

Nonselective Block by La^{3+} of *Arabidopsis* Ion Channels Involved in Signal Transduction

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Abstract. Lanthanide ions such as La^{3+} are frequently used as blockers to test the involvement of calcium channels in plant and animal signal transduction pathways. For example, the large rise in cytoplasmic Ca^{2+} concentration triggered by cold shock in *Arabidopsis* seedlings is effectively blocked by 10 mM La^{3+} and we show here that the simultaneous large membrane depolarization is similarly blocked. However, a pharmacological tool is only as useful as it is selective and the specificity of La^{3+} for calcium channels was brought into question by our finding that it also blocked a blue light (BL)-induced depolarization that results from anion channel activation and believed not to involve calcium channels. This unexpected inhibitory effect of La^{3+} on the BL-induced depolarization is explained by our finding that 10 mM La^{3+} directly and completely blocked the BL-activated anion channel when applied to excised patches. We have investigated the ability of La^{3+} to block noncalcium channels in *Arabidopsis*. In addition to the BL-activated anion channel, 10 mM La^{3+} blocked a cation channel and a stretch-activated channel in patches of plasma membrane excised from hypocotyl cells. In root cells, 10 mM La^{3+} inhibited the activity of an outward-rectifying potassium channel at the whole cell and single-channel level by 47% and 58%, respectively. We conclude that La^{3+} is a nonspecific blocker of multiple ionic conductances in *Arabidopsis* and may disrupt signal transduction processes independently of any effect on Ca^{2+} channels.

Key words: Lanthanum — La^{3+} — *Arabidopsis* — Ion channels — Cold shock — Anion channel

Introduction

The perception of environmental stimuli and their transduction into cellular responses enables plants to adapt

and respond to diverse environmental conditions. Several such transduction pathways include a change in cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_{\text{cyt}}$) as a necessary step in the process linking signal to response. Inhibition of the response by Ca^{2+} -channel blockers is commonly used to test this possibility. When combined with measurements of $[\text{Ca}^{2+}]_{\text{cyt}}$, such pharmacological agents may produce evidence that a signal effects a response by increasing $[\text{Ca}^{2+}]_{\text{cyt}}$. However, the strength of this approach relies heavily upon the specificity of the Ca^{2+} -channel blocker used.

Developments in recent years should urge caution on this pharmacological approach to demonstrating signal-transducing roles for Ca^{2+} channels in plants. Of great significance is that doubt has been cast on the selectivity of La^{3+} for Ca^{2+} channels as studies have reported that La^{3+} inhibits K^{+} channels in characean algae (Smith, Walker & Smith, 1987), cultured corn cells (Ketchum & Poole, 1991), *Amaranthus* cotyledon cells (Terry, Findlay & Tyerman, 1992), and xylem parenchyma cells of barley root (Wegner, DeBoer & Raschke, 1994). Also, it has been reported that La^{3+} reduces a current attributed to anion efflux in the alga *Chara* (Tyerman, Findlay & Paterson, 1986). Concern over using La^{3+} arises if this result holds true in vascular plants, where it is now appreciated that the activation of anion channels is key to many diverse signal transduction pathways (Ward, Pei & Schroeder, 1995). For example, abscisic acid activates anion channels in guard cells to induce stomatal closure (Pei et al., 1997), auxin alters the voltage dependence of an anion channel in guard cells and opens stomata (Marten, Loshe & Hedrich, 1991), and blue light activates an anion channel in *Arabidopsis* hypocotyl cells to inhibit their growth (Cho & Spalding, 1996) and induce anthocyanin accumulation (Noh & Spalding, 1998). With various types of ion channels participating in such diverse signaling pathways, it is crucial to fully understand the specificity of the pharmacological agents used to dissect these pathways.

The situation is made more complex by the fact that

some responses to environmental signals activate both Ca²⁺ and anion channels to induce a response. A classic example is the action potential of characean algae in which an initial influx of Ca²⁺ through plasma membrane channels increases [Ca²⁺]_{cyt} sufficiently to activate Cl⁻ channels, which further depolarizes the membrane. The same general mechanism appears to be responsible for the rapid membrane depolarization that accompanies cold shock in cucumber roots (Minorsky & Spanswick, 1989) and *Arabidopsis* seedlings engineered to report [Ca²⁺]_{cyt} in real time (Lewis et al., 1997).

There is no question that La³⁺ inhibits Ca²⁺ channels and the flux of Ca²⁺ across the plasma membrane of plant cells. Its effectiveness in this regard has been well documented (Knight, Smith & Trewavas, 1992; Knight, Trewavas & Knight, 1996; Piñeros & Tester, 1995; Gelli & Blumwald, 1997). However, this fact alone is insufficient justification for its use *in planta* to identify Ca²⁺ channels as components of signal transduction chains. In the absence of an assessment of the specificity of La³⁺ for Ca²⁺ channels, especially in species where the majority of current work on signal transduction is being performed, the possibility of misinterpreting results is very real. The effects of La³⁺ on a variety of ion channels and [Ca²⁺]_{cyt} in *Arabidopsis* presented here, especially its effects on anion channels, indicate that it is not useful as a tool for studying signal transduction in plants.

Materials and Methods

Ca²⁺ AND SURFACE POTENTIAL MEASUREMENT

In planta changes in [Ca²⁺]_{cyt} were measured in *Arabidopsis thaliana* (Columbia ecotype) seedlings transformed with the gene encoding aequorin and treated with its substrate coelenterazine as previously described (Lewis et al., 1997). Groups of etiolated seedlings were grown on agar for 4–5 days in complete darkness and handled in a darkroom lit by 0.002 μmol·m⁻²·sec⁻¹ green safelight. For some experiments, they were completely submerged in either 0.01, 0.1, 1 or 10 mM LaCl₃ or GdCl₃ for 6 hr, after which the trivalent ion solution was removed and the seedlings allowed to recover for at least 2 hr. Control seedlings were submerged in solutions of 15 mM MgCl₂, 30 mM KCl, or H₂O and otherwise treated identically. After the recovery period, agar blocks containing 40–60 seedlings were removed from the petri plates and placed upright into luminometer cuvette tubes before experimental treatments were performed using a luminometer (Picolite, United Technologies, Packard, Downers Grove, IL). Cold shock was administered by injecting 1 mL of 0°C distilled water or 1 mM KCl, 1 mM CaCl₂ solution through the luminometer injection port.

Experiments monitoring changes in surface potential (V_s) and [Ca²⁺]_{cyt} (aequorin luminescence) with a photomultiplier tube (Model PM2710, International Light, Newburyport, MA) were performed in complete darkness as previously described (Lewis et al., 1997). Control experiments using either room temperature distilled H₂O or air elicited no change in [Ca²⁺]_{cyt}. Recordings of depolarizations in response to a 20-sec pulse of BL (450 nm, 50-nm bandwidth, 100 μmol·m⁻²·sec⁻¹) were performed as previously described (Cho &

Spalding, 1996). The signal reported by two surface-contact electrodes (V_s) changes with the same magnitude and time course but opposite polarity, compared to membrane potential measured with an intracellular microelectrode (Spalding & Cosgrove, 1989, 1993).

PROTOPLAST PREPARATION

Protoplasts were prepared from the hypocotyls of *Arabidopsis* seedlings grown for 4 to 5 days in darkness as previously described (Cho & Spalding, 1996). To obtain root protoplasts, seeds (Ws ecotype) were planted on a 1.5% agar solution with 1 mM KCl and 1 mM CaCl₂ and maintained at 4°C for 4 days. Following this treatment, seedlings were placed into continuous light and grown for 4 to 5 days on vertically oriented plates at room temperature, after which root protoplasts were prepared by cutting the root tissue approximately 150 μm distal to the root tip with a sharp razor. Entire cut seedlings, minus the root tip, were placed into an enzyme solution containing 12.0 mg Cellulase, 2.0 mg Pectinase and 5.0 mg BSA dissolved in a solution consisting of 10 mM KCl, 1 mM CaCl₂, 5 mM Mes, and 300 mM sorbitol (pH 5.2 with BTP) and vacuum infiltrated 4 times for a total of 60 sec. Seedlings were maintained in this enzyme solution for 2 hr then rinsed 3 times with an identical solution, less the enzymes and buffers and maintained at 4°C until patch-clamp experiments were performed.

PATCH CLAMPING

Kimax-51 glass capillaries (Kimble Products, Vineland, NJ) were pulled and fire polished with a horizontal puller (P87; Sutter Instruments, Novato, CA). The resulting patch pipettes were filled with 130 mM CsCl, 5 mM HEPES, 10 μM Ca²⁺ (1.9 mM CaCl₂, 2 mM EGTA), 2 mM MgCl₂, and 4 mM Mg-ATP (pH 7.2 with BTP). Protoplasts were placed into a 0.5 ml recording chamber containing a sealing solution consisting of 30 mM CsCl, 5 mM HEPES, 10 mM CaCl₂, and 35 mM sorbitol, (pH 5.7) and a giga-ohm seal obtained. After obtaining outside-out patch configuration, the bathing solution plus La³⁺ was rapidly perfused into the recording chamber using a switchable manifold that released the selected solution.

For the root whole-cell experiments, protoplasts were placed into a 0.5 mL recording chamber containing a sealing solution consisting of 30 mM KCl, 10 mM CaCl₂, 5 mM HEPES, and between 90–160 mM sorbitol for osmotic balance (pH 7.0 with BTP). Once whole-cell configuration was established, the pipette solution (130 mM K-glutamate, 2 mM EGTA, 5 mM HEPES, and 4 mM Mg-ATP, pH 7.0) was allowed to equilibrate with the cytoplasm of the protoplasts for at least 2 min before the application of voltage protocols. The plasma membrane was clamped at a holding potential of -80 mV for 1.6 sec, followed by a voltage step to -40 mV for 2.4 sec before returning to -80 mV. In each of eight subsequent repetitions, the test voltage was 20 mV more positive than the one previous to it. Rapid exchange of the bath solution with one containing 10 mM La³⁺ was achieved as described above. Currents were recorded and analyzed using hardware, software, and methods all previously described (Cho & Spalding, 1996). All chemicals were obtained from Sigma (St. Louis, MO).

Results

La³⁺ INHIBITS THE COLD-INDUCED INCREASE IN [Ca²⁺]_{cyt} AND MEMBRANE DEPOLARIZATION

Application of cold shock to etiolated *Arabidopsis* seedlings induces a large increase in [Ca²⁺]_{cyt} and triggers rapid membrane depolarization. Figure 1A (top) displays

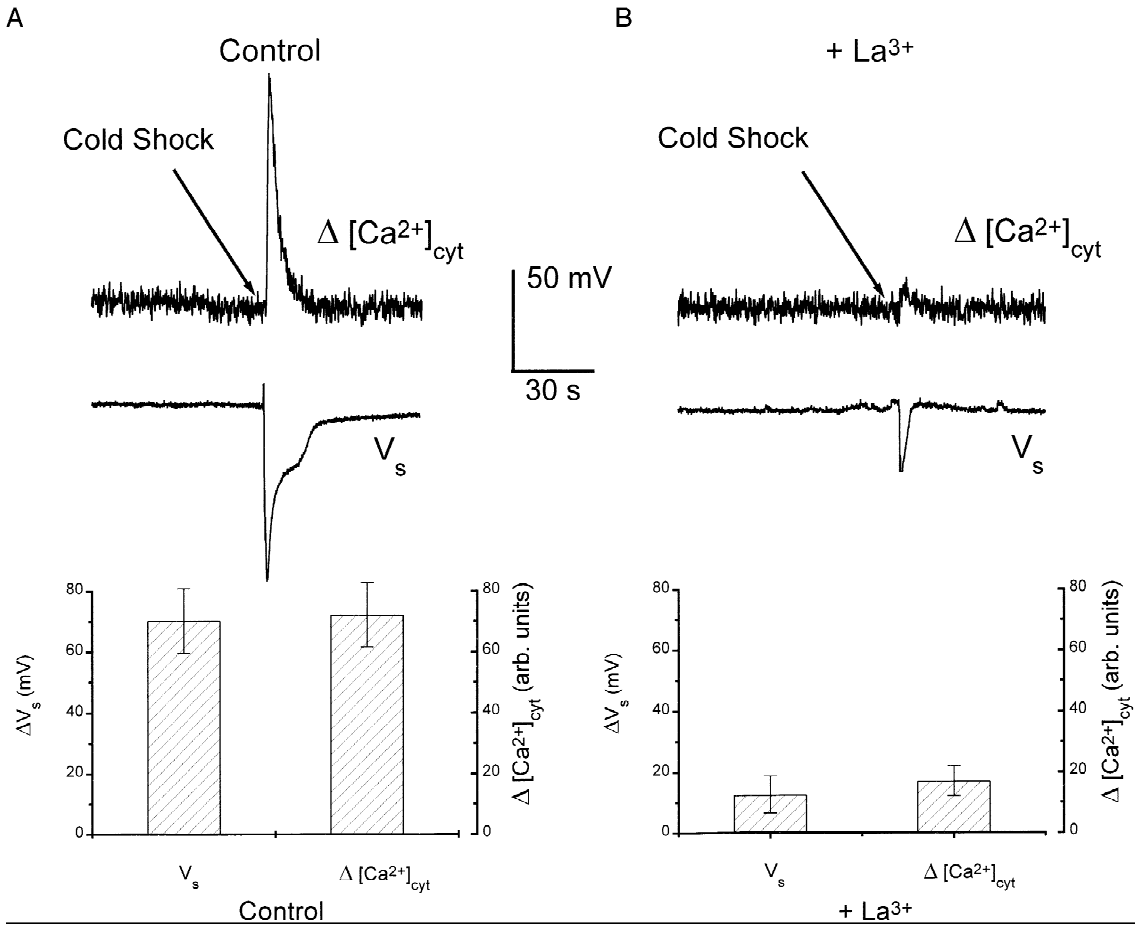


Fig. 1. La^{3+} inhibits the cold-induced increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ and membrane depolarization. (A) Top. Simultaneous recording of $[\text{Ca}^{2+}]_{\text{cyt}}$ (upper trace) and V_s (lower trace) in response to cold shock (arrow). Bottom. Changes in V_s and $[\text{Ca}^{2+}]_{\text{cyt}}$ induced by cold shock. (B) Top. Simultaneous recording of $[\text{Ca}^{2+}]_{\text{cyt}}$ (upper trace) and V_s (lower trace) in response to cold shock (arrow) in seedlings pretreated with 10 mM La^{3+} . Bottom. Average changes in V_s and $[\text{Ca}^{2+}]_{\text{cyt}}$ (\pm SE, $n = 9-10$) induced by cold shock in seedlings pretreated with 10 mM La^{3+} .

a simultaneous recording of $[\text{Ca}^{2+}]_{\text{cyt}}$ (upper trace, reported as aequorin luminescence, in arbitrary units) and surface potential (V_s , lower trace) induced by cold shock treatment. Figure 1A (bottom) shows the average change in V_s and $[\text{Ca}^{2+}]_{\text{cyt}}$ in response to cold shock ($n = 10$ for each experiment). La^{3+} has been used as a blocker of cold-induced increases in $[\text{Ca}^{2+}]_{\text{cyt}}$ in tobacco and *Arabidopsis* seedlings (Knight, Smith & Trewavas, 1992; Knight, Trewavas & Knight, 1996). Consistent with those results, Fig. 1B (top) shows that pretreatment of the seedlings with 10 mM La^{3+} significantly reduced both the cold-induced increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ and the accompanying membrane depolarization. Figure 1B (bottom) graphically displays the average change in V_s and $[\text{Ca}^{2+}]_{\text{cyt}}$ in response to cold shock following La^{3+} treatment ($n = 9$). The ability of different concentrations of La^{3+} to block the cold-induced increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ was tested and the results shown in the dose-response curve in Fig. 2. The half-maximal concentration of both LaCl_3 and GdCl_3 was 0.1 mM. None of the La^{3+} treatments had detrimen-

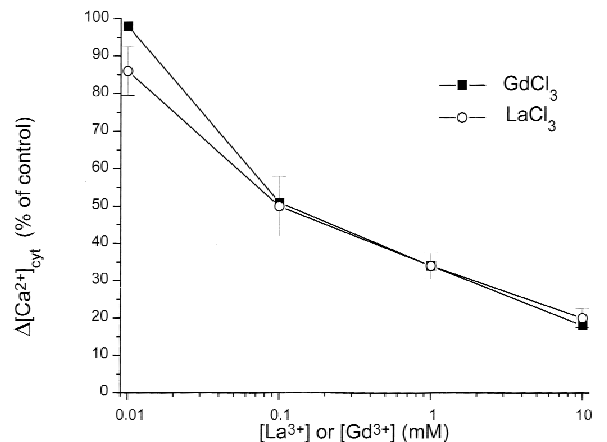


Fig. 2. Dose-response curve showing the effect of La^{3+} and Gd^{3+} on the cold-induced increase in $[\text{Ca}^{2+}]_{\text{cyt}}$. Plotted is the peak luminescence in response to cold shock relative to that of control seedlings. Each point represents the average of 2-4 experiments using independent groups of seedlings.

tal effects on the health of the etiolated seedlings as they produced green cotyledons, continued to grow indistinguishably from controls after being transferred to light (*data not shown*), and the resting membrane potential (-148 ± 5 mV) of hypocotyl cells was not affected (*data not shown*). The maximal effect of the trivalent ions was seen at 10 mM, and that concentration was used for the remainder of the experimental procedures.

La^{3+} BLOCKS THE BL-INDUCED MEMBRANE DEPOLARIZATION

Previous work in our laboratory demonstrated that blue light activates an anion channel, resulting in membrane depolarization in etiolated *Arabidopsis* seedling hypocotyl cells (Cho & Spalding, 1996). Because no role for Ca^{2+} in the BL-induced depolarization could be demonstrated (Spalding & Cosgrove, 1992; Lewis et al., 1997), we reasoned that La^{3+} should not affect the BL-induced depolarization, in contrast to its inhibitory effect on the cold response (Fig. 1B). Figure 3A (upper trace) shows a typical BL-induced depolarization of a control seedling in response to a 20-sec pulse of BL. Interestingly, Fig. 3A (lower trace) shows that pretreatment with 10 mM La^{3+} completely blocked the membrane depolarization induced by a 20-sec pulse of BL. Figure 3B shows the average BL-induced membrane depolarization in control and La^{3+} pretreated seedlings ($n = 9$).

La^{3+} DIRECTLY BLOCKS BL-ACTIVATED ANION CHANNELS IN *ARABIDOPSIS*

The inhibitory effect of La^{3+} on the BL-induced depolarization was not consistent with our previous results that the depolarization induced by BL is triggered via a Ca^{2+} -independent pathway (Lewis et al., 1997). One possible explanation is that La^{3+} directly inhibited the anion channels activated by BL through a Ca^{2+} -independent manner, which would suggest that it does not selectively block Ca^{2+} channels. We tested this hypothesis with the patch-clamp technique to determine what effect, if any, La^{3+} had on the activity of anion channels previously found to be responsible for the BL-induced depolarization. Figure 4A shows recordings of channel activity in an outside-out patch of membrane excised from a hypocotyl protoplast, at different clamped potentials. Measuring the channel current at different clamped potentials allowed the construction of the open channel I_o - V_m plot in Fig. 4B. A sigmoidal fit of the data in Fig. 4B crossed the voltage axis within 7 mV of the predicted equilibrium potential for Cl^- , indicating that Cl^- and not Cs^+ carried most of this current. The anion channel had a maximum conductance of 44 pS similar to the 46 pS measured for the BL-activated anion channel and had a comparable shaped I_o - V_m curve under similar conditions (Cho & Spalding, 1996). The long open times (Fig. 4A)

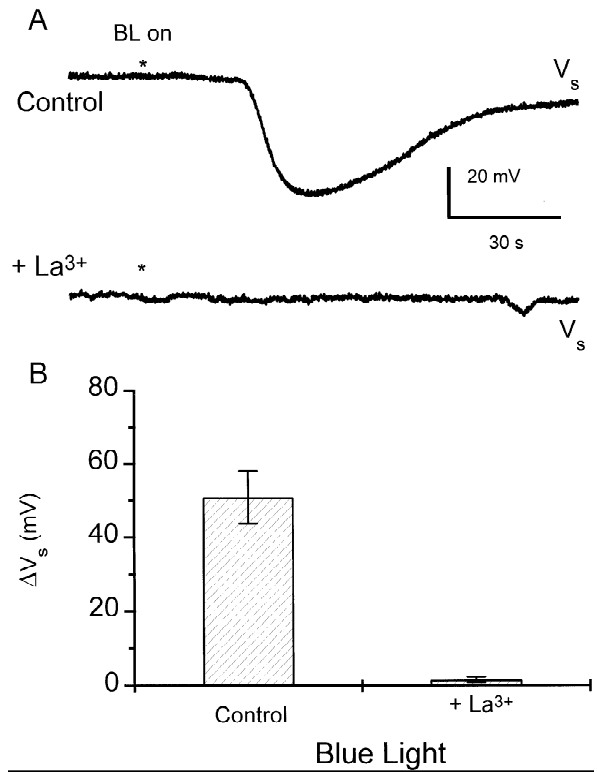


Fig. 3. La^{3+} inhibits the blue light-induced depolarization. (A) Surface electrode recording of changes in membrane potential induced by BL (20-sec pulse at asterisk) in control (upper trace) and seedlings treated with 10 mM La^{3+} (lower trace). Data shown are representative of 9–10 independent experiments. (B) Average magnitude of membrane depolarizations in response to a 20-sec pulse of BL in the presence or absence of 10 mM La^{3+} . Depolarizations are reported as means \pm SE of 9–10 independent experiments.

and the presence of subconductance states (*not shown*) are also characteristics of the BL-activated anion channel (Cho & Spalding, 1996). The combination of these channel characteristics identified the anion channel in Fig. 4 as the BL-activated anion channel previously characterized in *Arabidopsis* hypocotyls (Cho & Spalding, 1996). Figure 4C is a continuous recording of anion channel activity in an outside-out patch. The recording shows that anion channel activity is high until the addition of 10 mM La^{3+} (arrow) to the bathing solution (extracellular face of the plasma membrane). After a lag time on the order of seconds, anion channel activity was completely inhibited. The complete block of the anion channel by La^{3+} was not reversible (*not shown*). The inhibition of anion channels by La^{3+} shown in Fig. 4C is representative of 4 independent experiments showing qualitatively similar results.

La^{3+} DIRECTLY BLOCKS SEVERAL OTHER ION CHANNELS IN *ARABIDOPSIS*

The unexpected but important result that La^{3+} directly blocked the anion channel prompted an investigation of

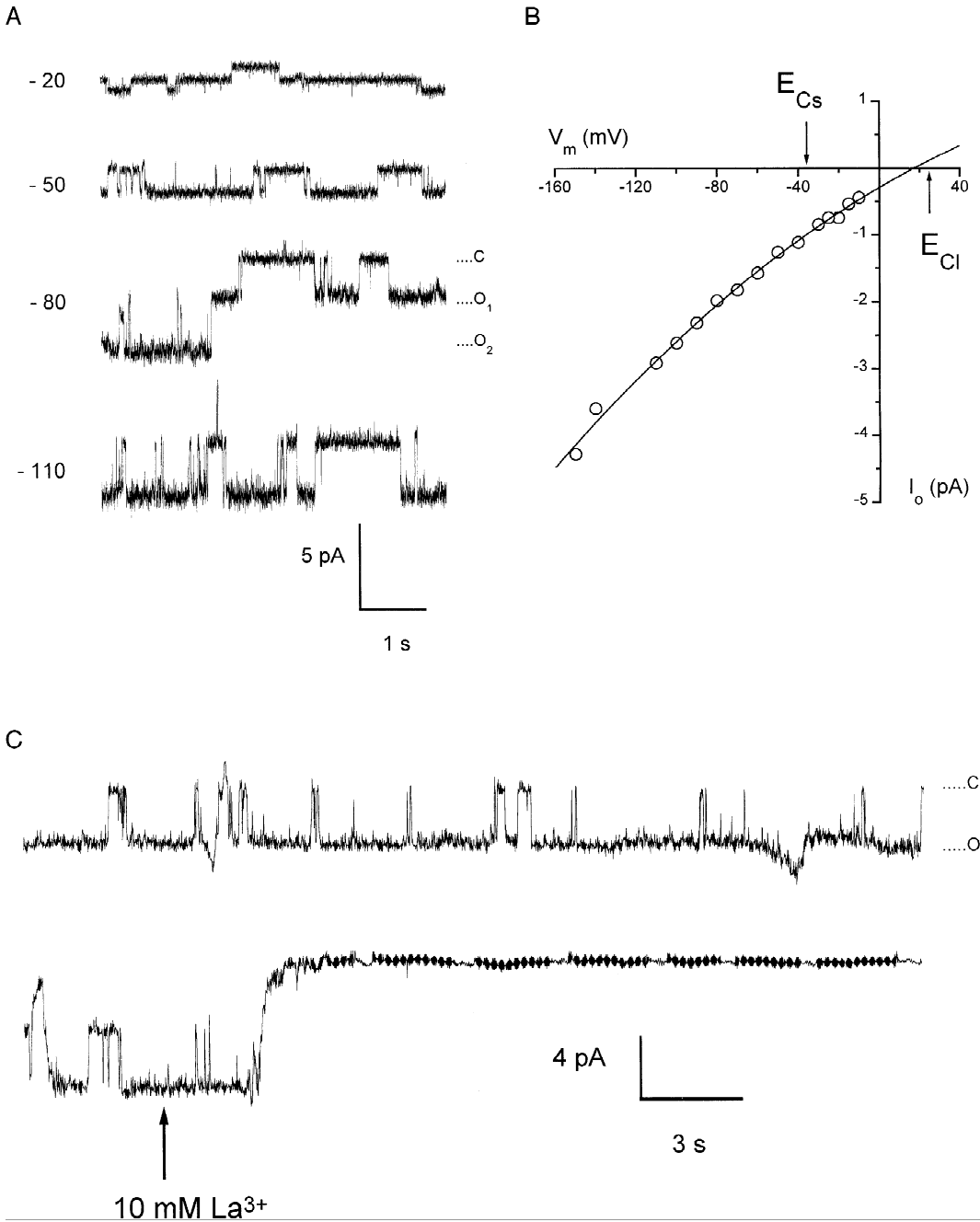


Fig. 4. La^{3+} directly inhibits anion channel activity. (A) Recordings of channel activity at each indicated clamp potential in an outside-out patch of hypocotyl plasma membrane. Closed and open states of the channel are denoted with a "C" and "O." Data were filtered at 500 Hz and digitized at 1 kHz. (B) Current-voltage relationship of the anion channel in (A). The membrane was clamped at the indicated potentials and the open-channel current of the major conductance state was measured. (C) Continuous recording of anion channel activity in an outside-out patch. 10 mM La^{3+} was perfused into the recording chamber at the arrow. Open and closed states of the channel are represented with a "O" and "C." Membrane potential was clamped at -110 mV. The recording shown is representative of 4 independent experiments showing qualitatively similar results. Data were filtered at 62.5 Hz and digitized at 125 Hz.

whether it could block other non- Ca^{2+} channels. Figure 5A shows a recording of ionic currents across an outside-out patch of a membrane excised from an *Arabidopsis* hypocotyl protoplast. An I_o - V_m plot was constructed and is shown in Fig. 5B. A linear fit of the data crosses

the voltage axis within 6 mV of the predicted equilibrium potential for Cs^+ , identifying this channel as a cation channel of unknown selectivity. The slope conductance of the cation channel was 21 pS between +40 and +120 mV, clearly distinguishing this channel from the anion

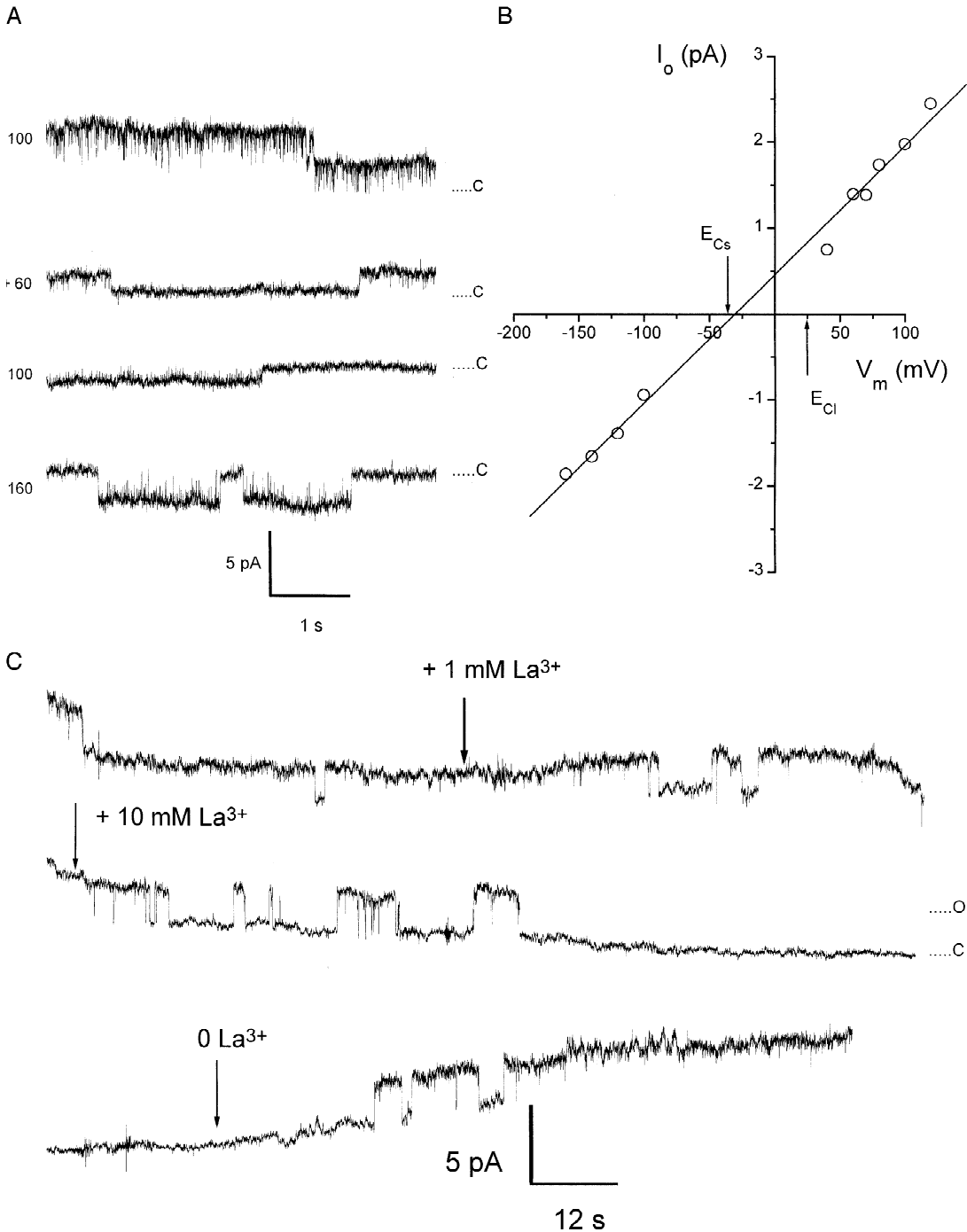


Fig. 5. La^{3+} inhibits cation channel activity. (A) Recordings of channel activity for at each indicated potential in an outside-out patch of hypocotyl plasma membrane. Closed states of the channel are represented with a ‘‘C.’’ Data were filtered at 500 Hz and digitized at 1 kHz. (B) Current-voltage relationship of the cation channel in (A). The membrane was clamped at the indicated potentials and the open-channel current of the major conductance state was measured. (C) Continuous recording of cation channel activity in an outside out patch. 10 mM La^{3+} was perfused into the recording chamber at the arrow. Open and closed states of the channel are represented with a ‘‘O’’ and ‘‘C.’’ Membrane potential was clamped at -100 mV. Data were filtered at 62.5 Hz and digitized at 125 Hz.

channel in Fig. 4. A continuous recording of cation-channel activity from an outside-out patch is shown in Fig. 5C. Exposure of the cation channel to 1 mM La^{3+} had no effect on cation channel activity (Fig. 5C, top

trace), but when the concentration was increased to 10 mM the cation channel was inhibited (middle trace, infusion was initiated at the arrow) although the time course of the inhibition was much longer than that of the

anion channel (Fig. 4C). The effect of La^{3+} on this channel's activity was reversed by rinsing the recording chamber with several milliliters of La^{3+} -free solution (Fig. 5C, bottom trace).

Figure 6 shows a different ion channel in *Arabidopsis* hypocotyl membranes, a nonselective stretch-activated channel that was only detectable when negative pressure was applied through the patch pipette. Figure 6A displays a recording of stretch-activated channel activity in an excised, outside-out patch of hypocotyl protoplast membranes at different clamped potentials. The I_o - V_m relationship of this channel is shown in Figure 6B. A sigmoidal fit of the data crosses the zero-current axis within 5 mV of the predicted equilibrium potential for Cs^+ , suggesting that this channel is also capable of conducting a cation current, although the cation selectivity of the channel is unknown. The slope conductance of this channel was 39 pS between -60 and -160 mV. The activity of this channel was markedly different from the other channels identified in this membrane. It displayed very short open times, possessed a "flickery" behavior at positive potentials, and was only present when the plasma membrane was subjected to negative pressure. Figure 6C shows a continuous recording of channel activity in an outside-out patch that was perfused with a solution containing 10 mM La^{3+} at the arrow. Similar to the anion channel, La^{3+} also completely blocked this channel after a lag time on the order of seconds. Unlike the anion channel however, the La^{3+} block was reversible, but only after tens of milliliters of La^{3+} -free solution had flowed through the recording chamber (*not shown*).

Figure 7 displays a recording of an outward-rectifying cation channel capable of conducting K^+ across the plasma membrane of root protoplasts. Fig. 7A shows time-dependent, outward currents in response to voltage steps imposed on a root protoplast under whole-cell patch clamp configuration ($n = 13$). Figure 7B shows that these whole-cell currents were inhibited approximately 47% when 10 mM La^{3+} was added to the recording chamber. Figure 7C displays current flowing through individual channels in an outside-out patch of membrane excised from a root protoplast and clamped at the indicated potentials. Figure 7D shows that 10 mM La^{3+} inhibited 58% of the K^+ -channel activity. The top trace in Fig. 7D shows that the K^+ channel had a high open-probability in the absence of La^{3+} ($nP_o = 3.1$) with as many as 6 channels open simultaneously. The addition of 10 mM La^{3+} rapidly inhibited K^+ -channel activity (middle trace), with only a maximum of 3 channels open at one time ($nP_o = 1.4$). Upon removal of La^{3+} , channel activity returned to its original level (lower trace) with an nP_o of 3.9. Open probabilities were calculated from 1.3 min recordings of K^+ channel activity before, during and after the La^{3+} treatment. The outward-rectifying single channels in Fig. 7C and D are probably the type responsible for the whole-cell currents in Fig. 7A and B because both were similarly inhibited by 10 mM La^{3+} . The cur-

rents measured at each potential in Fig. 7B were used to construct an I_o - V_m plot for this K^+ channel (*not shown*). The sigmoidal fit of those data crossed the voltage axis 30 mV more positive than the predicted equilibrium potential for a K^+ -selective channel under our recording conditions. The most plausible explanation for this discrepancy is that the channels in Fig. 7 are significantly Ca^{2+} -permeable (Schroeder & Hagiwara, 1990; Fairley-Grenot & Assmann, 1992).

Discussion

The traditional pharmacological use for La^{3+} in membrane physiology is as a Ca^{2+} -channel blocker. For example, 10 mM La^{3+} applied to intact plants blocks Ca^{2+} increases in response to cold shock (Fig. 1; Knight, Smith & Trewavas, 1992; Knight, Trewavas & Knight, 1996). The result showing that La^{3+} blocked the cold-induced membrane depolarization (Fig. 1B) would be consistent with a model maintaining that it results from an inward Ca^{2+} current and a subsequent Ca^{2+} -activated anion current (Lewis et al., 1997). However, this supporting evidence is not as persuasive as it would be if the blocker was specific for Ca^{2+} -channels.

The present work clearly demonstrates that La^{3+} does not block Ca^{2+} channels exclusively. Rather, it directly blocks many classes of ion channels including a nonspecific cation channel (Fig. 5C), a stretch activated channel (Fig. 6C), and outward rectifying K^+ channel (Fig. 7D) and, especially noteworthy, the BL-activated anion channel (Fig. 4C).

The significance of anion channel inhibition by La^{3+} is realized when one considers the large number of signaling pathways that include anion channel activation as a step (Schroeder & Hagiwara, 1989; Elzenga et al., 1995; Schmidt et al., 1995; Cho & Spalding, 1996; Pei et al., 1997). The effective block of anion channels by La^{3+} (Fig. 4C) will confuse the interpretation of results drawn from studies of signal transduction that use La^{3+} specifically as a Ca^{2+} -channel blocker.

The interpretation of data in this paper provide a case in point: La^{3+} inhibits both the BL-activated anion channel (Fig. 4C) and the resulting membrane depolarization (Fig. 3A). Without previous knowledge of the nonspecific effects of La^{3+} , the result that La^{3+} blocks the BL-induced depolarization (Fig. 3) could be wrongly interpreted as evidence of a role for Ca^{2+} in that process, when in fact it appears to be Ca^{2+} -independent (Spalding & Cosgrove, 1992; Lewis et al., 1997). Our molecular level demonstration of the irreversible, inhibitory effect of La^{3+} on this particular anion channel (Fig. 4C) extends to vascular plants the initial observations by Tyerman et al. (1986) showing inhibition of an anion current by La^{3+} in *Chara*.

In addition, because La^{3+} inhibits both the Ca^{2+} influx (Fig. 1B) and the anion currents responsible for the cold-induced depolarization (Fig. 4C; Lewis et al.,

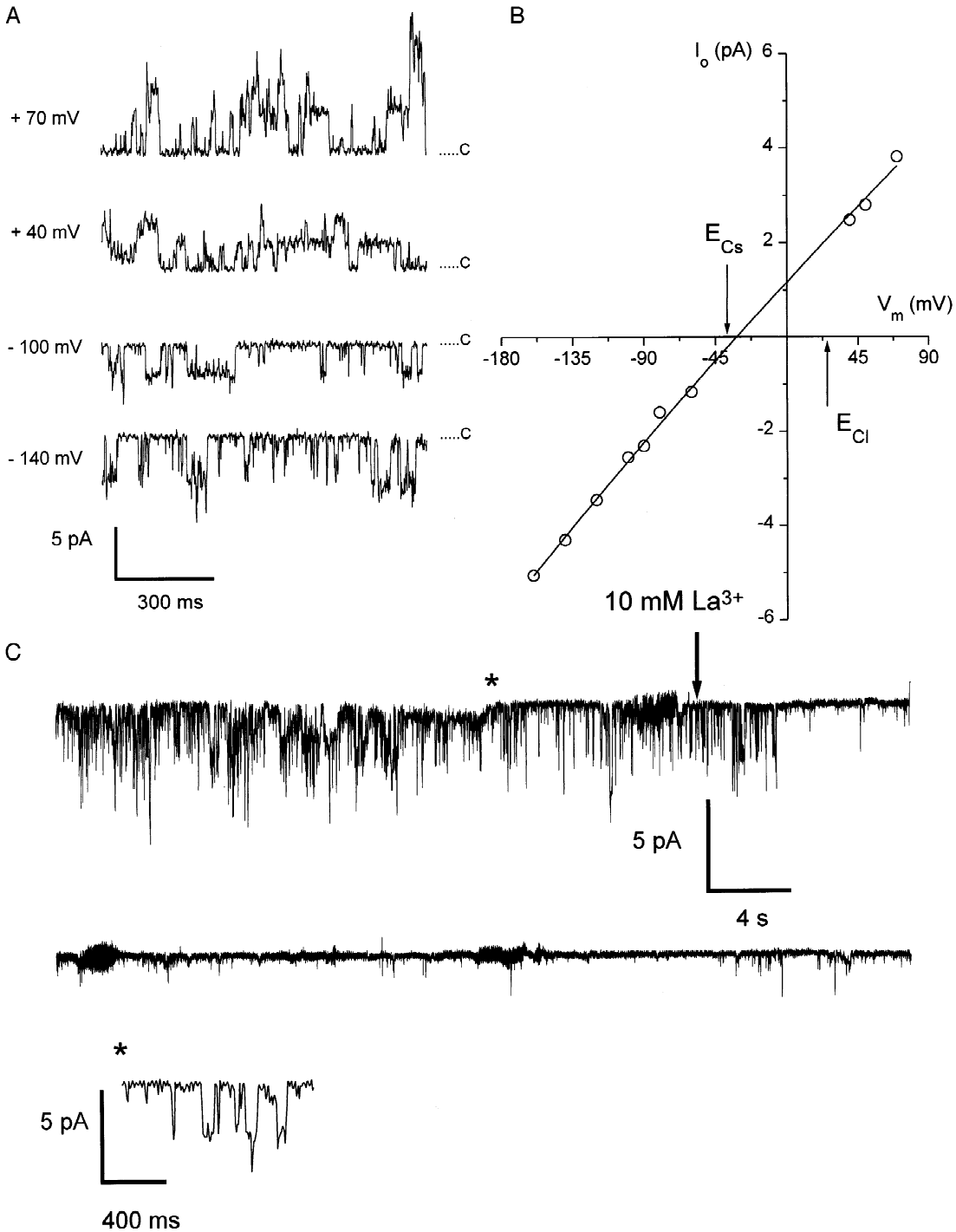


Fig. 6. La^{3+} reversibly inhibits a stretch activated cation channel. (A) Recordings of channel activity for at each indicated potential in an outside-out patch of hypocotyl plasma membrane. Closed states of the channel are represented with a "C." Data were filtered at 500 Hz and digitized at 1 kHz. (B) Current-voltage relationship of the cation channel in A. The membrane was clamped at the indicated potentials and the open-channel current of the major conductance state was measured. (C) Continuous recording of stretch activated channel in an outside-out patch. 10 mM La^{3+} was perfused into the recording chamber at the arrow. Open and closed state of the channel are represented with a "O" and "C." The inset is a 1-sec stretch of channel activity from the recording in Fig. 6C (marked with an asterisk). Membrane potential was clamped at +100 mV. Data were filtered at 62.5 Hz and digitized at 125 Hz.

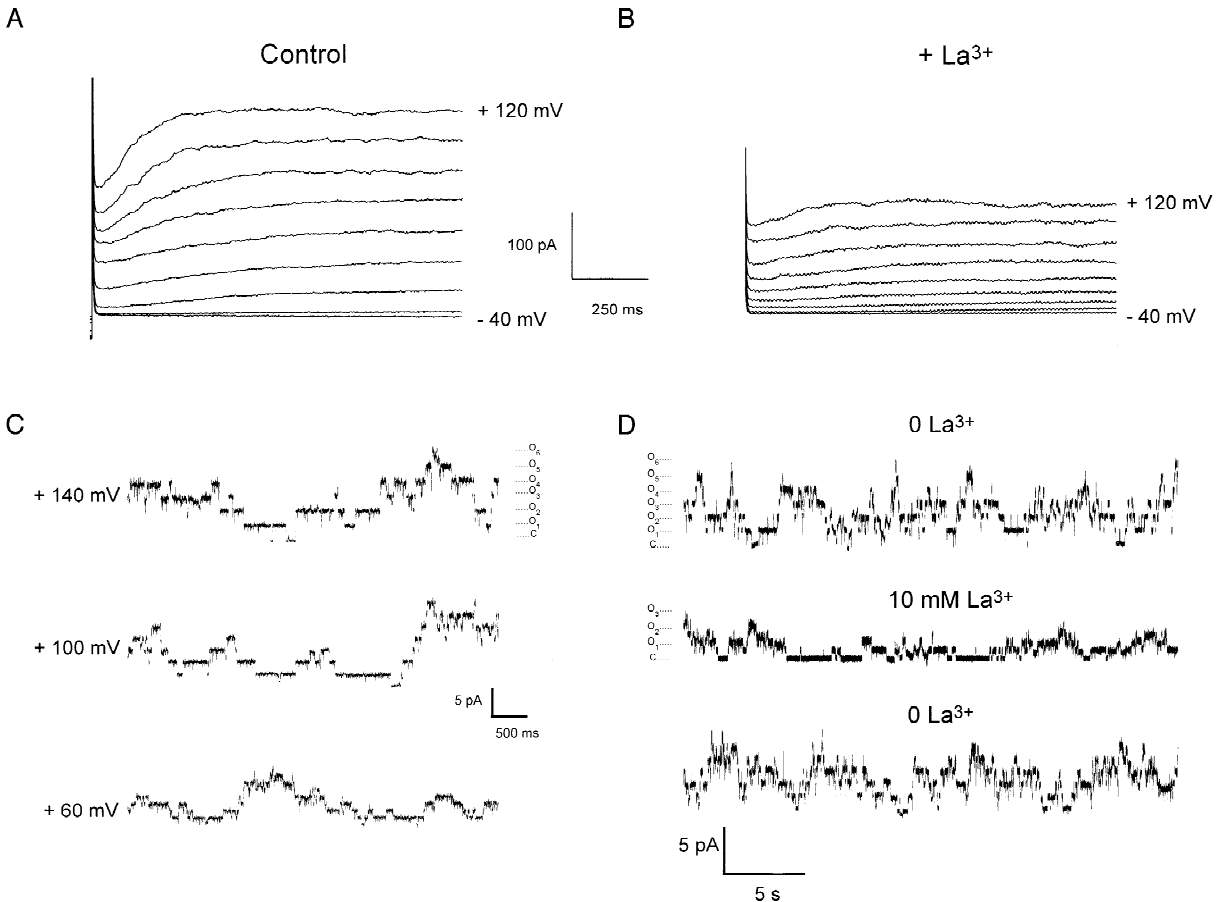


Fig. 7. La^{3+} inhibits root K^+ -channels. Outward K^+ currents from root protoplasts under whole-cell patch clamp in the absence (A) and presence of 10 mM La^{3+} (B). These recordings were filtered and digitized at 1 kHz. (C) K^+ channel activity in an outside-out patch of root plasma membrane at the indicated clamped potentials. (D) 30-sec recordings showing K^+ -channel activity in the absence (top trace), presence (middle trace), and after washout of 10 mM La^{3+} (bottom trace). Membrane potential was clamped at +80 mV. Closed and open states of the channel in (C) and (D) are represented with a "C" and "O." Single channel recordings in (C) and (D) were filtered at 500 Hz and digitized at 1 kHz.

1997), we can not rule out the possibility that cold shock simultaneously and independently activates both currents. In fact, results of these experiments raise an interesting question: although the increase of $[\text{Ca}^{2+}]_{\text{cyt}}$ into the micromolar range by cold shock can activate anion channels (Lewis et al., 1997), is Ca^{2+} required to activate anion channels in the cold-induced depolarization? Because we now understand the important inhibitory effect of La^{3+} on anion channels (Fig. 3A and Fig. 4C), we cannot interpret the La^{3+} block of the cold-induced depolarization (Fig. 1B) as evidence that inhibiting Ca^{2+} influx prevents anion-channel activation. Further experiments to test the ability of cold to activate anion channels are required to fully answer this question.

The nonspecific inhibition of ionic fluxes by La^{3+} is not without precedent. Several studies report inhibition of whole-cell K^+ current by La^{3+} including corn protoplasts (Ketchum & Poole, 1991), *Amaranthus* cotyledon protoplasts (Terry et al., 1992), and root xylem paren-

chyma cells (Wegner et al., 1994). Two possible explanations of these results have been advanced. Either La^{3+} inhibits whole-cell K^+ currents by preventing an increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ that is required for Ca^{2+} -dependent, K^+ -channel activation or La^{3+} (and other Ca^{2+} -channel blockers) inhibit K^+ channels directly. Consistent with the second explanation, our results show that 10 mM La^{3+} inhibited the whole-cell K^+ current (Fig. 7B) and directly blocked the relevant single K^+ channels (Fig. 7D) in root cells demonstrating that the effect of La^{3+} was not to inhibit K^+ current by preventing an increase in $[\text{Ca}^{2+}]_{\text{cyt}}$, but by blocking K^+ channels directly.

It has been established that La^{3+} blocks Ca^{2+} channels (Tester & MacRobbie, 1990; Piñeros & Tester, 1995; Klüsener et al., 1995; Gelli & Blumwald, 1997) and that application of La^{3+} to plant systems has the expected inhibitory effects on Ca^{2+} fluxes (Rengel, 1994), induction of the *TCH* genes (Polisensky & Braam, 1996), membrane depolarization (Minorsky & Span-

swick, 1989), and the increase in $[Ca^{2+}]_{\text{cyt}}$ triggered by cold (Fig. 1; Knight et al., 1992, 1996). However, the present work leaves little doubt that La³⁺ inhibits more than just Ca²⁺ channels. This nonspecific inhibition of multiple ion channels, especially anion channels known to be important in several signal transduction pathways, must be considered when interpreting results of experiments that use La³⁺ as a specific Ca²⁺-channel blocker.

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