Studies of the Uptake of Nitrate in Barley

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ABSTRACT

Transmembrane electrical potential differences (Δψ) of epidermal and cortical cells were measured in intact roots of barley (Hordeum vulgare L. cv. Klondike). The effects of exogenous NO3− on Δψ (in the concentration range from 100 micromolar to 20 millimolar) were investigated to probe the mechanisms of nitrate uptake by the high-affinity (HATS) and low-affinity (LATS) transport systems for NO3− uptake. Both transport systems caused depolarization of Δψ, demonstrating that the LATS (like the HATS) for NO3− uptake is probably mediated by an electrogenic cation (H+)? cotransport system. Membrane depolarization by the HATS was “inducible” by NO3−, and saturable with respect to exogenous [NO3−]. By contrast, depolarization by the LATS was constitutive, and first-order in response to external [NO3−]. H+ fluxes, measured in 200 micromolar and in 5 millimolar Ca(NO3)2 solutions, failed to alkalize external media as anticipated for a 2 H+:1 NO3− symport. However, switching from K2SO4 solutions (which were strongly acidifying) to KNO3 solutions at the same K+ concentration caused marked reductions in H+ efflux. These observations are consistent with NO3− uptake by the HATS and the LATS via 2 H+:1 NO3− symports. These observations establish that the HATS for nitrate uptake by barley roots is essentially similar to those reported for Lemma and Zea mays by earlier workers. There are, nevertheless, distinct differences between barley and corn in their quantitative responses to external NO3−.

Nitrates absorption by several plant species has been shown to be biphasic (see ref. 16 and references therein). At low external NO3− concentration ([NO3−]o), net NO3− uptake and 15NO3− influx are typically saturable (with Km values in the range from approximately 10 to 100 μM), inducible by exogenous nitrate and thermodynamically active (1, 3, 12, 16, 17). The mechanism of energy coupling for active transport by HATS for NO3− absorption has been investigated in a limited number of species by means of electrophysiological studies. Ullrich and Novacky (19) documented that NO3− absorption by Lemma was associated with depolarization of Δψ. Furthermore, the extent of depolarization of Δψ was increased (“induced”) by prior exposure of NO3−-deprived plants to NO3− solutions in the same way that NO3− uptake is enhanced (“induced”) by exposure to NO3−. Depolarization of Δψ was followed by gradual repolarization of the electrical potential difference (in the presence of exogenous NO3−), presumably through activation of the proton pump. Removal of NO3− caused hyperpolarization of Δψ. A mechanism for NO3− absorption involving a 2 H+:1 NO3− symport was proposed to account for these observations, whereby the energy for active NO3− absorption is derived from the proton gradient (ΔψH+en) generated by the plasma membrane H+−ATPase.

By contrast, Thibaud and Grignon (18) reported that NO3− absorption by corn roots was associated with hyperpolarization of Δψ. Furthermore, the failure of diethylstilbestrol (an inhibitor of H+−translocating ATPases) to inhibit this hyperpolarization led these authors to propose that NO3− absorption in corn roots occurred by means of a 2 NO3−:1 OH− antiport. More recently, McClure et al. (9), also using corn roots, have established that hyperpolarization of Δψ, associated with NO3− uptake, was preceded by a rapid and small (approximately 10 mV) depolarization. This depolarization was absent in NO3−-starved roots, and was virtually independent of [NO3−]o, even at [NO3−]o as high as 20 mM. However, the extent of the hyperpolarization of Δψ was followed depolarization was a function of [NO3−]o. Below 1 mM, hyperpolarization was saturable, with a half-saturation concentration (Km) that was similar to the Km for NO3− uptake. Beyond 1 mM, hyperpolarization was a first-order function of [NO3−]o.

The kinetics of NO3− influx in barley roots have been studied extensively by use of 15NO3− (5, 6, 15–17). Siddiqi et al. (16, 17) and Glass et al. (2) have recently characterized 15NO3− influx in the high concentration range (>1 mM) by the LATS as constitutive, first-order with respect to [NO3−]o, and relatively insensitive to metabolic inhibition. They suggested that NO3− uptake by LATS may occur via NO3−−specific channels. The present studies were undertaken (a) to compare the electrical properties of the HATS for NO3− in barley to that of corn and Lemma, and (b) to investigate energy coupling in the LATS for nitrate by examining the

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2 Abbreviations: HATS, high-affinity transport system; Δψ, transmembrane electrical potential difference; LATS, low-affinity transport system.
effects of external $[\text{NO}_3^-]$ on the electrical properties of this transport system.

**MATERIALS AND METHODS**

**Growth of Plants**

Seeds of barley (*Hordeum vulgare* L. cv. Klondike) were germinated in moistened sand contained in polyethylene cups as described previously (9, 15). After 3 d at room temperature (22°C), roots had penetrated the plastic gauze that covered the lower end of the cups to a length of 2 to 3 cm. Adhering sand was washed from the roots with deionized water and the seedlings, supported by their polyethylene cups, were transferred to inorganic nutrient solutions contained in black polyethylene vessels (4). These contained 2.4 L of nutrient solution. Nutrient solutions consisted of 200 $\mu$M CaSO₄ or 1/80 strength modified Johnson’s medium (15), plus or minus 100 $\mu$M NO$_3^-$ (as the Ca salt). Each cup contained three or four seedlings and each vessel contained four cups. Hence, depletion of the inorganic nutrients was not a significant problem during the 3 to 4 d of hydroponic growth. Nevertheless, solutions in the polyethylene vessels were completely replaced on alternate days.

Plants were exposed to NO$_3^-$ for up to 3 d prior to measurement of $\Delta \psi$ to induce the HATS for NO$_3^-$ uptake. In our first experiments, we followed the protocol of McClure et al. (9), in which plants were pretreated with NO$_3^-$ for 3 d. Subsequently, shorter exposures (18–24 h) were chosen because they result in larger NO$_3^-$ fluxes (15) and larger depolarizing effects of applied NO$_3^-$ on $\Delta \psi$.

**Electrical Measurements**

All impalements for the measurement of $\Delta \psi$ were in epidermal or cortical cells of roots of intact plants. Seedling plants were secured with a single seminal root positioned over platinum pins in a narrow Plexiglas chamber. The chamber was mounted to the stage of an Olympus BH-2 microscope (Spectra Services, Rochester, NY) as described previously (9). Roots targeted for impalement were held in place by small lengths of tygon tubing positioned roughly 5 mm on either side of the platinum pins that supported the root. Hence, during impalement, which was typically in a region approximately 3 to 4 cm from the root tip, displacement of the root was virtually impossible. Impalement of epidermal or cortical cells was achieved using a hydraulically driven Narashige micromanipulator (model M0–204; Narashige U.S.A., Green vale, NY) mounted onto the microscope stage. Membrane potentials were measured using a WPI model KS-750 amplifier (World Precision Instruments, Inc., New Haven, CT), and microelectrodes (tip diameter approximately 0.5 $\mu$m) were made from a single-barreled borosilicate glass tube filled with 3 M KCl (adjusted to pH 2 to reduce tip potentials). Reference electrodes were made in an identical manner and placed within a chamber adjacent to and continuous with the root chamber to minimize any contamination of the solution bathing the root by K$^+$. Membrane potentials were recorded on a strip chart recorder.

Solutions bathing intact roots during impalement were supplied from 1-L reservoirs through 1/8 inch tygon tubing. Compositions of these solutions are provided in the text but typically consisted of 200 $\mu$M CaSO$_4$ plus or minus various concentrations of Ca(NO$_3$)$_2$ or 1/80 strength Johnson’s nutrient solution plus or minus Ca(NO$_3$)$_2$. The latter solution was chosen because all our previous $^{15}$NO$_3^-$ flux work (2, 16, 17) was undertaken in this solution. The protocol used in these experiments was to impale the roots in 200 $\mu$M CaSO$_4$ solution or 1/80 strength Johnson’s solution (minus NO$_3^-$). When a stable reading of $\Delta \psi$ was obtained, the solution bathing the root was replaced by 200 $\mu$M CaSO$_4$ plus Ca(NO$_3$)$_2$ or by 1/80 strength Johnson’s solution plus Ca(NO$_3$)$_2$.

In other experiments, a different protocol was adopted: roots were first impaled in CaSO$_4$ solution, which was replaced by Ca(NO$_3$)$_2$ solution at the same strength. The depolarizations obtained by the latter method were due solely to the differences in the electrical effects of NO$_3^-$ and SO$_4^{2-}$ without contributions from Ca$^{2+}$. A second method was used to examine NO$_3^-$ effects in complete isolation from those due to Ca$^{2+}$. This involved titrating 15 mM Mes-Tris to pH 5.2 with HNO$_3$ and to pH 6 with Ca(OH)$_2$. For each ion (NO$_3^-$ or Ca$^{2+}$), roots were first equilibrated in 15 mM Mes-Tris, before exposure at the same pH, to the particular ion. The buffers alone typically caused approximately 20 mV depolarization of $\Delta \psi$, which repolarized spontaneously.

Data shown in Figures 1, 3, 4, and 6 are representative traces from experiments repeated several times on a particular day with a series of roots and repeated on two or three separate days with different batches of roots. Data for the experiment shown in Figure 7 were obtained from a single experiment repeated three times with three separate roots to ensure that NO$_3^-$ uptake by the HATS was not induced during the course of the experiment. The quantitative data shown in Figures 2 and 5 are means of three replicate measurements at each NO$_3^-$ concentration using a single root. The data are representative of experiments repeated at least three times.

**Measurement of H$^+$ Fluxes by Means of Ion-Selective Microelectrodes**

Liquid membrane-type neutral carrier-based H$^+$-selective microelectrodes (tip diameter = 0.5 $\mu$m) were constructed as previously detailed (8) using Fluka H$^+$-selective cocktail (catalog No. 95291, Fluka Chemical Co., Ronkonkoma, NY).

The root and vertically positioned microelectrodes were viewed at 60 to 150 $\times$ magnification with the Olympus microscope. To measure net H$^+$ fluxes, the appropriate experimental solution was passed through the chamber until the previous solution was displaced, and then flow was stopped to allow the development of ion gradients. Subsequently, H$^+$ activities in the unstirred layer were measured at 50 and 100 $\mu$m from the root surface and the net ionic fluxes at the root surface were determined from the following equation derived from diffusion analysis of the spatial symmetry of the H$^+$ activity gradients:

$$J_i = \frac{2\pi D_i (C_i - C_j)}{\ln(R_i/R_j)}$$

where $J_i$ is the net ionic flux (in $\mu$mol cm$^{-2}$ s$^{-1}$), $D_i$ is the self-diffusion coefficient for the ion of interest (in cm$^2$ s$^{-1}$), $C_i$ and $C_j$ are the ion activities measured at the two radial...
Figure 1. Depolarization and repolarization patterns at different concentrations of external NO$_3^-$; a, 200 µM NO$_3^-$; b, 100 µM NO$_3^-$; c, 10 mM NO$_3^-$; d, 15 mM NO$_3^-$. All plants had been induced by pretreatment with 100 µM NO$_3^-$ for 18 h.

positions, and $R_1$ and $R_2$ are the respective distances from the positions where the ion activities were measured to the center of the root (10). The above flux equation yields a flux normalized for a 1-cm long root segment (thus the units of µmol cm$^{-1}$ s$^{-1}$). Subsequently, the flux value calculated from the above equation was divided by the mass of the 1-cm segment of root (cross-sectional area of root in cm$^2$ × root density in g cm$^{-3}$ × 1-cm root length) and multiplied by the appropriate unit's conversion factor (3600 s h$^{-1}$) to obtain a net flux in units of µmol g$^{-1}$ h$^{-1}$. The net H fluxes determined in this study were measured approximately 2 cm back from the root apex.

RESULTS

$\Delta \psi$ in the range from $-200$ to $-260$ mV were recorded when roots were impaled in solutions containing 200 µM CaSO$_4$. Occasionally, values as low as $-300$ mV were recorded; these were less common and usually arrived at some time into a single impalement after several cycles of CaSO$_4$/Ca(NO$_3$)$_2$ treatment. Data shown in Figure 1a are for plants exposed to 100 µM NO$_3^-$ for 24 h before brief equilibration in 200 µM CaSO$_4$ solution containing no NO$_3^-$. The provision of 200 µM NO$_3^-$ [in the form of Ca(NO$_3$)$_2$] caused a rapid depolarization of $\Delta \psi$ from $-261$ to $-202$ mV, which was virtually complete within 2 min. The extent of depolarization due to external NO$_3^-$ was found to vary from root to root and according to NO$_3^-$ pretreatment, but was particularly reproducible for a single root or a single impalement. In this particular example (Fig. 1a), depolarization of $\Delta \psi$ in the presence of external NO$_3^-$ was followed rapidly by repolarization to within a few mV of the original value of $\Delta \psi$.

During the course of greater than 100 impalements undertaken in this series of experiments, the patterns of repolarization, when evident, appeared to fall into four categories:

1. As in Figure 1a, depolarization by low [NO$_3^-$], was followed rapidly by repolarization, restoring $\Delta \psi$ to within a few mV of its original value.

2. In many instances, particularly at low external [NO$_3^-$], repolarization was slow and the original value of $\Delta \psi$ was not restored even after 5 to 10 min (Fig. 1b).

3. At high external [NO$_3^-$], in the range beyond 5 mM, repolarization was extremely rapid, restoring $\Delta \psi$ to its original value within several minutes (Fig. 1c).

4. At the highest [NO$_3^-$] investigated (20 mM), repolarization appeared to commence when depolarization reached its maximum value but was short-lived and only achieved a partial recovery (5–10 mV) before a second much smaller depolarization was evoked. This biphasic pattern of depolarization was not followed by repolarization (Fig. 1d).

In those cases where repolarization was incomplete in Ca(NO$_3$)$_2$, replacement of the Ca(NO$_3$)$_2$ solution by 200 µM CaSO$_4$ solution caused a rapid onset of full repolarization and, typically, a small hyperpolarization. Even when complete repolarization had occurred in Ca(NO$_3$)$_2$ solution, CaSO$_4$ solution still caused a small hyperpolarization, followed over a period of 2 to 5 min by a return to $\Delta \psi$ values typical of those recorded prior to CaSO$_4$ exposure.

The effect of NO$_3^-$ concentration on $\Delta \psi$ is shown in Figure 2. The method used to obtain these results involved replacing 0.2 mM CaSO$_4$ solution by 0.2 mM CaSO$_4$ plus the appropriate concentration of Ca(NO$_3$)$_2$. The plot of depolarization versus [NO$_3^-$]$_o$ assumed the form of a rectangular hyperbola for roots that had been induced by NO$_3^-$ pretreatment for 18 h.

Figure 2. Concentration dependence of NO$_3^-$-mediated depolarization of $\Delta \psi$ by the HATS in plants induced for NO$_3^-$ uptake by pretreatment with 100 µM NO$_3^-$ for 18 h.
electrical responses to $\text{NO}_3^-$ in barley roots

Figure 3. Depolarization of $\Delta \psi$ by 200 $\mu$M and 10 mM $\text{NO}_3^-$ as a function of time in plants that had (initially) received no $\text{NO}_3^-$ exposure. a, 0 h; b, +1 h; c, +2.5 h; d, +5 h of exposure to $\text{NO}_3^-$.

(Fig. 2). The half-saturation value for this depolarization (analogous to a $K_m$ value) was 60 $\mu$M, whereas the maximum depolarization (analogous to a $V_{\text{max}}$) was 38 mV. These values were obtained by a direct fit of the data to the Michaelis-Menten equation by an iterative computer program. The $r^2$ for regression was 0.96. The effect of $\text{NO}_3^-$ concentration was also evaluated by switching from a given concentration of CaSO$_4$ to the identical concentration of Ca(NO$_3$)$_2$. The recorded depolarization under these conditions eliminated any effect of Ca$^{2+}$. The results of these experiments (data not shown) were virtually indistinguishable from those shown in Figure 2.

The electrical effects of $\text{NO}_3^-$ exposure were also examined in a complete inorganic nutrient solution ($\text{NO}_3^-$ strength Johnson’s modified medium) rather than in 200 $\mu$M CaSO$_4$. The depolarizing effects of $\text{NO}_3^-$ in Johnson’s solution were virtually identical to those observed in CaSO$_4$ solution. However, membrane potentials were typically less negative in the Johnson’s solution.

Time Course of Induction of $\text{NO}_3^-$ Response

Roots that had received no prior exposure to $\text{NO}_3^-$ (“uninduced plants”) gave little or no electrical response to Ca(NO$_3$)$_2$ in the low concentration range (approximately 5–100 $\mu$M). In an experiment designed to compare the electrical responses of uninduced roots to $\text{NO}_3^-$ exposures in the low (200 $\mu$M) to those in the high (10 mM) concentration range, as a function of duration of $\text{NO}_3^-$ exposure, plants were alternately exposed to these two concentrations of NO$_3^-$ for a period of 5 h. It is evident from Figure 3 that, although 200 $\mu$M NO$_3^-$ caused no significant depolarization at first exposure, the 10-mM NO$_3^-$ solution caused a strong depolarization only minutes later. Over the 5-h period, the depolarizing effect of 200 $\mu$M NO$_3^-$ increased gradually. Likewise, depolarization by 10 mM NO$_3^-$ increased to an extent that corresponded with the gradually increasing depolarization due to the HATS (at 200 $\mu$M) (Fig. 4). It is apparent that depolarizations due to the two transport systems were additive, resulting in a very large depolarization by 10 mM NO$_3^-$ by 5 h.

The effect of NO$_3^-$ pretreatment over longer durations was examined by exposing plants to 100 $\mu$M NO$_3^-$ for 1, 2, or 3 d. The depolarizations caused by 200 $\mu$M NO$_3^-$ actually diminished when plants were induced for longer than 24 h, declining from 37 ± 4 mV after 24 h of NO$_3^-$ exposure to 23 ± 4 mV after 48 h and 20 ± 0.9 mV after 72 h.

LATS

Unlike the depolarization due to the high-affinity system for $\text{NO}_3^-$ uptake, depolarization by [NO$_3^-$] values beyond 0.5 mM (the concentration range in which the LATS becomes evident) appeared to be constitutive (Figs. 3 and 4). Moreover, depolarization in this concentration range showed no indication of saturation, even at 10 to 20 mM NO$_3^-$ (Fig. 5). It should be emphasized that NO$_3^-$ was provided throughout these experiments as the Ca salt.

Because cations such as K$^+$ or Ca$^{2+}$ may themselves be depolarizing at quite moderate concentrations, it was important to distinguish between Ca$^{2+}$ and NO$_3^-$ as the source of the large depolarizations observed in the presence of high [NO$_3^-$]. Two methods were used to investigate this question. In the first, uninduced roots were exposed to CaSO$_4$ solutions in the high concentration range (>1 mM); these solutions typically caused significant depolarizations (Fig. 6) that were not accompanied by repolarization. When depolarization by CaSO$_4$ had caused $\Delta \psi$ to reach a new steady value, the solution bathing the root was replaced by an identical concentration of Ca(NO$_3$)$_2$. Thus, the Ca$^{2+}$ concentration remained unchanged, but NO$_3^-$ replaced SO$_4^{2-}$. The effect of this treat-

Figure 4. A plot of depolarization of $\Delta \psi$ by 200 $\mu$M (O) and 10 mM NO$_3^-$ (•) as a function of duration of NO$_3^-$ exposure. Plants were initially uninduced for NO$_3^-$ uptake.

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ment was to cause a further large depolarization. In the second method, buffered solutions (15 mM Mes-Tris) were employed to titrate Ca(OH)₂ or HNO₃ to a desired pH. By this method, it was possible, in a background of Mes-Tris (which itself caused approximately 20 mV depolarization), to evaluate the effect of Ca²⁺ and NO₃⁻ independently. Figure 7 demonstrates that the NO₃⁻ anion was strongly depolarizing; Ca²⁺ also caused a substantial (28 mV) depolarization. The pattern of the depolarizing effect of Ca²⁺, however, was distinctly different from that caused by NO₃⁻. The Ca-induced depolarization was smaller than that due to NO₃⁻ (28 mV compared with 42 mV) and took much longer to develop (approximately 16 min to reach the 28 mV reported compared to 5 min for NO₃⁻). In neither case was spontaneous repolarization evident, but upon returning roots to the buffered solutions minus Ca²⁺ or NO₃⁻, strong repolarizations brought Δψ back to their original values.

To evaluate the hypothesis that a NO₃⁻/H⁺ symporter was responsible for the depolarizing effects of NO₃⁻, H⁺ fluxes were measured close to the root surface during exposure to different salt solutions (Table I). It is evident that only when the K⁺ fluxes had been down-regulated by 3-d exposure to K⁺ was NO₃⁻ exposure accompanied by alkalinization of external media (Table IB). Nevertheless, whenever NO₃⁻ was provided to the roots of induced plants, the extent of acidification caused by K⁺ salts was substantially diminished. This was particularly true at high external [K⁺]. This response was evident in uninduced plants only at high external [NO₃⁻].

**DISCUSSION**

The experiments described above demonstrate that exposure to Ca(NO₃)₂ solutions may cause large depolarizations of Δψ in barley roots. At low Ca(NO₃)₂ concentrations, the reported values for depolarization are almost exclusively due to the absorption of NO₃⁻. This is demonstrated by the fact that 200 μM Ca(NO₃)₂ caused virtually no depolarization in uninduced plants (e.g. Fig. 3). Yet, within 1 to 5 h (which corresponds to the time scale for the induction of increased NO₃⁻ uptake), exposure to the same concentration of Ca(NO₃)₂ caused >20 mV depolarization. Also, the extent of depolarization diminished after 24 h, corresponding to the reduction of ¹⁵NO₃⁻ influx, which has been reported for prolonged exposure to NO₃⁻ in barley roots (15). Two other arguments support this conclusion. (a) In switching experi-
Table I. Fluxes of H⁺ Associated with Various Ion Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H⁺ Fluxes* (µmol g⁻¹ h⁻¹)</th>
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<tbody>
<tr>
<td>A.</td>
<td></td>
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<tr>
<td>200 µM CaSO₄</td>
<td>-0.41 ± 0.05</td>
</tr>
<tr>
<td>100 µM Ca(NO₃)₂</td>
<td>-0.42 ± 0.06</td>
</tr>
<tr>
<td>100 µM K₂SO₄</td>
<td>-2.05 ± 0.2</td>
</tr>
<tr>
<td>200 µM KNO₃</td>
<td>-1.24 ± 0.1</td>
</tr>
<tr>
<td>200 µM CaSO₄</td>
<td>-0.39 ± 0.06</td>
</tr>
<tr>
<td>5 mM Ca(NO₃)₂</td>
<td>-0.07 ± 0.01</td>
</tr>
<tr>
<td>100 µM K₂SO₄</td>
<td>-2.74 ± 0.2</td>
</tr>
<tr>
<td>100 µM K₂SO₄ + 5 mM Ca(NO₃)₂</td>
<td>-0.43 ± 0.06</td>
</tr>
<tr>
<td>B.</td>
<td></td>
</tr>
<tr>
<td>200 µM CaSO₄</td>
<td>-4.8 ± 0.3</td>
</tr>
<tr>
<td>100 µM CaSO₄ + 100 µM Ca(NO₃)₂</td>
<td>+0.1 ± 0.02</td>
</tr>
<tr>
<td>100 µM CaSO₄ + 100 µM K₂SO₄</td>
<td>-7.35 ± 0.25</td>
</tr>
<tr>
<td>100 µM CaSO₄ + 200 µM KNO₃</td>
<td>-3.64 ± 0.13</td>
</tr>
<tr>
<td>C.</td>
<td></td>
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<tr>
<td>200 µM CaSO₄</td>
<td>-0.61 ± 0.06</td>
</tr>
<tr>
<td>200 µM CaSO₄ + 100 µM K₂SO₄</td>
<td>-6.68 ± 0.19</td>
</tr>
<tr>
<td>200 µM CaSO₄ + 200 µM KNO₃</td>
<td>-8.75 ± 0.19</td>
</tr>
<tr>
<td>200 µM CaSO₄ + 5 mM K₂SO₄</td>
<td>-14.06 ± 0.60</td>
</tr>
<tr>
<td>200 µM CaSO₄ + 10 mM KNO₃</td>
<td>-10.54 ± 1.75</td>
</tr>
</tbody>
</table>

*H⁺ fluxes reported are the means of five separate measurements.

ments where CaSO₄ solution was replaced by the same concentration of Ca(NO₃)₂, the results were virtually identical to those in which Ca(NO₃)₂ was added in a background of CaSO₄ solution (see "Materials and Methods"). (b) In most of the experiments reported here, plants had been grown for 3 or 4 d in 200 µM CaSO₄. This treatment produces Ca²⁺- and SO₄²⁻- rich plants that would be expected to express low rates of Ca²⁺ and SO₄²⁻- uptake.

Thus, the HATS for NO₃⁻ influx in barley roots, like those of *Lemma* and corn roots, operate with concomitant depolarization of ΔΨ (9, 19). Like *Lemma* and corn, depolarization was commonly followed by repolarization and, sometimes, by a modest hyperpolarization. However, unlike corn, depolarization in barley was [NO₃⁻]-dependent and saturable in a manner similar to the uptake of NO₃⁻. Indeed, the Kₘ for the uptake of NO₃⁻ was 60 µM. Siddiqi *et al.* (16) found that the influx of 13NO₃⁻ was also saturable below 1 mM, and the Kₘ for influx varied between 30 and 80 µM, depending on NO₃⁻ pretreatment. According to McClure *et al.* (9), corn, by contrast, showed only a small depolarization of approximately 10 mV in response to NO₃⁻. This was independent of [NO₃⁻] even at concentrations as high as 10 mM. In this regard, barley more closely resembles *Lemma*. Like *Lemma* and corn, depolarization by low [NO₃⁻] is evident only after induction by NO₃⁻ pretreatment. As shown in Figure 3, the extent of depolarization by the HATS increased rapidly over a period of 5 h. This corresponds well with the induction of 13NO₃⁻ influx in this barley variety (15) as well as the induction of net NO₃⁻ uptake (our unpublished results). Likewise, the reduced depolarization of ΔΨ caused by 200 µM NO₃⁻ after 2 and 3 d of NO₃⁻ pretreatment (compared with 1 d of NO₃⁻ pretreatment) mirrors the reduction of 13NO₃⁻ influx that occurs between 24 and 96 h after the first exposure to NO₃⁻ (15). This effect has been interpreted as the result of negative feedback from internal NO₃⁻ or reduced N (7).

Repolarization and hyperpolarization following depolarization is thought to reflect stimulation of H⁺ pumping by the plasma membrane ATPase. Clearly, the extent of the depolarizing current (due to the proposed 2 H⁺:1 NO₃⁻ symport), balanced or exceeded by the activity of the H⁺ pump, will determine, at any instant, the measured value of ΔΨ. The small depolarization of ΔΨ, independent of [NO₃⁻], observed in corn may indicate narrow tolerance limits for depolarization in this species. Hence, a small depolarization may result in activation of the H⁺ pump. In barley, by contrast, large depolarizations (up to 60 mV) appear to be tolerated before the H⁺ pump is sufficiently activated to produce repolarization. As discussed above, the pattern of repolarization was variable and sometimes complex in barley roots. The clear concentration dependence of hyperpolarization observed in corn was rarely evident in barley. Generally, repolarization was sufficient to restore ΔΨ to values close to the depolarized value. Hyperpolarization was usually small, a matter of 5 to 10 mV.

The depolarizing effect of NO₃⁻ in the high concentration range (>1 mM), especially in roots of plants that had received no previous NO₃⁻ exposure, is particularly important. It demonstrates that NO₃⁻ absorption by the LATS, which has been examined in some detail by means of 13NO₃⁻ studies (2, 16, 17), is not a diffusive flux, as has been proposed by various other workers (11, 14, 19). A diffusive NO₃⁻ flux would not be depolarizing. Moreover, a major difficulty with a diffusive model, as indeed with a channel-mediated flux, which we proposed earlier (16, 17), is that according to the Nerst potential, with ΔΨ values as low as -200 to -300 mV, cytoplasmic [NO₃⁻] would have to be in the nanomolar range for the NO₃⁻ flux to be diffusive.

We have recently suggested (17) that in cells actively engaged in the reduction of nitrate, particularly epidermal cells, [NO₃⁻] values may be much lower than in inner cortical cells, which have been reported to contain but a small proportion of root nitrate reductase activity (13). However, even if [NO₃⁻] values were sufficiently low to accommodate a channel-mediated flux in thermodynamic terms, the depolarizing effect of high NO₃⁻ still remains to be accounted for. The latter observation suggests that the LATS system for transport is a cation (possibly H⁺) cotransport system. Considering the importance of this finding, it was critical to be certain that the depolarizing effects of high concentrations of Ca(NO₃)₂ could be ascribed to NO₃⁻ influx and not to effects of high [Ca²⁺]. Moreover, because treatments involving high [NO₃⁻] might include the depolarizing effects of the HATS, it was important to evaluate the depolarizing effects of the LATS using uninduced plants where the HATS for NO₃⁻ is virtually absent.

The depolarizing effect of HNO₃ presented in 15 mM MesTris (pH 5.2) to uninduced roots (Fig. 7) effectively addresses the concerns described above. The extent of depolarization shown (42 mV) was typical of three independent determinations (mean = 39 ± 2.5 mV). To check that exposure to NO₃⁻
during the course of the experiment had not induced the HATS, the Ca(NO₃)₂ solution was displaced by flowing 200 μM CaSO₄ solution through the root chamber for 5 min. Roots were then exposed to 200 μM CaSO₄ plus 100 μM Ca(NO₃)₂. The very small depolarizations (2–4 mV) demonstrated that the roots had not been induced by the short exposure to high concentrations of NO₃⁻.

CaSO₄/Ca(NO₃)₂ switching experiments with uninduced roots (e.g. Fig. 6) also demonstrated that high [NO₃⁻] caused a large depolarization of Δψ, over and above that caused by CaSO₄. Because the [Ca²⁺] was held constant in this protocol, the depolarization arising from a switch from CaSO₄ to an identical concentration of Ca(NO₃)₂ represents the extent to which the NO₃⁻ depolarization exceeded that due to SO₄²⁻. The observed depolarization (Fig. 6) is therefore an underestimate of the NO₃⁻ effect because SO₄²⁻ itself may cause a significant depolarization.

Therefore, it was decided to investigate the concentration dependence of the depolarization of Δψ by the LATS by supplying Ca(NO₃)₂ in a background of 200 μM CaSO₄. The reported depolarizations (Figs. 3–5) are therefore the sum of the Ca²⁺ and NO₃⁻ effects on Δψ. This method suffers from this shortcoming, but because the Ca²⁺ effect was relatively small by comparison with the NO₃⁻ effect and relatively slow to reach its maximum effect (Fig. 7), it was considered to be a satisfactory compromise. By contrast, the switching method, although useful at low Ca(NO₃)₂ concentrations where SO₄²⁻ has little depolarizing effect, suffers a greater disadvantage at higher CaSO₄ because of the potential depolarizing effects of SO₄²⁻.

The observed depolarizing effect of high [NO₃⁻] in uninduced plants is also important because it indicates that the NO₃⁻ flux due to the LATS traverses the plasma membranes of epidermal or cortical cells. It is not represented by NO₃⁻ entering the stele through undifferentiated endodermal regions, passage cells, or breaks of the endodermis associated with lateral roots. The existence of the LATS in unicellular organisms (11, 14) also provides evidence that this flux does not depend upon a complex tissue organization. The constitutive LATS for NO₃⁻ should be acknowledged as a physiologically meaningful transport system at the cellular level. The use of higher concentrations of NO₃⁻ to observe the nature of the concentration dependence or to obtain stronger (more definitive) NO₃⁻ responses does not deny the expression of the LATS at much lower concentrations. In Skeletonema, a linear dependence of NO₃⁻ uptake was apparent at [NO₃⁻] <20 μM (14). In higher plants, the system becomes apparent between 200 and 500 μM [NO₃⁻], (16).

The existence of a constitutive LATS, given our knowledge of an inducible HATS, nevertheless is perplexing. However, we fail to comprehend the biological significance of this system should not lead to its dismissal as irrelevant. Indeed, in microbial systems, constitutive LATS that operate at high external ion concentration, together with derepressible HATS at low ion concentration, are typical. One explanation for its existence may be to facilitate very rapid induction of the HATS during seasonal flushes of NO₃⁻. These are well documented in marine as well as in terrestrial environments. As [NO₃⁻] decreases due to initial (constitutive) absorption and further uptake associated with growth stimulation, the presence of a fully induced HATS might confer significant advantages in competition for the diminishing resource.

The concentration dependence of the depolarization by high [NO₃⁻] is entirely consistent with the reported concentration dependence of ¹⁵NO₃⁻ influx at high [NO₃⁻], in barley (16). Moreover, the depolarizations due to the HATS and LATS are apparently additive. The experiments in which CaSO₄ solution was replaced by Ca(NO₃)₂ at the same Ca²⁺ concentration, as well as the experiments with Ca(OH)₂ or HNO₃ buffered solutions, clearly establish that, notwithstanding a depolarizing effect of Ca²⁺, there is an even more pronounced effect of NO₃⁻.

The most likely explanation for the depolarization of Δψ by high [NO₃⁻] is that NO₃⁻ uptake by the LATS is thermodynamically uphill even in those (epidermal) cells that, by virtue of the localized nitrate reductase activity (13), may have lower cytoplasmic [NO₃⁻] than inner cortical cells. As a consequence, cotransport with H⁺ may provide the necessary driving force for this flux. This explanation, in light of the present electrophysiological data, clearly demands that the earlier proposal of a channel-mediated NO₃⁻ influx localized in epidermal cells (17) be rejected.

Attempts to demonstrate H⁺ influx associated with provision of NO₃⁻ were complicated by the net H⁺ efflux in CaSO₄ solution and results were not entirely unequivocal (Table I). However, when H⁺ efflux was stimulated by application of K₂SO₄ solution, the extent of this efflux was strongly reduced by transfer to equimolar KNO₃. The simplest interpretation of this observation is that NO₃⁻ uptake by the HATS is mediated by a 2 H⁺:1 NO₃⁻ symport. When plants were pretreated with 100 μM K⁺ for 3 d to reduce cation uptake, substitution of 200 μM CaSO₄ by 100 μM Ca(NO₃)₂ plus 100 μM CaSO₄ caused a net influx of H⁺ (Table IB). Similarly, when 100 μM K₂SO₄ plus 100 μM CaSO₄ was replaced by 200 μM KNO₃ plus 100 μM CaSO₄, H⁺ efflux declined from 7.35 to 3.64 μmol g⁻¹ h⁻¹ (Table I).

These experiments were undertaken with plants that had been induced for NO₃⁻ uptake by pretreatment with 100 or 200 μM NO₃⁻. Higher [NO₃⁻], typical of the [NO₃⁻] for absorption by the LATS, caused a much stronger reduction of H⁺ efflux. For example, Table I indicates that the substitution of 100 μM K₂SO₄ by 200 μM KNO₃ reduced H⁺ efflux from 2.05 to 1.24 μmol g⁻¹ h⁻¹. When roots acidifying in 100 μM K₂SO₄ were treated with 5 mM Ca(NO₃)₂ in addition to the 100 μM K₂SO₄, H⁺ efflux declined from 2.74 to 0.43 μmol g⁻¹ h⁻¹.

Table IC shows data for H⁺ fluxes by uninduced plants. It is evident that exposure to 200 μM NO₃⁻ failed to reduce the extent of H⁺ efflux in these plants, consistent with the low level of expression of the HATS for nitrate uptake in NO₃⁻ deprived plants (16). By contrast, 10 mM NO₃⁻ was able to bring about significant reduction of H⁺ fluxes (from 14.06 to 10.54 μmol g⁻¹ h⁻¹). This observation is consistent with the documented constitutive character of the LATS for nitrate influx (16) and for depolarization of Δψ reported in the present paper. These results indicate that NO₃⁻ uptake by the LATS, like HATS, is probably mediated by a 2 H⁺:1 NO₃⁻ symport.

In summary, the electrophysiological experiments described above establish that both the high- and low-affinity NO₃⁻ transport systems of barley roots are electrically depo-
larizing. The electrical properties of these transport systems conform in all aspects examined (concentration dependence, negative feedback effects, inducibility) to the transport properties examined earlier by means of $^{15}$NO$_3$ (15, 16). The use of uninduced plants enabled us to isolate the LATS and to demonstrate unequivocally its constitutive capacity for NO$_3$ absorption and plasma membrane depolarization. The measurement of H$^+$ fluxes associated with NO$_3$ absorption by both the HATS and LATS suggest that 2 H$^+:$1 NO$_3^-$ symports may represent the means for driving NO$_3^-$ “uphill” against a sizable electrochemical potential gradient.

**LITERATURE CITED**