uptake proceeds in the absence of Na⁺. Therefore, a Na⁺-independent high-affinity K⁺-transport mechanism must be present and functioning in all species tested. The presence of Na⁺ in the assay either fails to alter K⁺-transport characteristics or has an inhibitory action. Inhibition by Na⁺ may reflect some degree of Na⁺ binding by the K⁺ transporter, resulting in competitive inhibition of high-affinity K⁺ transport by Na⁺ as described by Rains and Epstein (1967).

As mentioned earlier, most plants will encounter some level of Na⁺ in their surroundings. Therefore, the virtual absence of Na⁺ in our “minus Na⁺” growth conditions may bear little resemblance to a natural environment and may repress Na⁺-coupled transport mechanisms. Growth of plants in the presence of 1 mM NaCl, a concentration sufficient to create a substantial driving force but too low to induce salinity stress, did slightly enhance growth rates of Arabidopsis and wheat and improved growth in barley by approximately 20 and 40% for plants grown at pH 6.0 and 9.0, respectively. Also, Rb⁺ uptake in Na⁺-grown plants was lower than that in plants grown without Na⁺. Both of these Na⁺-induced effects probably originate from a K⁺-substituting role of Na⁺, especially as an osmoticum, which would ameliorate K⁺-starvation conditions. Nevertheless, as observed in plants grown without Na⁺, no increased Rb⁺ uptake occurred when Na⁺ was present in the assay solution. These characteristics were confirmed by electrophysiological experiments with Na⁺-grown plants in which K⁺-induced depolarizations in the absence of Na⁺ were comparable to those in the presence of Na⁺.

Alternatively, a low degree of Na⁺-coupled K⁺ uptake may occur but remains undetected against a high background of protons. The electrochemical gradient for protons and thus limit K⁺:H⁺-symport-mediated transport. Increasing the external pH will decrease the electrochemical gradient for protons and thus limit K⁺:H⁺-symport-mediated K⁺ transport. With an external pH of 6.0, an internal pH of 7.5, and a membrane potential of ~150 mV, K⁺:H⁺ symport is capable of accumulating K⁺ in excess of 10⁶-fold (Maathuis and Sanders, 1994). However, at an external pH of 9.0, with the other parameters unchanged, the K⁺-accumulating capacity would decrease to approximately 300. Although hyperpolarization of the membrane (which is up to 100 mV in wheat when the external pH is increased from 5.5 to 9.5) will counteract this decrease in driving force, a reduction in the K⁺-accumulating capability may induce activation of alternative mechanisms to energize high-affinity K⁺ uptake. In all species tested, a pH of 9.0 reduced relative growth rates but did not lead to any noticeable stress symptoms. In plants grown at a lower pH the presence of Na⁺ in either the growth medium or the assay solution failed to affect K⁺ transport, other than in an inhibitory fashion. Even with an external pH as high as 9.8 in the assay medium, we could not detect any effect of Na⁺ on the K⁺-induced depolarization of the membrane in both wheat and Arabidopsis.

This study of Na⁺-coupled K⁺ transport includes the wheat variety Atlas 66, which is the original source of HKT1. The conclusive evidence presented by Rubió et al. (1995) that HKT1 co-transport micromolar Na⁺ and K⁺ in yeast and oocyte cells points to a similar function of HKT1 in plants. However, we found no evidence of K⁺:Na⁺-co-transport in this wheat variety (Walker et al., 1996), and if HKT1 does function as a K⁺:Na⁺ co-transporter in plants, its physiological role appears to be noncrucial and additional mechanisms must be present to explain the observed K⁺ uptake in the absence of Na⁺. Obviously, it cannot be excluded that certain growth conditions not tested in this study might invoke a more dominant role for HKT1-mediated transport. Alternatively, HKT1 may function as either a Na⁺:K⁺ or H⁺:K⁺ co-transport, depending on the prevalent H⁺ and Na⁺ gradients, as has been described for Na⁺,K⁺-ATPases and H⁺,K⁺-ATPases in animal cells (Polvani and Blostein, 1988; Polvani et al., 1989). At this stage it also cannot be ruled out that HKT1 in wheat codes for an endo-membrane transporter rather than for a plasma membrane transporter.

In conclusion, this study used several techniques to screen for Na⁺-coupled high-affinity K⁺ transport. In Egeria, Elodea, and Vallisneria and in charophytes, observations support the idea that such coupling takes place. Conversely, in Arabidopsis, wheat, barley, maize, and Riccia, our investigations failed to provide any evidence for Na⁺ coupling. Therefore, our results strongly suggest that in terrestrial species such as Arabidopsis, wheat, barley, and maize Na⁺-coupled K⁺ transport has no or limited physiological relevance. Furthermore, this type of secondary transport energization may be limited to certain aquatic angiosperms and charophytic species only.

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