Plant Physiology, July 1999, Vol. 120, pp. 849–857, www.plantphysiol.org © 1999 American Society of Plant Physiologists

Inhibition of Water Channels by $HgCl_2$ in Intact Wheat Root Cells[1]

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To assess the extent of water flow through channels in the membranes of intact higher plant cells, the effects of $HgCl_2$ on hydraulic conductivity (L_P) of wheat (*Triticum aestivum* L.) root cells were investigated using a pressure probe. The L_P of root cells was reduced by 75% in the presence of 100 μM $HgCl_2$. The K^+-channel blocker tetraethylammonium had no effect on the L_P at concentrations that normally block K^+ channels. $HgCl_2$ rapidly depolarized the membrane potential (V_m) of the root cells. The dose-response relationship of inhibition of L_P and depolarization of V_m were not significantly different, with half-maximal inhibition occurring at 4.6 and 7.8 μM, respectively. The inhibition of L_P and the depolarization of V_m caused by $HgCl_2$ were partially reversed by β-mercaptoethanol. The inhibition of L_P by $HgCl_2$ was similar in magnitude to that caused by hypoxia, and the addition of $HgCl_2$ to hypoxia-treated cells did not result in further inhibition. We compared the L_P of intact cells with that predicted from a model of cortical cells incorporating water flow across both the plasma membrane and the tonoplast using measured values of water permeability from isolated membranes, and found that $HgCl_2$ has other effects in addition to the direct inhibition of water channels.

The cloning and functional expression of aquaporins from higher plants (Maurel et al., 1993, 1997a; Daniels et al., 1994, 1996; Kammerloher et al., 1994; Yamada et al., 1997; Weig et al., 1997; Chaumont et al., 1998; Johansson et al., 1998) has indicated that water flow across intact higher plant membranes could be predominantly through aquaporins. The biophysical evidence for this in higher plants has lagged behind the molecular work, but recent studies have shown that biophysical characteristics of water transport are consistent with water flow occurring predominantly through channels in some membranes. This evidence includes the following: (a) the ratio of osmotic to diffusional water permeability is greater than unity (Niemietz and Tyerman, 1997); (b) the activation energy is low (Maurel et al., 1997b; Niemietz and Tyerman, 1997); and (c) water permeability is sensitive to sulfhydryl reagents, in particular $HgCl_2$ (Maurel et al., 1997b; Niemietz and Tyerman, 1997).

In the membranes of intact giant charophyte cells, high diffusional water permeability, low activation energy, and inhibition by sulfhydryl reagents have been well established (Wayne and Tazawa, 1990; Henzler and Steudle, 1995; Steudle and Henzler, 1995; Tazawa et al., 1996; Schütz and Tyerman, 1997). The frictional interactions between the transport of water and highly permeant molecules (Tyerman and Steudle, 1982; Steudle and Henzler, 1995; Hertel and Steudle, 1997) are also indicative of water movement through aqueous pores in the membranes of characean cells.

Inhibition by mercurials of water flow through most (Maurel, 1997; Tyerman et al., 1999) but not all (Daniels et al., 1994) aquaporins has prompted experiments testing this effect in whole organs of plants (tomato roots, Maggio and Joly, 1995; wheat roots, Carvajal et al., 1996; barley roots, Tazawa et al., 1997). The strong inhibition that is often observed at high concentrations of $HgCl_2$ has been interpreted as a direct block of water channels and has prompted the view that aquaporins could be involved in the regulation of water flow across roots. However, there is no direct evidence to show that the hydraulic conductivity (L_P) of individual root cells is sensitive to $HgCl_2$. There is also no direct evidence to exclude the possibility that $HgCl_2$ inhibition may be indirect via metabolic inhibition, and recent studies have shown that $HgCl_2$ rapidly depolarizes the membrane potential (V_m) of *Chara corallina* cells (Tazawa et al., 1996; Schütz and Tyerman, 1997).

The possibility of an indirect metabolic effect is especially relevant, since Niemietz and Tyerman (1997) found that the water permeability of isolated plasma membrane extracted from wheat roots was not inhibited by $HgCl_2$. Previously, Zhang and Tyerman (1991) had shown that hypoxia and azide both substantially inhibit the L_P of intact wheat root cells. Moreover, the evidence for water-channel-mediated water flow in isolated plasma membrane vesicles was overall not very strong (Niemietz and Tyerman, 1997). In contrast, the water permeability of tonoplast-enriched membrane vesicles was strongly inhibited by $HgCl_2$ and other evidence pointed to water channels being active in the tonoplast (Niemietz and Tyerman, 1997). Qualitatively identical results were obtained by Maurel et al. (1997b) for plasma membrane and tonoplast from tobacco suspension-cultured cells, adding weight to the possibility that aquaporins are not significant in determining water flow in native plasma membranes.

At variance with these results, Kaldenhoff et al. (1998) recently demonstrated that expression of an antisense gene

[1] This study was supported in part by the Australian Research Council.

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Abbreviation: TEA^+, tetraethylammonium ion.

for PIP1b, a putative plasma membrane aquaporin in Arabidopsis, resulted in an increased root-to-shoot ratio and an apparently reduced water permeability of leaf mesophyll protoplasts. These results are consistent with PIP1b being involved in water flow. There is clearly a need to bridge the gap between measurements at the whole organ level and those at the isolated membrane level by investigating the effects of mercurials on single intact cells and the possibility of indirect effects of $HgCl_2$ on water permeation.

There has also been a lack of attention to the possibility that K^+ channels in the plasma membrane and tonoplast may also mediate water flow across the membranes, as suggested by work on *C. corallina* (Wayne and Tazawa, 1990; Homblé and Véry, 1992). The K^+ outward and inward channels in the plasma membrane, which accommodate K^+ efflux and influx, respectively, when activated, have been identified in various higher plant cells, including the protoplasts derived from wheat root cells (Schachtman et al., 1992; Findlay et al., 1994; Gassmann and Schroeder, 1994). However, whether the K^+ channels could contribute to the L_P of root cells remains unknown.

To address these issues, we investigated the effect of $HgCl_2$ and a K^+-channel blocker, TEA^+, on the L_P of cortical cells of wheat roots using a pressure probe. We also investigated the effect of $HgCl_2$ on the V_m of cortical cells to compare it with the inhibition of L_P. Finally, we compared measured water flow in intact cells with modeled water flow using measured water permeabilities of isolated membrane vesicles from wheat roots (Niemietz and Tyerman, 1997).

MATERIALS AND METHODS

Plant Material

Wheat (*Triticum aestivum* L. cv Machete) seeds were germinated in the dark for 48 h at 25°C on filter paper soaked with 0.5 mM $CaSO_4$. The seedlings were then grown hydroponically in fully aerated one-half-strength Hoagland solution under controlled conditions, as described previously (Zhang and Tyerman, 1991).

Pressure Probe Measurements

The roots of 4- to 8-d-old plants were used in the pressure probe experiments. Measurements were made on the second to fourth layer of cortical cells 10 to 20 mm from the root apex. An excised root was held in a Perspex chamber positioned on the specimen stage of a light microscope. A glass capillary attached to the pressure probe was introduced into the root cortical cells through a small opening on one side of the chamber. The chamber was flushed with aerated one-half-strength Hoagland solution.

Details of the pressure probe measurements have been given previously (Zhang and Tyerman, 1991; Zhang et al., 1996). Once the pipette filled with silicone oil was introduced into a cortical cell, there was a sudden movement of cell sap into the micropipette, forming a meniscus between the oil and the sap. By moving the meniscus to a position adjacent to the root surface with the pressure probe, a stationary turgor pressure (P) output was recorded. The half-time for water flow equilibration ($t_{1/2}$) induced by rapid changes in P was determined from the P relaxation curves recorded with a chart recorder and later digitized using an optical scanner and the program UnGraph (version 2.0, Biosoft, Cambridge, UK). For some experiments relaxation curves were fitted to both single- and double-exponential equations using the Prism program (GraphPad Software, San Diego, CA), which uses the Levenberg-Marquardt method to minimize the sum of squares. The equation that gave the best fit to the data was deduced by performing an F test within the Prism program that takes into account the difference in df from having different numbers of variables in the two equations.

The L_P of the cells was calculated using the equation:

$$L_p = \frac{V \cdot \ln 2}{A t_{1/2}(\epsilon + \pi_i)} \quad (1)$$

where V is the cell volume, A is the surface area, π is the intracellular osmotic pressure that is approximated to initial P because of the low π of the bathing solution, and ϵ is the volumetric elastic modulus determined independently by measuring changes in V (ΔV) and corresponding changes in P (ΔP):

$$\epsilon = V\Delta V/\Delta P \quad (2)$$

For each cell, measurements of ϵ and $t_{1/2}$ were first performed in the absence of $HgCl_2$ or TEA-Cl, and then the same measurements were repeated in the presence of $HgCl_2$ or TEA-Cl. The cell was delineated by injecting silicon oil from the pressure-probe pipette at the end of the measurements, allowing for more accurate determinations of cell dimensions. The cells were approximated to cylinders.

Measurements of V_m

The V_m of the root cells was measured as described by Zhang and Tyerman (1997). The roots were bathed with aerated solution containing 1 mM KCl, 0.1 mM $CaSO_4$, and 1 mM Hepes, pH 7.0. Microelectrodes were pulled from borosilicate glass capillaries with solid filaments (Clark Electromedical Instruments, Reading, UK). The micropipettes were filled with 1 M KCl. A reference electrode was filled with the same electrolyte solution as the micropipette plus 2% agar. The electrical potential difference was measured with an amplifier (model 1600 Neuroprobe, A-M Systems, Carlsborg, WA) with an input impedance of 10^{13} Ω.

RESULTS

Effect of $HgCl_2$ on Water Relations of the Cells

No significant changes in P or ϵ of wheat root cells were found when the roots were treated with $HgCl_2$ concentrations up to 100 μM for 1 h (Fig. 1A). However, there was a significant increase in the $t_{1/2}$ of the cells when 10 and 100 μM $HgCl_2$ were added to the bathing medium (Fig. 1B).

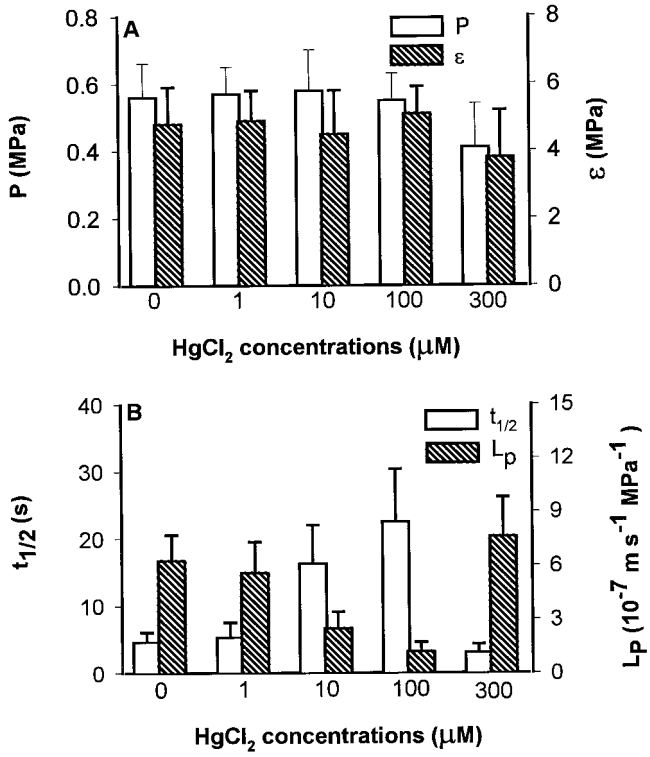

Figure 1. Effects of $HgCl_2$ on cellular water relations in wheat roots. The values are means ± SD of 6 to 10 cells measured before $HgCl_2$ addition and after 30 min.

Because there was no significant change in ϵ, this increase in $t_{1/2}$ indicates that there was a decrease in the L_P (Fig. 1B). When the roots were treated with 300 μM $HgCl_2$, an increase in L_P (decrease in $t_{1/2}$) was often observed (Fig. 1B). This increase in L_P and a concurrent decrease in P suggest that the membranes become leaky. Figure 2 shows a time course of changes in L_P ($t_{1/2}$) of a cell upon adding 100 μM $HgCl_2$ to the bathing medium. The reduction of L_P by $HgCl_2$ was not reversed when 1 mM β-mercaptoethanol was used to replace the $HgCl_2$ (data not shown). However, when $HgCl_2$ was washed out with 5 mM β-mercaptoethanol, about 60% of the inhibition was recovered (Fig. 2).

To rule out the possibility that the reduction of L_P in the presence of $HgCl_2$ could result from a coincidental time-dependent change in L_P, measurements of L_P ($t_{1/2}$) of the root cells were repeatedly made on the root cells bathed in the control solution. No time-dependent change in the $t_{1/2}$ (L_P) of the cells was found (Fig. 2). The ratio of L_P for endosmotic and exosmotic water flow, $L^{en}{}_P/L^{ex}{}_P$, was 1.12 ± 0.11 (n = 21) for the cells in control solution and 1.02 ± 0.08 (n = 8) for the cells treated with 100 μM $HgCl_2$. Therefore, $HgCl_2$ reduced the L_P of both endosmotic and exosmotic water flow.

A marked decrease in L_P of wheat root cells was found when they were exposed to low-O_2 treatments (Zhang and Tyerman, 1991); therefore, it would be interesting to determine whether the reduction of L_P by $HgCl_2$ and hypoxia is caused by a similar mechanism. The roots were pretreated

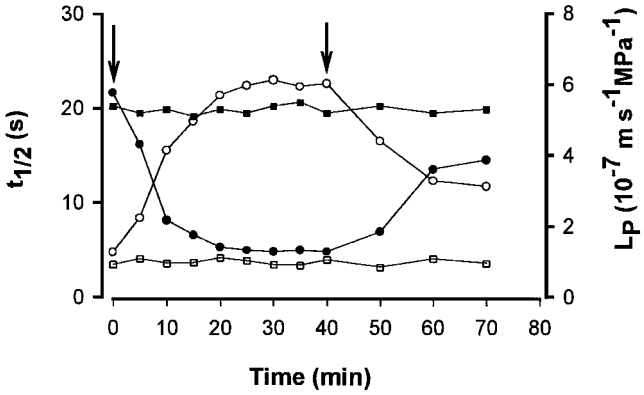

Figure 2. Time course of changes in $t_{1/2}$ (□, ○) and L_P (■, ●) of one root cell in response to 100 μM $HgCl_2$ in the bathing medium (circles) or in control solution (squares). The first arrow indicates addition of 100 μM $HgCl_2$ to the bathing medium, and the second arrow indicates removal of $HgCl_2$ by 5 mM β-mercaptoethanol.

with low O_2 for 1 h, and the effect of $HgCl_2$ on the L_P of the cells was then examined under hypoxia. As shown in Table I, the hypoxia-treated cells had a low L_P, and the addition of 100 μM $HgCl_2$, a concentration that saturates the effect on L_P, did not significantly change the L_P of the cells (P = 0.12, t test).

Effect of TEA^+ on L_P of Wheat Root Cells

In contrast to the $HgCl_2$ treatments, when TEA^+ at concentrations of 1 and 10 mM was added to the bathing medium for up to 1 h, no significant change in the L_P of the cells was found (Table I), which could have been due to the K^+ channels not being activated under our experimental conditions. In protoplasts of wheat root cells, a K^+ outward channel is activated when the V_m becomes more positive than the equilibrium potential for K^+ (E_K) (Schachtman et al., 1992). The wheat root cells were rapidly depolarized by hypoxic treatments to a V_m more positive than E_K (Zhang and Tyerman, 1997). The hypoxia-elicited membrane depolarization would be expected to activate the K^+ outward channels. However, the L_P of the cells was only slightly changed upon addition of 10 mM TEA^+ to the hypoxic bathing medium (Table I).

Table I. *Effect of 100 μM $HgCl_2$, TEA-Cl (1 and 10 mM) on L_P of wheat root cells in aerated (control) and hypoxic solutions*

Values are means ± SD; n is the number of the cells measured for each treatment.

Treatment	L_P (10^{-7} m s^{-1} MPa^{-1})	n
Control	5.6 ± 1.6	21
Hypoxia	1.8 ± 0.7	6
$HgCl_2$	1.2 ± 0.5	8
Hypoxia + $HgCl_2$	1.6 ± 0.8	5
TEA (1 mM)	5.2 ± 2.1	8
TEA (10 mM)	5.5 ± 1.8	6
Hypoxia + TEA (10 mM)	2.1 ± 1.2	4

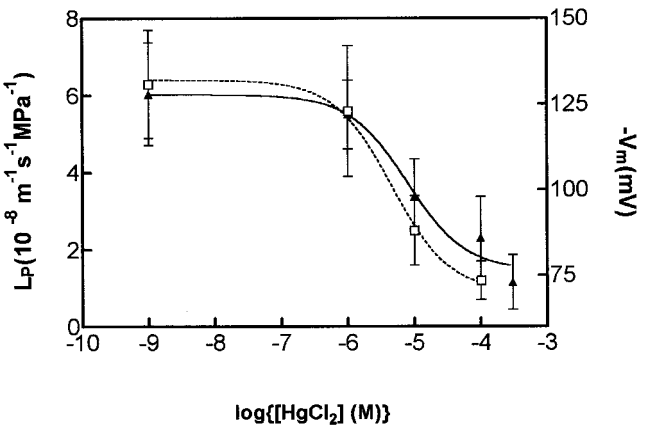

Figure 3. Dose-response curves of V_m (▲) and L_P (□) to $HgCl_2$ concentrations in the bathing medium for root cortex cells. The values are means ± SD of 6 to 10 cells and have been fitted by sigmoidal dose-response curves of the form: $y = y_{max} + (y_{max} - y_{min})/(1 + 10^{(\log EC_{50} - \log[HgCl_2\ concentration])})$. The half-maximal inhibition constants (EC_{50}) for L_P and V_m were 4.6 and 7.8 μM, respectively.

Effect of $HgCl_2$ on V_P

When $HgCl_2$ was added to the bathing solution, the root cells showed a substantial membrane depolarization following an initial small hyperpolarization. The depolarization increased with increasing $HgCl_2$ concentrations. These results have been plotted as a dose-response curve in Figure 3 so that they can be compared with the dose-response curve for $HgCl_2$ inhibition of L_P shown on the same graph. The control values without added $HgCl_2$ where plotted against an arbitrarily small amount of $HgCl_2$ to accommodate plotting the results on a logarithmic abscissa. The dose-response curves that were fitted gave half-maximal inhibitory constants of 4.6 μM $HgCl_2$ for L_P and 7.8 μM $HgCl_2$ for V_m, which are not significantly different within 95% confidence limits. The membrane depolarization was not fully reversed when $HgCl_2$ was washed out with mercaptoethanol. For the four cells examined, 100 μM $HgCl_2$ depolarized V_m from −112 ± 15 to −81 ± 7 mV. Upon removal of $HgCl_2$ and addition of 5 mM mercaptoethanol, V_m hyperpolarized to −92 ± 9 mV.

Modeling the Effect of $HgCl_2$ on Tonoplast and Plasma Membrane L_P

To solve for the change in P as a function of time while taking into account the volume flows across the tonoplast and plasma membrane, the coupled differential equations for volume flow across the tonoplast and plasma membrane were solved using an iterative procedure (Eulers method). The procedure was performed in the program Mathcad (version 7.0, MathSoft, Cambridge, MA) based on the method outlined by Wendler and Zimmermann (1985). The finite difference equations used for the iteration were:

$$\Delta V_{c\to o} = A^P L_P{}^P [P + (\pi_o - \pi_c)]\,\Delta t \quad (3)$$

$$\Delta V_{v\to c} = A^t L_P{}^t (\pi_c - \pi_v)\,\Delta t \quad (4)$$

$$\Delta\pi = \pi \frac{\Delta V}{V + \Delta V} \quad (5)$$

$$\Delta P = \epsilon \frac{\Delta V_{c\to o}}{V_t} \quad (6)$$

where $\Delta V_{c\to o}$ and $\Delta V_{v\to c}$ are the changes in V caused by water flow across the plasma membrane and tonoplast, respectively, over a small increment in time Δt; A^P and A^t are the surface areas of the plasma membrane and tonoplast, respectively; $L_P{}^P$ and $L_P{}^t$ are the L_P of the plasma membrane and tonoplast, respectively; π_c and π_v are the π of the cytoplasm and vacuole, respectively. Equation 5 was used to adjust π_c and π_v with the relevant compartment volume and changes in V. It is assumed that ϵ and L_P are constant with P over a small change in P and that the tonoplast and plasma membranes are effectively impermeable to the solutes that make up the total osmotic pressures in the compartments. The volume of the vacuole (V_v), cytoplasm (V_c), and cell (V_t) and the P were calculated as a function of time by iteration with small Δt (0.01 s) in the following order of calculations, $\Delta V_{c\to o}$ and $\Delta V_{v\to c}$, then adjustment to P, π_v, V_v, ΔV_c, π_c, and V_c. The calculations were essentially the same as that described by Wendler and Zimmermann (1985). The iteration was tested for variations in the size of the step size Δt and found to be stable for Δt less than 0.1 s.

Using the P_{os} measured by Niemietz and Tyerman (1997) for the tonoplast and plasma membrane, and converting to units of L_P, P as a function of time generated from the model was compared with P relaxation kinetics measured with the pressure probe (Fig. 4). The other parameters used in the model ($\pi = \pi_c = \pi_v$, ϵ, V, A, P [$t = 0$]) were taken from measurements made with the pressure probe on individual cells. The cytoplasm was assumed to be 2 μm in thickness.

For all six cells examined, the measured kinetics of the P relaxations were more rapid than predicted from the L_P values obtained by Niemietz and Tyerman (1997). To obtain good fits for cells before $HgCl_2$ treatment the plasma membrane L_P had to be increased by between 1.2- and 10-fold (Fig. 4; Table II). The tonoplast L_P did not have a significant effect on the kinetics except in one cell, in which it had to be increased by a factor of 3 before a reasonable fit could be obtained.

Niemietz and Tyerman (1997) found that the P_{os} of the plasma membrane was not inhibited by $HgCl_2$, but that the tonoplast-enriched fraction was significantly inhibited. Incorporating the saturation inhibition by $HgCl_2$ of the tonoplast L_P (to 30% of control) but no inhibition of the plasma membrane L_P into the model resulted in the half-time for equilibration being reduced (Fig. 4, dotted line), but not sufficiently to match the inhibition observed at 100 μM $HgCl_2$ in pressure-probe experiments on intact cells. To fit the intact cell data both the plasma membrane and tonoplast L_P had to be reduced from control values (Fig. 4; Table II). The relaxation of P was more often fitted by a double-exponential equation in the presence of 100 μM $HgCl_2$ (Table II).

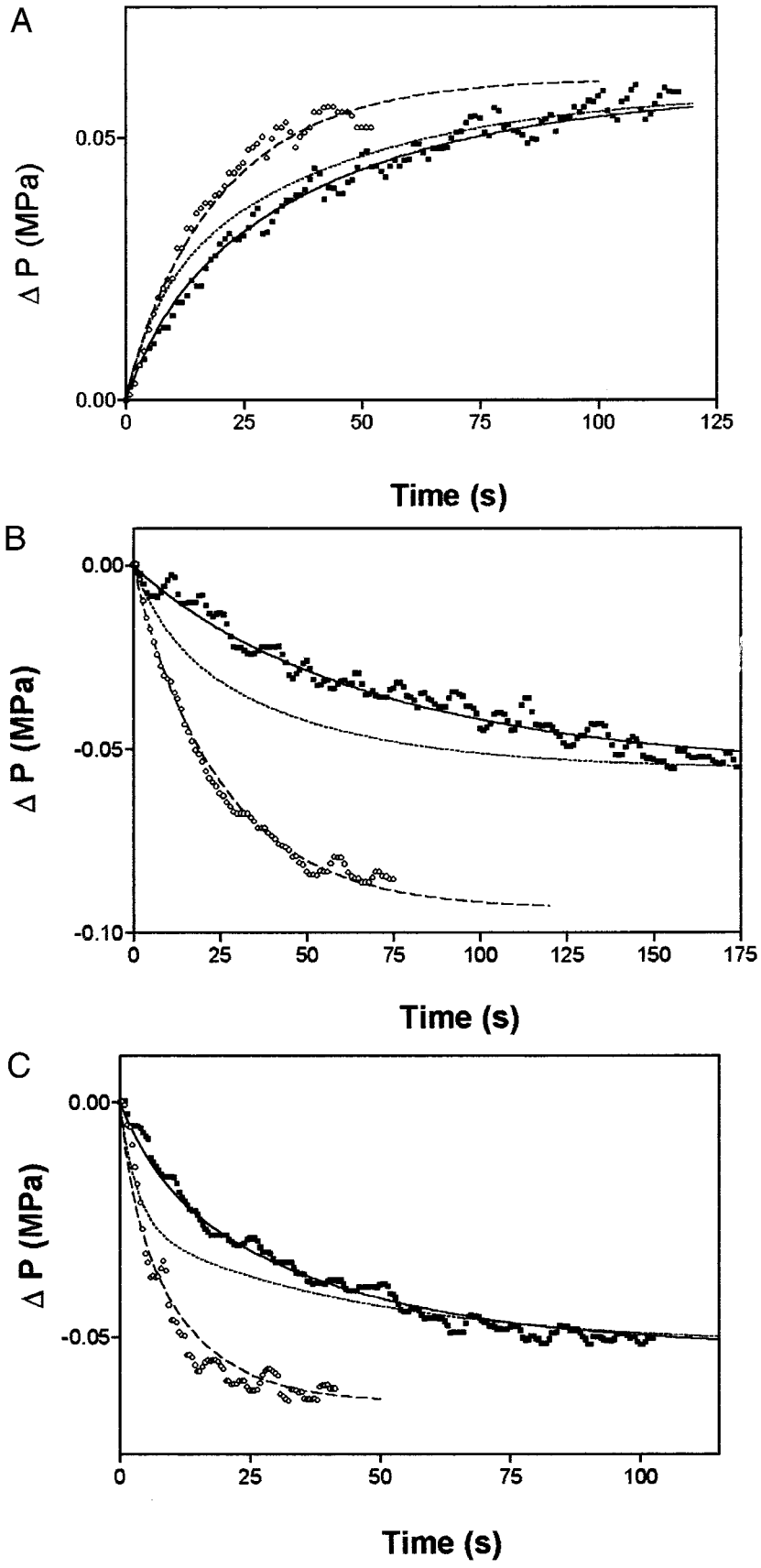

Figure 4. Examples of *P* relaxation curves for three cells before and after $HgCl_2$ treatment. A, Cell no. 1412; B, cell no. 287; and C, cell no. 58. The fitted lines were generated from the three-compartment model of Wendler and Zimmermann (1985). The cell numbers correspond to those in Table II. Open symbols, Control; closed symbols, plus $HgCl_2$; dashed lines, control fit; dotted lines, tonoplast inhibited; solid lines, plasma membrane and tonoplast inhibited.

Table II. *Tonoplast and plasma membrane L_P required to fit the pressure relaxations of individual cortical cells from wheat roots*

The three-compartment model of Wendler and Zimmerman (1985) was used, and the starting values of the tonoplast and plasma membrane L_P were set at those obtained for isolated membrane vesicles from wheat roots of similar age obtained by Niemietz and Tyerman (1997). The values presented in the table are the multiplying factors used on the Niemietz and Tyerman (1997) L_P values to obtain a good fit for each cell. L_P^t, 6.3×10^{-7} m s^{-1} MPa^{-1}; L_P^P, 9.2×10^{-8} m s^{-1} MPa^{-1}. Also given is whether the kinetics of the pressure relaxation were best fit by a single- (s) or double-exponential (d) equation. The other parameters for fitting to the model were set to the values measured with the pressure probe on the individual cells, assuming that the cytoplasm was 2 μm in thickness.

Cell No.	Control			$HgCl_2$		
	$\times L_P^t$	$\times L_P^P$	Kinetics	$\times L_P^t$	$\times L_P^P$	Kinetics
237	1	1.18	s	0.25	0.65	d
58	1	5.50	s	0.20	1.80	d
287a	1	1.35	s	0.30	0.50	s
287b	1	5.00	s	0.20	1.40	d
1412	1	2.30	s	0.30	1.60	d
297	3	10.0	s	1.50	1.40	s

DISCUSSION

We demonstrated, using a pressure probe, that $HgCl_2$ induced a rapid and significant decrease in the L_P of wheat root cortical cells. This reduction in L_P was comparable to that found in *C. corallina* internodal cells (Henzler and Steudle, 1995; Tazawa et al., 1996; Schütz and Tyerman, 1997), which was interpreted as an inhibition of the water channels. However, treatments of wheat root cells that cause general metabolic inhibition also reduce L_P to a similar extent as that caused by $HgCl_2$ treatment (Zhang and Tyerman, 1991). Furthermore, as shown in this study, there was no additional effect of $HgCl_2$ treatment on the L_P of cells already metabolically compromised by hypoxia treatment. This indicates that $HgCl_2$ could reduce L_P via general metabolic inhibition that may affect various water flow pathways, rather than by a direct block of water channels. This is further supported by the similarity between the dose response of cell V_m and L_P to $HgCl_2$.

The inhibition of L_P by $HgCl_2$ was only partly recovered when $HgCl_2$ was replaced with the reducing agent mercaptoethanol. A similar effect was observed with V_m. In contrast, for *C. corallina* internodal cells, the effect of $HgCl_2$ on L_P could be fully reversed with mercaptoethanol (Henzler and Steudle, 1995; Schütz and Tyerman, 1997). This difference could arise if $HgCl_2$ inhibition in wheat root cells were through a variety of different mechanisms, including direct blockage of water channels and metabolic inhibition. The L_P of root cells treated with 0.3 mM $HgCl_2$ increased rather than decreased, and this increase corresponded to a decrease in P, suggesting that the cell membranes become leaky in the presence of high concentrations of $HgCl_2$. This finding highlights the potential nonspecific and detrimental effect of $HgCl_2$ on the membranes of plant cells. Therefore, a low $HgCl_2$ concentration is recommended for future studies, which should also take into account the nonspecificity of $HgCl_2$ on L_P in intact plant cells.

It is possible that a substantial water flow occurs through plasmodesmata when pressure is altered in one cell within the symplast, as occurs with pressure-relaxation and pressure-clamp experiments (Murphy and Smith, 1998). The reduction of L_P of cortical cells in wheat caused by metabolic inhibition has been suggested to be due to closure of plasmodesmata (Zhang and Tyerman, 1991). However, further investigations showed an increase in the solute size able to permeate plasmodesmata with anaerobic stress (Cleland et al., 1994) and no change in the cell-to-cell electrical resistance under hypoxia (Zhang and Tyerman, 1997). Therefore, to account for the reduced cell L_P, either the water permeability of cell membranes is reduced under metabolic inhibition, or water and solutes take different pathways through plasmodesmata and metabolic inhibition reduces the L_P of the water pathway.

The overall L_P of cells measured in the pressure-probe experiments is most likely dominated by the L_P of a composite membrane consisting of the plasma membrane and plasmodesmata in parallel, and the cytoplasm and tonoplast in series (Steudle, 1989; Maurel, 1997; Murphy and Smith, 1998). It is assumed that the tip of the pressure probe is located in the vacuole, because upon stabbing the cell, sap gushes into the capillary. Also, the osmotic volume of cells measured with the pressure-clamp technique was never significantly smaller than the geometric volume (Zhang and Tyerman, 1991), a result inconsistent with the tip of the microcapillary being situated in the cytoplasm (Murphy and Smith, 1998). A reduction of overall cell L_P by $HgCl_2$ could result from a decrease in the L_P of the plasma membrane plus the plasmodesmata, the tonoplast, or both.

Recent studies using isolated membrane vesicles have shown that the L_P of the tonoplast, measured as P_{os}, is much higher than that of the plasma membrane and is dominated by water flow through channels (Maurel et al., 1997b; Niemietz and Tyerman, 1997). The tonoplast L_P, in contrast to that of the plasma membrane, is sensitive to $HgCl_2$ (Maurel et al., 1997b; Niemietz and Tyerman, 1997). It has been suggested that the higher water permeability of the tonoplast allows the vacuole to effectively buffer the cytoplasm, thereby minimizing the magnitude of short-term volume transients in the cytoplasm that might have detrimental effects on the cytoskeleton and metabolism (for modeled cell, see Tyerman et al., 1999).

Using the L_Ps for the tonoplast and plasma membrane measured by Niemietz and Tyerman (1997), we could not reconstruct the pressure relaxations observed in the present study. First, the plasma membrane L_P had to be increased significantly to fit the pressure relaxations of intact cells. Despite the tonoplast and plasma membrane L_Ps becoming more similar in magnitude, the model still indicated that water flow was dominated mostly by flow across the plasma membrane. This is indicated by the pressure relaxations being fit best by a single exponential equation, and is supported by the results of Oparka et al. (1991), who found that the $t_{1/2}$ of turgor relaxation curves is not significantly different with the pressure probe located in either the cytoplasm or in the vacuole. Second, the inhibition of L_P in the intact cells caused by $HgCl_2$ could not be entirely accounted for by the inhibition of tonoplast L_P. In all cases the plasma membrane L_P had to be reduced to fit the pressure relaxations of inhibited cells. This is in contrast to the finding of Niemietz and Tyerman (1997) that the L_P of isolated plasma membranes is not sensitive to $HgCl_2$.

A possible explanation for these results is that the plasma membrane does contain functional water channels in intact cells that are inactivated in some way by treatments that disrupt the cells or inhibit metabolism. Perhaps during the plasma membrane isolation procedures used by Maurel et al. (1997b) and Niemietz and Tyerman (1997), the water channels also become inactivated by metabolic inhibition. This would reconcile the biophysical observations of lack of water channel activity in isolated plasma membrane (Maurel et al., 1997b; Niemietz and Tyerman, 1997) with the observations that aquaporins are located in the plasma membrane (Chrispeels and Maurel, 1994) at very high densities (Johansson et al., 1996). Phosphorylation of aquaporins seems to be a likely mechanism for the regulation of water permeation (Maurel, 1997). The plasma membrane aquaporin PM28A of spinach leaf is a major phosphoprotein (Johansson et al., 1996), and its water permeability is reduced upon dephosphorylation (Johansson et al., 1998). Therefore, reduced phosphorylation of the root cell aquaporins caused by metabolic inhibition provides one possible explanation for the reduction of the plasma membrane L_P of wheat root cells under metabolic inhibition. It may also account for the observation that L_P of isolated plasma membranes is less than the L_P of plasma membranes of intact cells.

An alternative explanation is that plasmosdesmatal L_P is reduced by metabolic inhibition. This would also explain the lack of agreement between the L_P of isolated plasma membranes and the L_P of the intact composite membrane of cells in tissues (plasma membranes plus plasmodesmata) required to fit the pressure relaxations. However, as outlined above, to fit the available evidence this explanation requires that solute and water take different pathways through plasmodesmata, and it begs the question of what the aquaporins are actually doing in the plasma membrane.

A reduction in cortical cell L_P by $HgCl_2$ may have a different effect on the overall root L_P, depending upon the pathways of water flow across the root. Radial water flow within the root can in principle occur in three parallel pathways: apoplastic, symplastic via plasmodesmata, and transcellular pathways (Steudle, 1998). It is difficult to separate the symplastic from the transcellular (Murphy and Smith, 1998); therefore, the two pathways are generally considered as a cell-to-cell pathway (Steudle, 1998). If water flow is dominated by an apoplastic pathway, water flow across the root may not be controlled directly by water-channel activity and water channels may only facilitate local equilibrium of water with the apoplast in the pathway.

Since the exodermis could be a major hydraulic barrier for water flow due to the formation of suberin lamellae (Zimmermann and Steudle, 1998), it is expected that the aquaporins in the exodermal cells may be involved in regulating the root L_P. However, if water flow through the root occurs via the cell-to-cell pathway, an inhibition of water channels in the cortical cells would have a marked effect on the L_P of the whole root. In this context, an inhibition of L_P of whole roots by $HgCl_2$ has been shown in several plant species (wheat root, Carvajal et al., 1996; barley root, Tazawa et al., 1997; and tomato root, Maggio and Joly, 1995). For example, 50 μM $HgCl_2$ reduced the wheat root L_P by 66% (Carvajal et al., 1996), and the L_P of barley root was reduced by 90% in the presence of 100 μM $HgCl_2$ (Tazawa et al., 1997). It should be noted that higher concentrations of $HgCl_2$ (0.5 mM) and mercaptoethanol (60 mM) were used in the study of $HgCl_2$ effects on the L_P of tomato roots (Maggio and Joly, 1995). It is conceivable that such high $HgCl_2$ concentrations may have profound effects on root physiology in addition to the inhibition of water channels.

The inhibition of L_P of individual wheat root cortical cells by $HgCl_2$ is comparable to that found in whole wheat roots (Fig. 1; Carvajal et al., 1996) and provides an explanation for the reduction of the L_P in wheat roots by $HgCl_2$ (Carvajal et al., 1996). However, it cannot be assumed that this inhibition is caused exclusively by direct blockade of water channels; although it is likely that water channels are inhibited by $HgCl_2$, this could be an indirect effect (especially for the plasma membrane). The average L_P of the plasma membrane of root cells, which was deduced from fits to the three-compartment model of Wendler and Zimmermann (1985) in the absence of $HgCl_2$, was 3.9×10^{-7} m s^{-1} MPa^{-1} (Table II). This value corresponds to a P_{os} of 5.8×10^{-5} m s^{-1}, which is about 2 times higher than the P_d of wheat root protoplasts determined by NMR (Zhang and Jones, 1996). A P_{os}/P_d higher than unity is an indication of the involvement of water channels in water flow across the membranes (Finkelstein, 1987; Verkman, 1992).

The presence of functional water channels in root cells could be of importance in the regulation of water flow in response to environmental and developmental signals. A decrease in root L_P seems to be a general phenomenon when plants are grown under unfavorable conditions such as salinity, hypoxia (Steudle, 1998), and nutrient deficiency (e.g. N and P) (Carvajal et al., 1996). Roots of N- and P-deficient wheat plants exhibited a whole-root L_P similar to those treated with $HgCl_2$, and the root L_P of nutrient-deficient plants was no longer sensitive to $HgCl_2$ (Carvajal et al., 1996). Since nutrient deficiency may not directly

affect metabolism (Carvajal et al., 1996), mechanisms other than metabolic control are expected to be responsible for the regulation of water-channel activity.

The effect of $HgCl_2$ on the L_P of plant cells may not be a general phenomenon, as Rygol and Lüttge (1984) showed no effect of 0.1 mM $HgCl_2$ on the L_P of subepidermal cells of pepper fruits. This would indicate that the involvement of water channels in water flow through the cell membranes of plants is restricted to certain types of cells, and probably depends on physiological roles of the cells, as demonstrated in algae (Gutknecht, 1967; Wayne and Tazawa, 1990; Henzler and Steudle, 1995; Schütz and Tyerman, 1997; Tazawa et al., 1996) and animal cells (for review, see Verkman, 1992). This explanation may also account for the large variations in the L_P of plant cells so far determined by the pressure probe (Steudle, 1989).

In contrast to $HgCl_2$, the K^+-channel blocker TEA^+ showed no effect on the L_P of wheat root cells (Table I), possibly due to K^+ channels being closed during the TEA^+ treatment. However, no significant effect of TEA^+ on the L_P of cells exposed to low-O_2 treatments (Table I) seems to discount this possibility, as the V_m of the cells is depolarized to be more positive than the equilibrium potential of K^+ under the hypoxic treatments (Zhang and Tyerman, 1997), and the K^+ outward channels are likely to be activated at this depolarized V_m (Schachtman et al., 1992). The lack of effect of TEA on L_P is unlikely to result from changes in V_m and consequently the voltage-gated K^+ channels, as TEA^+ had little effect on the V_m of wheat root cells (Zhang and Tyerman, 1997). Therefore, the extent of water flow through TEA-sensitive K^+ channels, as far as can be determined from blocker studies, is probably minor.

In summary, the L_P of intact wheat root cells is sensitive to $HgCl_2$. The inhibition of L_P by $HgCl_2$ is comparable to that after hypoxia treatment and the inhibitions are not additive. $HgCl_2$ rapidly depolarized the plasma membrane V_m at a similar half-maximal concentration to that causing inhibition of L_P. These results suggest that cell metabolism may have a major effect on the activity of water channels in intact cells, which makes it difficult to attribute the effect of $HgCl_2$ as direct blockage or blockage of water channels in intact cell or organ systems.

ACKNOWLEDGMENT

We wish to thank Dr. Christa Niemietz for comments concerning the manuscript.

Received January 4, 1999; accepted April 1, 1999.

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