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Opposing Effects of Aluminum on Inward-Rectifier Potassium Currents in Bean Root-Tip Protoplasts

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Abstract. Inward currents in root cap protoplasts of the aluminum-tolerant cultivar, Dade, of *Phaseolus vulgaris* L. were investigated using the whole-cell patch-clamp technique. The properties of these currents were similar to those seen in inward rectifying K^+ channels in other plant tissues. Replacing bath K^+ with Na^+ nearly abolished the observed currents. Higher bath K^+ concentrations increased inward currents. $AlCl_3$ in pH 4.7 bath solutions caused inward K^+ currents to activate more rapidly and at more positive voltages when compared with $AlCl_3$ free solutions. In 10 μM $AlCl_3$ the activated inward K^+ currents were significantly larger than in the $AlCl_3$-free solution at all voltages except at the most negative voltage of −174 mV and the least negative of −74 mV. In contrast, in 80 μM Al^{3+}, when hyperpolarizing voltages were most negative, the inward K^+ currents were inhibited relative to the currents in 10 μM $AlCl_3$. Enhancement of inward K^+ currents by $AlCl_3$ is consistent with Al^{3+} binding to the external surface of the root cap protoplast, decreasing the surface charge, thus causing the channels to sense a more negative membrane potential. Inhibition of inward K^+ currents with higher $AlCl_3$ concentrations and more negative voltages is consistent with Al^{3+} block of K^+ channels.

Key words: K^+ channels — Bean — Root tips — Aluminum

Introduction

Inwardly rectifying K^+ channels in higher plant cells were first reported by Schroeder, Rashke and Neher (1987). These authors used whole-cell and single-channel "patch clamp" techniques of Hamill et al. (1981) with guard cell protoplasts of *Vicia faba*. The channels activated slowly with a hyperpolarizing voltage pulse, remained activated until the voltage pulse was removed, then deactivated rapidly. The channels were selective to K^+ compared to Na^+. In guard cells, K^+ influx through these channels promotes the swelling of guard cells and the opening of stomata by increasing the osmotic pressure of the solution in the guard cells. Aluminum, (Al^{3+}), a phyto-toxic metallic cation in acid solutions, present in many acid soils (Foy, Chaney & White, 1978, Kochian, 1995), inhibited K^+ current through these channels at concentrations between 10 and 1000 μM (Schroeder, 1988).

Gassman and Schroeder (1994) investigated inwardly rectifying K^+ channels in protoplasts from root hairs. These channels are associated with K^+ uptake by roots when external K^+ concentrations are relatively high and the membrane potential is more negative than the E_K. The channels close when external K^+ is low, thus preventing the loss of K^+ from cells (Gassman & Schroeder, 1994). In *Arabidopsis* roots, protoplasts derived from cortical protoplasts have inward-rectifier K^+ channels that are coded by the K^+ channel gene AKT1. Mutant plants that have this gene disrupted have much reduced inward K^+ currents and the plants lacking this gene have much reduced K^+ uptake (Hirsch et al., 1998; Spalding et al., 1999). Significant K^+ uptake can occur via this channel even when the external K^+ concentration is as low as 10 μM. This is in part due to the very negative membrane potential (−219 mV) and the fact that the channel is not shut off at this very negative voltage and very low K^+ concentration (Spalding et al., 1999). In protoplasts derived from root hairs of wheat, Gassman and Schroeder (1994) also found

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that Al^{3+} inhibited inward K^+ currents at Al^{3+} activities between 0.3 and 30 μM. Al^{3+} could thus inhibit K^+ uptake by root hairs where much of the K^+ initially enters the root before being translocated to the rest of the plant. The inhibition of K^+ uptake by Al^{3+} could be involved in Al^{3+} toxicity responses in plants (Gassman & Schroeder, 1994).

In the present study, we used patch-clamp techniques to investigate the effect of Al^{3+} on K^+ currents in protoplasts isolated from root caps of an Al^{3+} tolerant cultivar, 'Dade' (Foy et al., 1967), of *Phaseolus vulgaris* L., the snap bean, and found that low concentrations of $AlCl_3$ increased inward K^+ currents. High $AlCl_3$ concentrations decreased inward K^+ currents relative to the low concentration when voltages were most negative.

Materials and Methods

Plant Material

Seeds of the Dade cultivar of *Phaseolus vulgaris* L. provided by Asgrow Seed Company were germinated in the dark at about 25°C. Seedlings grew for two days in moistened paper towel "rag dolls" that allowed the roots to grow vertically (Cumming, Cumming & Taylor, 1992). About 12 seedlings were placed on a perforated plastic holder above an aerated nutrient solution contained in a 1 L Pyrex beaker. The nutrient solution composition in μM was 100 $Ca(NO_3)_2$, 50 NH_4NO_3, 50 $MgCl_2$, 10 KH_2PO_4, 45 K_2SO_4, 2 $MnCl_2$, 6 H_3BO_4, 0.5 $ZnSO_4$, 0.15 $CuSO_4$, 0.1 $(NH_4)_2$ Mo_7 O_{24}, 10 Fe,Na-EDTA, pH 4.7. Seedlings grew at 25°C for an additional two days in a growth chamber under fluorescent and incandescent light with a 14 h light, 10 h dark photoperiod. The light intensity, measured with a Biospherical Instruments QSL-100 meter having a light probe with spherical geometry, was 366 μE m^{-2} s^{-1} PAR.

Protoplast Preparation

Fifty to 100 root tips were cut from larger secondary roots in a small Petri dish filled with distilled water. The dissection was done under high magnification of a dissecting microscope to ensure that mostly the root-cap zone was excised. The water was removed by aspiration. Root caps were rinsed once in distilled water. Two ml of wall-digesting enzyme solution (Amtmann, Jelitto & Sanders, 1999) were added to the Petri dish that was covered and gently shaken for 2 h at 25°C. The protoplasts were filtered through nylon mesh and further purified by sucrose layering and centrifugation (Schachtman, Tyerman & Terry, 1991). Protoplasts were kept in the bath solution (*see* Experimental Solutions) on ice and used on the same day they were isolated.

Experimental Solutions

For all experiments, patch pipettes were filled with a filtered solution having the following composition in mM: 100 K glutamate, 10.4 KOH, 2 $MgCl_2$, 2 EGTA, 2 MgATP, 285 mannitol. The pH was 7.2. The osmolality was 514 milliosmole kg^{-1}. The bath solution composition, in mM, for the experiments shown in Fig. 1 was as follows: 100 KCl or 100 NaCl, 1 $CaCl_2$. The pH was 4.7 and the osmolality was adjusted with mannitol to 590 milliosmole kg^{-1}. The bath solution composition for the experiment shown in Fig. 2 was the same as for Fig. 1, except the KCl concentrations were either 1, 10, or 50 mM and the osmolality was adjusted with mannitol to 490 milliosmoles kg^{-1}. For the Al^{3+} experiments shown in Fig. 3, the sealing and aluminum-free solutions had the same composition. In mM, it was 10 KCl, 0.2 $MgCl_2$, 0.2 $CaCl_2$, 429 mannitol. The pH was adjusted with HCl to 4.7. The osmolality was 450 milliosmole kg^{-1}. Experimental solutions containing Al^{3+} were prepared by adding 0.1 M $AlCl_3 \cdot 6H_2 \cdot O$ solution to the sealing solution. To reduce the risk of creating A1 polymers (Bertsch, 1987), the pH was not adjusted after adding $AlCl_3$. The unadjusted pHs were as follows: 0 μM $AlCl_3$, pH 4.7; 10 μM $AlCl_3$, pH 4.7; 80 μM $AlCl_3$, pH 4.5.

Electrophysiology

Standard whole-cell patch-clamp methods were used for measuring whole-cell currents (Hamill et al., 1981). Protoplasts were added to the glass chamber that had been coated with 0.01% polylysine to promote adhesion. The patch electrode was coated with the same solution before filling to promote sealing. We used an Ag/AgCl, 2 M KCl, agarose salt bridge as the reference electrode to prevent voltage changes when bath solutions containing different concentrations of chloride were changed. Patch experiments were performed with an Axopatch 1B amplifier (Axon Instruments). The electrode resistance in the bath solution was 5 to 10 MΩ. Series resistance and capacitance were compensated. Protoplast capacitance varied between 12 and 24 pF. PClamp 6 (Axon Instruments) was used for collecting data and Clampfit 8.0 (Axon Instruments) was used for analysis. In the experiments shown in Figures 1 and 2, the holding potential was 0 mV and the data were not adjusted for junction potentials. In the $A1^{3+}$ experiments (Fig. 3), the holding potential was −54 mV after correcting for a measured junction potential of −14 mV (Fenwick, Marty & Neher, 1982). The data were collected at 333 Hz (167 Hz per channel). The filter frequency was 100 Hz. Currents for *I-V* plots in Figs. 2 and 3 were averages of the final 1 s of the 3.6-s traces. Traces in Figs. 1, 2 and 3 were leakage-compensated off-line using Clampfit 8 of pClamp. Leakage currents were obtained from a −10 mV, 300 ms pre-pulse. Leakage resistances were calculated using Ohm's law. In Fig. 2, the same leak adjustment was applied to all traces of a treatment. In Figs. 1 and 3, leak currents were measured for each trace and the leak adjustment was applied separately to each trace. In Figs. 1, 2, and 3, the baseline was adjusted to zero for all of the traces.

Results

Inward Currents are Specific for K^+ over Na^+ and Increase with Higher Concentrations of Bath K^+

Initial experiments were performed to test for the occurrence and specificity of inward rectifying K^+ channels in protoplasts isolated from root caps of an aluminum-tolerant cultivar, Dade, of *P. vulgaris*. In the whole-cell patch-clamp experiments shown in Fig. 1, the holding potential was 0 mV. Eight hyperpolarizing pulses in 20 mV steps and 3.6 s duration were given. The inward currents had characteristics similar to those of inwardly rectifying K^+ currents that were characterized in *V. faba* guard-cell protoplasts (Schroeder et al., 1987). The channels took about 1 s to fully activate when hyperpolarizing

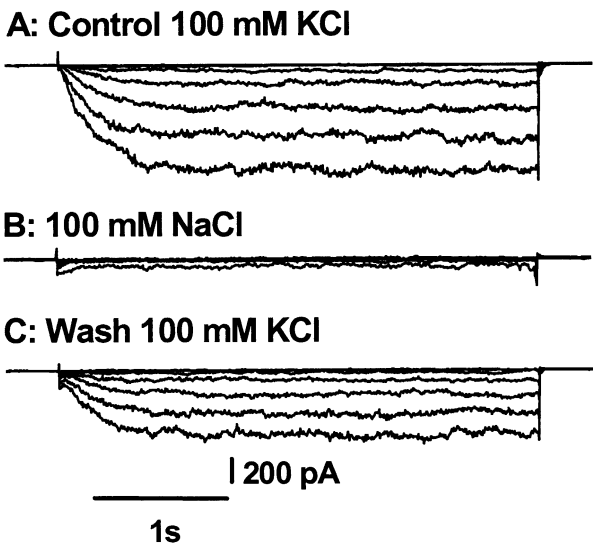

Fig. 1. Inward current recorded from an isolated root cap protoplast before (*A*) and after (*B*) substituting 100 mM NaCl in the bath for 100 mM KCl. The holding potential was 0 mV. Eight 3.6 s hyperpolarizing steps of 20 mV each were given to a maximum of −160 mV. Replacing the 100 mM bath solution with 100 mM KCl restored the inward current. Current traces were leak-adjusted individually, then adjusted to the baseline. Voltages were not corrected for junction potentials.

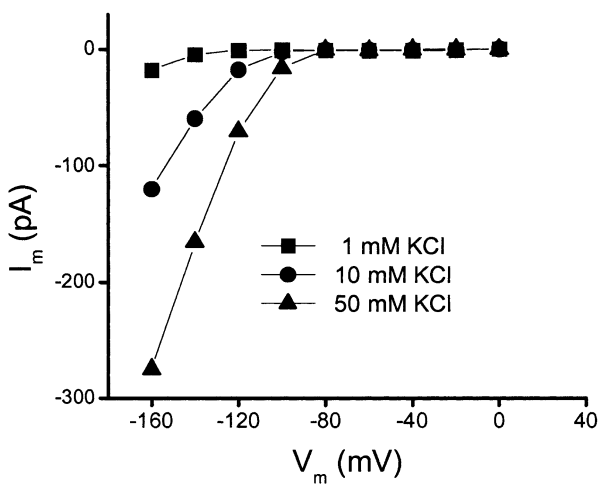

Fig. 2. Elevating the bath KCl concentration increased the amplitude of the inward currents in isolated root-cap protoplasts. The voltage protocol was the same as described in Fig. 1. Current traces were leak- and baseline-adjusted. Each point is the average of the final 1 s of the 3.6 s hyperpolarizing pulse.

voltages were applied and remained activated until the hyperpolarizing voltages were removed (Fig. 1*A*). The K^+ selectivity of the channel was ascertained by replacing K^+ with Na^+ in the bath solution. In the absence of K^+, currents were reduced by more than 90%. (Fig. 1*B*). The inward currents were restored when K^+ replaced Na^+ in the bath solution (Fig. 1*C*).

If the currents we observed were K^+ currents, we would also expect them to increase in higher bath K^+ concentrations. Consistent with this expectation, the amplitude of the inward currents increased in response to elevated external concentrations of KCl (Fig. 2).

Al^{3+} Activates Currents at More Positive Voltages and Inhibits Them at More Negative Voltages and Higher Al^{3+} Concentrations

Al^{3+} has been reported to reduce the inward K^+ currents in protoplasts from guard cells of *V. faba* and in protoplasts from root hair cells of wheat (Schroeder et al., 1987; Gassmann & Schroeder, 1994). We tested for effects of $A1^{3+}$ on K^+ channels in this Al^{3+}-tolerant cultivar, using the whole-cell patch-clamp technique with conditions similar to those described for Figs. 1 and 2. The bath solutions contained 10 mM KCl and low concentrations (0.2 mM) of $MgCl_2$ and $CaCl_2$. The pH was 4.7 (in 80 μM $AlC1_3$, the pH was 4.5) to increase the trivalent Al^{3+} relative to the divalent and monovalent hydrolysis products (Baes & Mesmer, 1976). Total $AlCl_3$ concentrations were 0, 10, and 80 μM. Precautions were taken to prevent formation of Al_{13} polymers (Bertsch, 1987). Figure 3*A–C* illustrates currents from a single protoplast activated by hyperpolarizing voltage pulses. The figure shows current traces when the bath solution contained 0, 10, and 80 μM $AlCl_3$, respectively. The holding potential was −54 mV after correcting for a −14 mV junction potential. Six hyperpolarizing pulses were applied in 20 mV steps up to −174 mV. In 0 μM $AlCl_3$ (Fig. 3*A*), three separate current traces are evident at the applied voltages of −134, −154, and −174 mV. The traces following the three less negative voltage pulses are clustered close to the baseline. In 10 μM $AlCl_3$ (Fig. 3*B*), six separate voltage traces are evident, as is the increased inward current at the most negative voltage. At the higher, 80 μM, concentration of $AlCl_3$ (Fig. 3*C*), the amplitude of the K^+ currents, relative to those in $AlCl_3$ -free solution, increased at the less negative step potentials and decreased at more negative potentials. Also in 80 μM $AlCl_3$, six separate current traces are evident, as is a relative reduction in the current at the more negative voltage when compared to 10 μM $AlCl_3$.

Average normalized data from five protoplasts are shown in the form of a current-voltage curve (Fig. 3*D*). The figure shows that the activation potentials of the K^+ currents were shifted to less negative voltages with both 10 and 80 μM $AlCl_3$. The results of a statistical analysis of the data shown in the normalized current voltage curves (Fig. 3*D*) are shown in Table 1. Paired *t*-tests at different applied voltages compared inward K^+ currents of control and aluminum treatments, or compared inward K^+ currents of the two aluminum concentrations. The

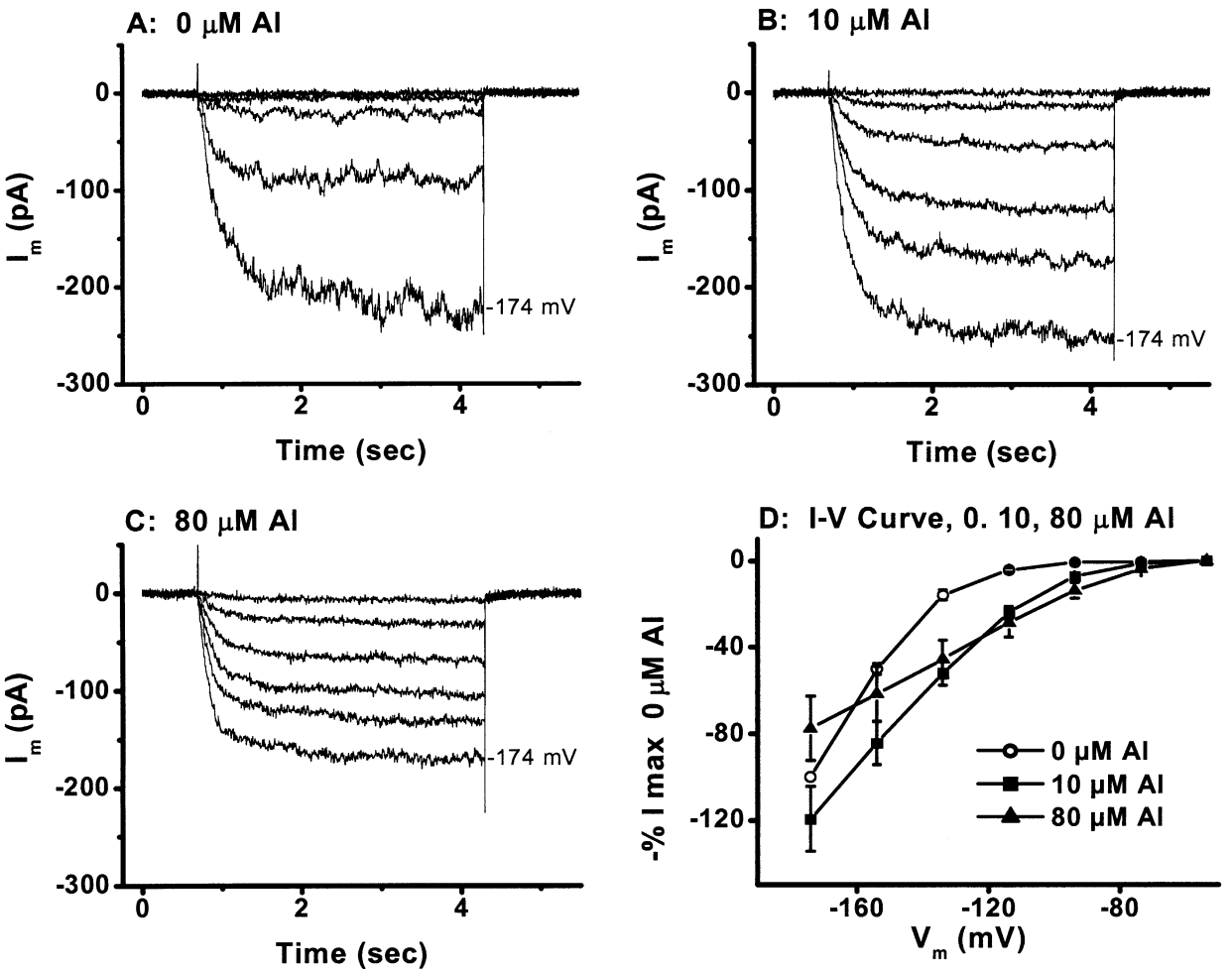

Fig. 3. Al^{3+} effects on inward currents in response to hyperpolarizing voltage pulses in one root-cap protoplast (*A–C*). Frames *A–C* show current traces in 0, 10, and 80 μM $AlCl_3$. The holding potential was −54 mV after correcting for a −14 mV junction potential. Voltage pulses were given in 20 mV increments to −174 mV. Currents were leak-adjusted individually, followed by a baseline adjustment. Each point is the average of the final 1 s of the 3.6 s hyperpolarizing pulse. Normalized *I/V* curves in frame *D* were based on averages of data from 5 protoplasts. For a given protoplast, currents at a given voltage and $AlCl_3$ concentration were divided by the currents at 0 μM $AlCl_3$ and −174 mV. This value was multiplied by −100 to provide curves analogous to *I/V* curves.

analysis shows that 10 μM $AlCl_3$ stimulated inward K^+ currents compared to 0 μM $AlCl_3$ at voltages from −94 to −154 mV. The more concentrated 80 μM $AlCl_3$ solution stimulated inward K^+ currents from −94 to −134 mV when compared with 0 μM $AlCl_3$. When compared to 10 μM $AlCl_3$ solutions, 80 μM $AlCl_3$ inhibited inward K^+ currents at −154 and −174 mV. Thus, $AlCl_3$ both stimulated and inhibited inward K^+ currents in root cap protoplasts of the aluminum-tolerant cultivar, Dade, of *P. vulgaris*. The inhibition occurred with 80 μM $AlCl_3$ and the most negative voltages.

Aluminum Decreases the Time for Voltage-Induced Activation of Inward Currents

The rise time for activation of inward K^+ currents decreases with more negative membrane potentials and with higher concentrations of $AlCl_3$ (Fig. 4). This is also apparent in the representative traces shown in Fig. 3*A–C*. The decrease in rise time is consistent with an aluminum-induced increase in the open probability of the inward rectifying K^+ channels.

The Aluminum-Induced Increase in Inward K^+ Currents is Consistent with a Reduction of the Negative Charge on the External Surface of the Membrane or K^+ Channel

At pH 4.7, Al^{3+} can bind to plant cell membranes and reduce their surface charge (Yermiyahu et al., 1997; Ahn et al., 2001). Changes in surface charge could account for the aluminum-induced increases in inward currents. The change in effective membrane potential seen by the K^+-channel voltage sensor was estimated by measuring the voltage of an $AlCl_3$ treatment that would give the same current as the

Table 1. Effect of aluminum on inward K^+ currents in *P. vulgaris* c.v. Dade root-cap protoplasts

Comparison	Difference in –mean % max control current					
	Applied Voltage (millivolts)					
	−74	−94	−114	−134	−154	−174
0 and 10 μM Al	0.92	6.50	19.6	36.1	34.3	19.5
P	0.142 (*ns*)	0.009	0.003	0.005	0.030	0.26 (*ns*)
0 and 80 μM Al	3.23	13.1	24.4	29.6	11.7	−22.5
P	0.061 (*ns*)	0.022	0.028	0.032	0.433 (*ns*)	0.203 (*ns*)
10 and 80 μM A1	2.31	6.60	4.82	−6.46	−22.7	−42.0
P	0.052 (*ns*)	0.062 (*ns*)	0.357 (*ns*)	0.207 (*ns*)	0.004	0.001

$N = 5$; *ns*, not significant.

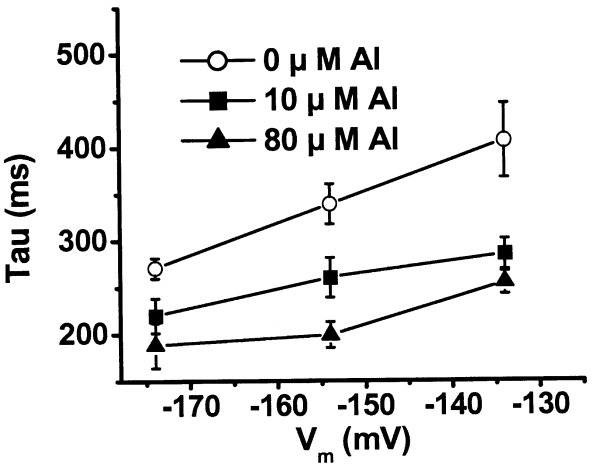

Fig. 4. The effect of 0, 10, and 80 μM Al^{3+} on the rise times of the K^+ currents at applied voltages of −134, −154, and −174 mV ($n=4$). The rise times (*Tau* values) were adequately fit with a single exponential, and decreased with increasing $AlCl_3$ concentrations and with more negative voltages. The *Tau* values were based on the first 1.5 s of a trace, beginning 18 ms after the beginning of the voltage pulse (immediately following the decay of the capacitance transient).

control (Fig. 5). Thus, in 10 μM $AlCl_3$, the effective voltage detected by the voltage sensors in the K^+ channels would be about 27 mV more negative than in the control solution. In 80 μM $AlCl_3$, the effective voltage change would be about 37 mV more negative. This would be consistent with a positive change of 27 mV in the surface potential in 10 μM $AlCl_3$ and a positive change of 37 mV in 80 μM $AlCl_3$. Changes in surface potentials of plant cell membranes by Al^{3+} based on binding studies (Yermiyahu et al., 1997) are similar to our estimates of $AlCl_3$-induced surface potential changes that are based on changes of the activation voltage of inwardly rectifying K^+ channels.

Discussion

The $AlCl_3$ enhancement of inward K^+ currents we observed in root-tip protoplasts of *P. vulgaris* is consistent with Al^{3+} binding to the external surface of the protoplast, reducing its negative charge, thus causing a hyperpolarization that would be detected by the channel voltage sensor (Hille, Woodhull & Shapiro, 1975). This would increase the number of open voltage-gated K^+ ion channels and decrease the time constant for the voltage-activated currents, as was seen in our data.

Micromolar concentrations of Al^{3+} bind to plasma membranes of plant root cells, significantly increasing their surface charge (Yermiyahu, Brauer & Kinraide, 1997; Yermiyahu et al., 1997; Ahn et al., 2001). In wheat-root plasma-membrane vesicles, the binding constant for Al^{3+} was 717 times that of Ca^{2+} and 9.8 times that of La^{3+} (Yermiyahu et al., 1997). Al^{3+} (1.97 μM activity) increased the surface potential by 24 mV at pH 4.5, 0.267 mM Ca^{2+} (Yermiyahu et al., 1997). In our patch-clamp experiments with bean root-tip protoplasts, 10 μM $AlCl_3$ added to a solution with 0.2 mM Ca^{2+}, 0.2 mM Mg^{2+}, 10 mM K^+, pH 4.7, increased K^+ currents consistent with a 27 mV increase in surface charge.

Binding sites for Al^{3+} on plant plasma membranes include the lipid bilayer and membrane proteins, including ion-channel proteins (Hille, 2001). Al^{3+} binds to liposomes composed of neutral phosphatidylcholine, which is abundant in plasma membranes of plant and animal cells, (Akeson, Munns & Burau, 1989). These authors calculated that 5 μM activity of Al^{3+} could change the surface charge of plant plasma membranes from −30 to +11 mV (Akeson et al., 1989). An example of direct ion binding to proteins occurs with H^+ binding to histidine sites on K^+ inward-rectifier channel proteins of potato guard-cell protoplasts. This binding activates inward K^+ currents (Hoth et al., 1997). With Al^{3+},

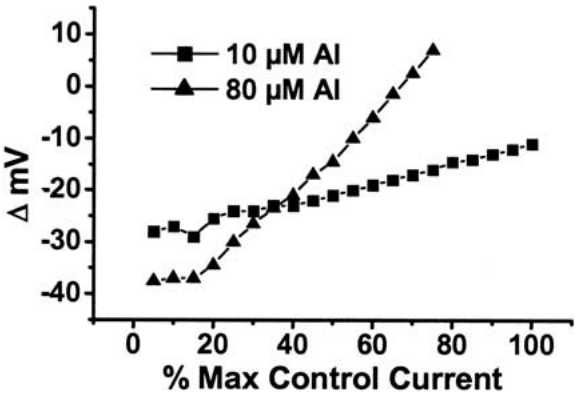

Fig. 5. Differences between applied voltages that give the same inward currents when $AlCl_3$-containing solutions are compared with control solutions. Data were derived from Fig. 3*D*. The *Y*-axis values are the voltage differences between the 0 $AlCl_3$ and the $AlCl_3$-containing solution at a given percent of the maximum current in 0 μM $AlCl_3$. For example, when the current in 10 μM $AlCl_3$ is 10% of the maximum current in 0 μM $AlCl_3$ and the current in 0 μM $AlCl_3$ is also 10% of the maximal current in 0 μM $AlCl_3$, the voltage in the 0 μM $AlCl_3$ solution minus the voltage of the 10 μM $AlCl_3$ solution (Δ mV) is −27 mV.

appropriate binding sites would be phosphate and carboxyl groups.

If Al^{3+} enhances inward currents by decreasing surface charge, then increased La^{3+}, Ca^{2+}, and H^+ concentrations would also be expected to decrease surface charge, which they do (Kinraide, Ryan & Kochian, 1992). The binding of these cations would also be expected to enhance inward rectifier K^+ currents. Indeed, in other plant tissues, enhanced inward K^+ currents occurred with La^{3+} (Wegner, De Boer & Rashke, 1994), Ca^{2+} (Blatt, 1992; Fairley-Grenot & Assmann, 1992), and H^+ (Blatt, 1992; Ilan, Schwartz and Moran, 1996; Hoth et al., 1997; Amtmann et al., 1999; Piñeros & Kochian, 2001).

If we detected enhanced inward-rectifier K^+ currents in bean root-tip protoplasts with $AlCl_3$, and others have detected enhanced inward-rectifier K^+ currents with La^{3+}, Ca^{2+}, and H^+, why haven't others seen this phenomenon with Al^{3+}? Possible explanations include (1) fundamental plant or tissue differences in the kinds and concentrations of membrane surface ligands that bind Al^{3+} in the vicinity of the channel voltage sensor (Yermiyahu et al., 1997); (2) high levels of Ca^{2+} or Mg^{2+} in the bath solution, which could dominate surface charge effects (Kinraide, 1998); (3) higher pH in the bulk solution, which would have reduced the effective Al^{3+} activity in the bulk solution (but could have increased it at the membrane surface) (Kinraide, 1991); (4) lower pH in the bath solution, which could have activated inward K^+ channels and masked the Al^{3+} activation; (5) high Al^{3+} activities, which could cause blocking of K^+ channels; (6) differences in the preparation of Al^{3+}-containing solutions, which could have altered the relative amounts of Al^{3+} and Al polymers in the bath solutions, particularly the toxic aluminum polymer, Al_{13}, (Bertsch, 1987; Kinraide and Parker, 1989; Kinraide, 1991).

Al^{3+} inhibition of inward rectifier K^+ channels occurs in other tissues (Schroeder, 1988; Gassmann & Schroeder, 1994; Ryan et al., 1997; Kollmeier et al., 2001; Piñeros & Kochian, 2001). We observed inhibition at a higher $AlCl_3$ concentration when hyperpolarizing voltages were more negative. Piñeros and Kochian (2001) also found that Al^{3+} inhibition of inward K^+ currents increased with higher Al concentrations and more negative membrane potentials. Similar inhibitions of inward K^+ currents have been reported for La^{3+} (Wegner et al., 1994) and Ca^{2+} (Blatt, 1992; Fairley-Grenot & Assmann, 1992; Wegner et al., 1994) at more negative voltages and at higher concentrations of La^{3+} and Ca^{2+}.

At the bath pH of 4.7 of our study, aluminum would occur as Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2{}^+$, and activities would be further reduced by ionic strength effects of Ca^{2+}, Mg^{2+}, and K^+ (Baes & Mesmer, 1976; Kinraide, 1991). Thus, while we cannot attribute the observed effects to any particular aluminum species, it appears that cationic aluminum increases K^+ channel activity consistent with a decrease in the negative charge on the cell surface.

Could an Activation of Inward Currents be Caused by Al^{3+} Activation of Inward Anion Currents or Other Inward Cation Currents?

Al^{3+} activates anion channels in wheat and maize (Ryan et al., 1997; Kollmeier et al., 2001; Zhang, Ryan & Tyerman, 2001). Al^{3+} activation of anion channels in plants other than members of the grass family have not been well studied, although the Al^{3+} activation of oxalate efflux from buckwheat root tips is inhibited by the anion channel blocker phenylglyoxal (Zheng, Ma & Matsumoto, 1998). Al^{3+} activates the secretion of citrate in the Al^{3+}-tolerant cultivar of *P. vulgaris* (Miyasaka et al., 1991), but the mechanism of this response is not known. To reduce the possibility of anion currents in our experiments, we replaced potassium chloride in the patch electrode with potassium glutamate. A small amount of chloride (4 mM) was associated with the added magnesium. Anion currents activate much more rapidly than inwardly rectifying K^+ currents (Ryan et al., 1997; Zhang et al., 2001). We checked for aluminum-activated anion currents in our system by noting changes in the current associated with the brief (300 ms, −10 mV) pre-pulses used for adjusting leak currents. The average inward current associated with the pre-pulses actually decreased from 1.99 pA to 1.1 pA when a bath solution containing 10 μM $AlCl_3$ replaced the $AlCl_3$ free bath solution, but increased to 3.22 pA when 80 μM $AlCl_3$ replaced 10 μM $AlCl_3$.

Therefore, it appears that the observed Al^{3+} -stimulated currents in *P. vulgaris* root cap protoplasts do not involve anion currents but are driven by K^+ channel activity.

We cannot rule out the possibility that $AlCl_3$ activated another kind of cation channel. Several kinds of non-selective cation channels (NSCCs) occur in plants and yeast (Bihler, Slayman & Bertl, 1998; Davenport & Tester, 2000; Bihler, Slayman & Bertl, 2002). Also, genetic homologies with known NSCCs in animals and other evidence support the presence of a variety of kinds of NSCCs in plants (Dennison & Spalding, 2000; Demidchik, Davenport & Tester, 2002; Sivaguru et al., 2003). Yeast protoplasts have an NSCC, NSC1, that is activated when Ca^{2+} concentrations are low (10 μM), inhibited by high Ca^{2+} (10 mM) and inhibited by low pH (pH 4) (Bihler et al., 1998). If the root-tip protoplasts used in our experiments had channels with similar properties, then Al^{3+} in the bath solution could have reduced Ca^{2+} and H^+ concentrations at the protoplast surface and possibly activated this kind of channel. These and other alternative explanations will need to be tested when more information becomes available on the properties of NSCCs in plants.

Physiological Significance of Al^{3+} Effects on Inward-Recitifier K^+ Channels

K^+ inward-rectifier channels are important in K^+ accumulation by plant cells when K^+ concentrations in the external solution are relatively high (Maathius et al., 1997) or even when the external K^+ concentrations are low, provided membrane potentials are more negative than E_K and the channels are not closed (Hirsch et al., 1998; Spalding et al., 1999). Blocking these channels with Al^{3+} would inhibit K^+ uptake in cells such as guard cells and root hairs (Schroeder, 1988; Gassmann & Schroeder, 1994). In contrast, if Al^{3+} caused more K^+ channels to open under these conditions, K^+ uptake could be promoted. When external K^+ concentrations are low and membrane potentials are more positive than the E_K, Al^{3+}-induced opening of these channels at more positive voltages could cause K^+ to leak out of cells. A reduction in cytoplasmic K^+ concentrations could reduce the amount of K^+ transport from roots to shoots.

Aluminum could also affect membrane potentials by either blocking or activating K^+ channels. When E_K is more negative than E_m, blocking the channels could depolarize the membrane potential. In the same cultivar of *P. vulgaris* used in these experiments, Olivetti, Cumming & Etherton (1995) noted that Al^{3+} depolarized membrane potentials of root cap cells of intact plants. The depolarization was consistent with an Al^{3+} block of K^+ channels. These authors hypothesized that the depolarization was part of a signaling mechanism associated with Al resistance (Cumming & Tomsett, 1992).

In conclusion, we propose that $AlCl_3$ can have dual effects on inwardly rectifying K^+ channels (stimulatory and inhibitory), depending on its concentration and the nature of the local surface charge in the vicinity of the channel.

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