Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): effects of ABA and of HgCl₂

Xianchong Wan¹, Ernst Steudle¹,* and Wolfram Hartung²

¹ Lehrstuhl Pflanzenökologie, Universität Bayreuth, D-95440 Bayreuth, Germany
² Lehrstuhl Botanik I, Universität Würzburg, D-97082 Würzburg, Germany

Received 22 August 2003; Accepted 3 November 2003

Abstract

Hydraulic properties (half-time of water exchange, $T_{1/2}$, and hydraulic conductivity, $L_p$; $T_{1/2} \sim 1/L_p$) of individual cells in the cortex of young corn roots were measured using a cell pressure probe for up to 6 h to avoid variations between cells. When pulses of turgor pressure of different size were imposed, $T_{1/2}$ ($L_p$) responded differently depending on the size. Pulses of smaller than 0.1 MPa, which induced a small proportional water flow, caused no changes in $T_{1/2}$ ($L_p$). Medium-sized pulses of between 0.1 and 0.2 MPa caused an increase in $T_{1/2}$ (decrease in $L_p$) by a factor of 4 to 23. The effects caused by medium-sized pulses were reversible within 5–20 min. When larger pulses of more than 0.2 MPa were employed, changes were not reversible within 1–3 h, but could be reversed within 30 min in the presence of 500 nM of the stress hormone ABA. Cells with a short $T_{1/2}$ responded to the aquaporin blocker mercuric chloride (HgCl₂). The treatment had no effect on cells which exhibited long $T_{1/2}$ following a mechanical inhibition by the large-pulse treatment. Step decreases in pressure resulted in the same inhibition as step increases. Hence, the treatment did not cause a stretch-inhibition of water channels and was independent of the directions of both pressure changes and water flows induced by them. It is concluded that inhibition is caused by the absolute value of intensities of water flow within the channels, which increased in proportion to the size of step changes in pressure. Probable mechanisms by which the mechanical stimuli are perceived are (i) the input of kinetic energy to the channel constriction (NPA motif of aquaporin) which may cause a conformational change of the channel protein (energy-input model) or (ii) the creation of tensions at the constriction analogous to Bernoulli’s principle for macroscopic pores (cohesion–tension model). Estimated rates of water flow within the pores were a few hundred μm s⁻¹, which is too small to create sufficient tension. They were much smaller than those proposed for AQP1. Based on literature data of single-channel permeability of AQP1, a per channel energy input of 200 kBT ($k_B$=Boltzmann constant) was estimated for the energy-input model. This should be sufficient to initiate changes of protein conformation and an inactivation of channels. The data indicate different closed states which differ in the amount of distortion and the rates at which they relax back to the open state.

Key words: ABA, aquaporins, energy/input model, hydraulic conductivity, mechanical gating, root, Zea mays.

Introduction

There is increasing evidence that water channels (aquaporins) play a key role in plant water relations (Steudle and Henzler, 1995; Maurel, 1997; Kjellbom et al., 1999; Tyerman et al., 1999; Steudle, 2000, 2001; Maurel and Chrispeels, 2001; Javot and Maurel, 2002; Tyerman et al., 2002). By contrast with ion channels, the gating behaviour of aquaporins is poorly understood. This is largely due to the fact that the permeation of water across aquaporins cannot be measured at a sensitivity comparable to that for ions. There are, to date, no techniques available which are as
sensitive as patch clamp and other electrophysiological
techniques. Pressure probe techniques are the most sensitive
for aquaporin studies using intact individual cells,
although there are other techniques that use protoplasts or vesicles.
Water channel activity can be affected (gated) by
phosphorylation, pH, pCa, osmotic pressure and salinity,
heavy metals, temperature, nutrient deprivation, or anoxia
and oxidative stresses (Johannson et al., 1996; Gerbeau
et al., 2002; Steudle and Tyerman, 1983; Azaižė et al.,
1992; Birner and Steudle, 1993; Carvajal et al., 1996;
Hertel and Steudle, 1997; Wan and Zwikaye, 1999; Zhang
and Tyerman, 1991, 1999; Henzler et al., 1999; Henzler
and Steudle, 2000). For ion channels, the mecha-
nosensitivity is well established (Rees et al., 2000; Hamill
and Martinac, 2001), but there are only a few observations
that this may also occur during the transport of water
across plasma membranes (Cosgrove and Steudle, 1981;
Steudle et al., 1982). This is astonishing because turgor
pressure is thought to play a key role during osmotic
adjustment in both the older and the more recent literature
(Steudle et al., 1982; Hohmann et al., 2000). Ye et al.
(2004) proposed a cohesion/tension mechanism for the
regulation of the water permeability in Chara which is
comparable to that proposed earlier for ion channels by
Zimmerberg and Parsegian (1986). In the model, a
reversible mechanical collapse of transport protein is
caused by negative pressures in channels. A different type
of a reversible mechanical inhibition is referred to here,
which was initiated when pulses of turgor pressure or water
flow were imposed on the plasma membrane of cortical
cells of young corn roots with the aid of a pressure probe.
Upon treatment, water channel activity decreased by a
factor of 4 to 23. Most interestingly, the water-stress
hormone ABA affected the process, stimulating a switch
back to the open state of channels inhibited by the input of
mechanical energy.

Materials and methods

Plant material

Corn (Zea mays L., Helix, Kleinwanzlebener Saatzucht AG,
Einbeck, Germany) seeds were germinated on filter paper soaked in
0.5 mol m⁻³ CaSO₄ for 3 d at 25 °C in the dark. Seedlings were then
grown hydroponically in continuously aerated nutrient solution as
described by Hose et al. (2000), in a growth chamber under the
following conditions: day/night 14 h photoperiod with 300 μmol m⁻²
s⁻¹ photosynthetically active radiation at the seedling level, 22/19 °C
(day/night) temperature. Roots of 6–8-d-old seedlings (including
germination) were used in the experiments.

Pressure probe measurements and pulse application

The pressure probe technique has already been successfully
employed at the cell level of higher plants for many years, and
also improved over time (Azaižė et al., 1992; Steudle, 1993;
Henzler et al., 1999). The same version of the pressure probe was
employed as used by Henzler et al. (1999). Excised or intact roots
were mounted on a metal sledge by magnetic bars. The nutrient
solution, as used for hydroponic culture of the seedlings, ran along
the roots using a circulating system. No difference in water uptake
was found between excised and intact roots over several hours (data
not shown). Thereafter, excised roots were usually used because of
easy mounting. A cell was punctured by an oil-filled microcapillary
and a meniscus formed between cell sap and oil. The meniscus was
then pushed close to the surface of the root to restore sap volume
close to the natural cell volume. Hydrostatic pressure relaxations
were performed on cortical cells 30–40 mm from the root apex
(mature region of main roots).

Small pressure differences (∆P, peak sizes less than 0.1 MPa)
were applied at the beginning of pressure–relaxation measurements,
until a steady half-time of pressure relaxations was reached
following the puncture shocks (see Results for detail). Then medium
or large-sized peaks of pressure relaxations were used to see how the
pressure pulses affected the cell permeability by monitoring the
change in Tₑ (calculated from pressure–relaxation curves, cf.
Steudle, 1993). Measuring changes in volume (∆V) and the
 corresponding changes in pressure (∆P) was usually performed
when Tₑ values were intentionally induced to higher levels by
applying large pressure pulses (see Results for detail). Ratios of
∆P/∆V were used to calculate the volumetric elastic modulus
(ε=V/ΔP/ΔV) at a given range of turgor pressure at which ν was
constant. It holds that the hydraulic conductivity Lp is given by
Lₚ=ln(2)/(ΔTₑ/(ε+π)), where V is the cell volume, A is the cell
surface area, and π is the cell osmotic pressure. Hence, at constant ν,
Tₑ is a direct measure of the hydraulic conductivity, Lₚ.

Application of ABA and of HgCl₂

After the steady-state Tₑ (base line, short) was reached with small
pressure pulses or, alternatively, the Tₑ was induced to relatively
stable longer values by large pressure pulses (Tₑ ′ long), ABA [(+/-)
cis-trans) was added to the circulating bathing solution (pH=5.5) at
a final concentration of 500 nM. Thereafter, pressure relaxations
were performed with small pressure differences to monitor any
change in cell permeability. When Tₑ values stabilized again, several
large pressure pulses were applied. The whole experimental process
ran for 4–6 h with individual cells. A similar procedure for ABA
application was employed during HgCl₂ treatment. 50 µM
mercurial was applied to cells with either high water permeability
(base line Tₑ), or where the Tₑ had been extended by large pressure
pulses (low Lₚ).

The data were compared using a paired t-test to determine any
effects on the Tₑ (Lₚ) values before and after treatments using the
same individual cells. Means and corresponding standard deviations
(SD) were presented. All statistically significant differences were
tested at the 0.05 level.

Results

Effects of impalement by pressure probe and duration
of experiments

Root cortical cells of layers 1–8 from the surface were
randomly punctured and cell hydraulic conductivity
(inversely proportional to half-time of water exchange)
was subsequently measured to see whether or not punctur-
ing had an effect on the open/closed state of water
channels. A typical recording is shown in Fig. 1. The figure
indicates that half-times were relatively long immediately
after puncturing (Tₑ =4 s), but became much shorter after
about 6 min. A more detailed analysis of the phenomenon
using a population of 8 cells showed that there were two
different types of response. In some cells, Tₑ was originally
short ($T_1 = 0.7$ s) and then became transiently long ($T_1 = 4$ s; Cell A in Fig. 2). In others, there was an immediate response to puncturing and a slow return to short half-times (Cell B in Fig. 2). The fact that short half-times were often measured in the beginning and that they were found to be stable over long periods of time suggested that short half-times rather than long ones represent the open state of channels usually present in the cell. However, this may vary in response to external conditions.

Constant turgor ($P=0.5–0.8$ MPa) and $T_1$ ($T_2^{-1}/Lp$) could be followed in individual cortical cells over periods of up to 6 h. The base line of half-times of untreated cells was $0.39\pm0.17$ s in all 64 cells used (Table 1). Elastic moduli of cells are required to calculate cell $Lp$. They were calculated in separate experiments during periods with long half-times to avoid errors due to the rapid efflux of water from the cells, which would tend to reduce the absolute value of calculated $\varepsilon$ artificially (Steudle et al.,

---

**Fig. 1.** Typical effect of puncturing of an individual root cortical cell of maize on half-time of water exchange ($T_2$). After introducing the tip of the microcapillary of the cell pressure probe into a cell, turgor drains, followed by pushing the meniscus (between the silicon oil and the cell sap) back to close to the surface of the root, by which the turgor pressure is rebuilt. These actions usually resulted in transiently high $T_2$ values due to the puncture shock. Pressure–relaxation curves in the figure show that shorter $T_1$ values were restored after about 20 min.

**Fig. 2.** Puncture shocks (as shown in Fig. 1) resulted in somewhat variable responses in restoration. In cell A, an example of a delayed response is shown with a peak in $T_2$ after about 10 min. Immediately after puncturing, $T_1$ was rather short. In cell B, there was an immediate response without a peak. The $T_2$ was found to be already quite long initially, and then reduced to a short steady-state. Every dot represents a pressure–relaxation measurement. Cell A was measured for more than 4 h. Applied pressure differences were all small (less than ±0.1 MPa), and $T_2$ values did not significantly change in the steady state.
1980). The average value of $\varepsilon$ was 5.6±2.7 MPa ($n=64$ cells). Using values of $\varepsilon$ of individual cells at given ranges of turgor and of the osmotic pressure of cells (calculated from steady-state turgor and from osmotic pressure of the medium), half-times were used to work out hydraulic conductivity, $L_p$ (see Materials and methods). The dimensions of the cells were obtained from cross and longitudinal sections. They were 30±6 mm (diameter) and 171±36 mm (length) (mean ±SD, $n=56$ cells). Half-times were among the shortest that have been reported so far for higher plant cells. They were comparable to those given for cells in growing tissue (epicotyl of pea or hypocotyl of soybean; Cosgrove and Steudle, 1981; Steudle and Boyer, 1985) or for the root cortex of bean (Steudle and Brinckmann, 1989). Exosmotic pressure relaxations frequently produced shorter $T_1$ than endosmotic pressure relaxations. It was 0.37 when $T_1$ was 0.41±0.16 versus 0.44±0.21 s ($n=8$ cells). When cells leaked, turgor pressure irreversibly declined and $T_1$ became long (see Fig. 5). Data from 10 cells showed that the $T_1$ changed from 0.68±0.42 to 9.8±5.3 s. The difference was very significant ($P<0.001$).

To the authors’ knowledge, this is the first time that these parameters could be measured over such long time periods for plant tissue cells. Usually, the longest periods for the measurement of water relations parameters of individual cells of higher plant tissue are 1–2 h (Zhu and Steudle, 1991; Zhang and Tyerman, 1999; Hose et al., 2000; T Henzler and E Steudle, unpublished data). For giant algal cells such as the internodes of Chara much longer periods for measurements have been reported (up to 7 d; Ye et al., 2004). Long-term measurements with tissue cells are difficult and require some patience, training, and precautions so as to avoid vibrations of the tip sitting in a cell or of the root tissue which would tend to cause leakages at the seal between the tip of the probe and the cell membrane (see below). Results from long-term measurements are most valuable for studying the effects on cell $T_1$ ($L_p$). Following effects on individual cells avoids

---

**Table 1. Effects of pressure pulses on the half-time of water exchange ($T_2$ in s)**

Control measurements of the pressure probe were conducted with small pressure pulses (below ±0.1 MPa). A steady-state $T_2$ was viewed with the small pressure pulses (Fig. 2). Medium pressure pulses (±0.1–0.2 MPa) sometimes (10/28 cells) induced longer $T_2$ which could be spontaneously reversed (6/10 cells). The induced longer $T_2$ by big pressure pulses (more than ±0.2 MPa) can not be spontaneously reversed during time intervals of up to 3 h. Different letters in the row refer to significant difference at $P=0.05$ level.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control $T_2$ [s]</th>
<th>Induced $T_2$ [s]</th>
<th>Recovered $T_2$ [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small pressure (64 cells)</td>
<td>0.39±0.17</td>
<td>No change</td>
<td>–</td>
</tr>
<tr>
<td>Medium pressure (6 cells)</td>
<td>0.61±0.26 a</td>
<td>5.9±4.4 b</td>
<td>1.4±1.4 a</td>
</tr>
<tr>
<td>Large pressure (24 cells)</td>
<td>0.59±0.29 a</td>
<td>5.0±2.7 b</td>
<td>No recovery, after 1–3 h</td>
</tr>
</tbody>
</table>

---

**Fig. 3. Effect of large (peak sizes >0.2 MPa) and middle (peak sizes=0.1–0.2 MPa) pulses on half-time of water exchange of individual cells ($T_2$). Pressure pulses are referred to a relatively rapid change of cell pressure. (A) Large pressure pulses; (B) medium pressure pulses.**
variation between cells, which can be substantial (Zhu and Steudle, 1991).

Effects of pressure pulses of different size and direction

During measurements with the pressure probe, turgor pressure has to be changed to measure $T_2$ ($L_p$). However, pressure pulses could cause a change in water permeability. The magnitude of applied pressure pulses affected the half-time of water exchange of individual cell’s $T_2$. When small pressure pulses (peak size <0.1 MPa) were applied, there were no changes in $T_2$ during the time period, when half-times had already assumed short values. After several large pressure relaxations (peak sizes >0.2 MPa), $T_2$ changed from $0.59 \pm 0.29$ to $5.0 \pm 2.7$ s ($n=24$ cells, $P < 0.0001$; Table 1). Since the cell elastic modulus ($e$) remained constant, this means that hydraulic conductivity decreased by the same factor presumably by a closure of water channels. Figure 3A shows a typical response of $T_2$ to large pressure pulses. Usually, changes in $T_2$ following large pulses were irreversible. Medium-sized peaks of 0.1–0.2 MPa also frequently induced longer $T_2$ values which could be spontaneously reversed after about 5–20 min. The latter was observed thereafter with small pressure relaxations (<0.1 MPa; Fig. 3B). So, there was a difference between the treatment with middle-sized pressure pulses (size: 0.1–0.2 MPa; Fig. 3B) and large pulses. Changes were statistically significant, from 0.61±0.26 to 5.9±4.4 s ($n=6$ cells, $P=0.029$) and back to 1.4±1.4 s ($n=6$, $P=0.048$). Small pressure relaxations did not change short $T_2$ at all, even when measuring an individual cell for several hours (Fig. 2; Table 1). Cells treated with large pulses often did not return to short values even after 3 h. The findings suggest that either the treatment with pressure pulses or the water flow induced by them affected water permeability, i.e. the change in water channel activity. The reversibility of the effect depended on the size of the mechanical stimuli.

In subsequent experiments, it was tested whether or not the sign of pressure pulses (i.e. an increase or decrease of pressure) or of water flows was important. Typical results are shown in Fig. 4. They indicate that the sign of the pulses (water flow) was not important. Pressure pulses did not induce ‘rapid’ leakages around the seal between the tip of the probe and the plasma membrane. This would have led to a step decline in turgor pressure. It is important to note that, for a given cell, turgor pressure could be kept constant during the long periods of time of up to several hours. When a ‘leakage’ did occur, the observed increases in half-time were similar to those found after treatment with large pressure pulses (Fig. 5). In some cells, however, the integrity of the cell membrane was partially lost. These cells continuously lost turgor over a period of 2 h. In cells with ‘slow’ leakages, half-times increased to reach values similar to those obtained in the presence of large pulses. However, half-times never returned to the short values even after a few hours of observation. The result suggests that, in leaky cells, there was either an efflux of cell solutes because of an increase in solute permeability or a reduced rate of active pumping, but the hydraulic conductivity (water permeability) was decreased and not increased in
spite of an increase of permeability for ions (see Discussion).

Effects of ABA

At least during time intervals of 4–6 h, changes in $T_2$ induced by large pressure pulses were not reversible. However, irreversible changes of $T_2$ tended to be either completely or partially reversible by adding 500 nM ABA to the root medium (Fig. 6). The times required for a switching to short $T_2$ were somewhat variable and ranged between 5–30 min for the five cells with long $T_2$ treated with ABA. ‘Reversed cells’ repeatedly switched to longer $T_2$ again by applying large pressure pulses (Fig. 7). On the other hand, no effect of ABA was found on the $T_2$ values when they were already short. Data from three cells and more than 136 measurements showed that there was no difference in the $T_2$ values from controls without ABA ($T_2=0.34\pm0.21$ s) and from cells treated with ABA ($T_2=0.35\pm0.22$ s) ($P=0.47$). The results indicated that ABA treatment affected the state of long $T_2$ (low $Lp$) rather than that of short $T_2$ (high $Lp$). This may suggest that in the former, water channels were closed by the treatment, and were reopened in the latter.

Closure of water channels by mercury treatment ($HgCl_2$)

If the hypothesis holds true that pressure pulses cause a reversible change of water channels, cells with long half-times should not react with substances which typically cause a closure of water channels such as $HgCl_2$ (Maurel and Chrispeels, 2001; Steudle and Henzler, 1995; Tazawa et al. 1996; Schütz and Tyerman, 1997; Zhang and Tyerman, 1999; Tyerman et al., 1999). When cells exhibiting a short $T_2$ were exposed to 50 µM $HgCl_2$, half-times became long after about 5–20 min (Fig. 8A) which is comparable to the findings of Zhang and Tyerman (1999) with cortical cells of wheat. However, when long half-times had been induced by large pressure pulses, there was no effect of mercury (Fig. 8B). The result supports the idea that pressure pulses reversibly affected water channel activity.

Discussion

To the authors’ knowledge, the present work is the first rigorous protocol with a large population of cells, that indicates the role of mechanical stimuli on the open/closed state of water channels. The long-term experiments employed allow effects to be followed on individual cells, thus avoiding variation between cells. Long-term experiments with the pressure probe, however, require considerable skill and experience with the technique and the strict avoidance of any vibrations which would have caused leakages and drops in turgor pressure. Hence, there are only a few examples in the literature of long-term experiments, the duration of which were 1–2 h at most. In the present paper, the time interval for experimentation was extended to 6 h at constant cell turgor. This indicates an enormous stability and integrity of cell membranes.

The effects observed are distinct from general phenomena of membrane leakiness or changes in the transport properties of plant cell membranes when punctured with the probe. For example, when cells were leaking in response to puncturing or large pressure pulses, this should
have resulted in an increase of cell $L_p$ rather than in a decrease. The decrease in turgor observed in some experiments could either have been the result of an increase of permeability for nutrient ions or was due to a decrease of active pumping or of both. It is hard to envisage that solute transport could have affected water transport directly, as both use different transporters and are on quite different time scales. Solute leakage occurred at a scale of several hours whereas half-times of water exchange were of the order of seconds or was even more rapid.

**Effects of gating have to be considered in measurements with the pressure probe**

The results show that the absolute value of turgor pressure, pressure pulses, and puncturing affected the water permeability differently. There are different states of water permeability in which the water channel blocker HgCl$_2$ affects cell $L_p$ differently. The stress hormone blocker ABA (500 nM) caused a recovery in water channels inhibited by pressure pulses. Some of the results resemble earlier observations on pressure-dependent changes in the transport of water across cells in the growing epicotyl of pea seedlings (Cosgrove and Steudle, 1981). It is not yet clear if the effect has a general significance or just relates to cells in the pea epicotyl or cortical cells of corn roots. In any case, the finding must be considered when measuring water permeabilities (hydraulic conductivities) of plant cells using the pressure probe, particularly in the presence of large step changes in turgor pressure (large pressure relaxations).

In part, a switching between short and long half-times may explain the finding that, in corn root cells, there was some scatter in the $T_{12}$ values which ranged from less than 1 s to 10 s (Zhu and Steudle, 1991). The finding is in accordance with the recent observation that, in corn, ABA caused transient increases in root cell $L_p$ (Hose et al., 2000). In these experiments, the addition of 100 nM ABA caused a significant increase of $L_p$ by a factor of 8 to 27, which, however, was transient. Measurements on individual cells could only be followed for up to 2 h. As usual, measurements required the application of pressure pulses to measure half-times. Values of $T_{12}$ were around 3 s after puncturing, and 20–30 min later they decreased to about 0.5 s in the presence of 100 nM ABA. This is in agreement with the findings of this paper. The switch to longer half-times would be due to the pulses applied during the subsequent measurement which caused a decrease in $L_p$ (increase in $T_{12}$). Hence, the earlier results are fully understandable in the light of the present results of the long-term experiments with individual cells.

**Molecular models that explain switching**

The effects of puncturing are, perhaps, nothing more than a kind of pressure pulsing whereby pulses are quite short and, hence, allow a more or less rapid recovery. It is interesting that the hydraulic conductivity was inhibited regardless of the direction of pulses (Fig. 4). This may

---

**Fig. 8.** Effects of HgCl$_2$ on half-time of water exchange of an individual cell ($T_{12}$). 50 $\mu$M HgCl$_2$ was added to the circulating medium, when the cell was in the steady-state of short $T_{12}$ (high cell $L_p$) (A). After inducing a long $T_{12}$ through large pressure pulses, there was no significant effect of HgCl$_2$ on the cell water permeability ($T_{12}$) (B). Means ±SD are shown ($n$=6 cells). Different letters in bars refer to significant difference at $P=0.05$ level.
indicate that it is not the pressure pulse which affects the state of water channels but the intensity of water flow passing through the channels which is proportional to the change in pressure. Differences in the effects of small and large pulses suggests some kind of a threshold for water flow intensity which has to be attained before channels close. It appears that a particular disturbance in the narrow inner part of the channel occurs where flow is constricted, according to present knowledge of the structure of water channels (Murata et al., 2000; Ren et al., 2001). It could be that water flow causes a change in the channel structure which results in channel closure (see below). Following this event it takes some time for the channel conformation to relax back to the original structure or conformation. Following puncturing events or medium-sized pressure pulses, the time required for a readjustment of channels is rather short. It seems to be much longer for larger pulses. Obviously, ABA has an ameliorative effect on the readjustment of the original structure of channels. The mechanisms by which the hormone affects this are not known. There are, however, indications from the literature that ABA affects the structure of lipid bilayers (Bürner et al., 1993). It is intriguing that, when channels were closed in the presence of large pressure pulses, they did not react to HgCl₂, as they did when channels were open. The usual interpretation of the effect of mercurials is that they bind to the SH groups of cysteine residues in the transport protein which are exposed to the surface of the membrane, thus causing a reversible change in the structure of the protein. When channels are already closed, the attachment of mercury will have no further effect. Hence, the test with the channel blocker HgCl₂ is crucial, showing that ABA reacts to HgCl₂, as they did when channels were open. The mechanism: energy-input model versus Bernoulli

Since the effect depended on the absolute value of pulses or water flow intensity rather than on their direction, two possible mechanisms may be envisaged to explain the phenomenon. In one mechanism, a high rate of water flow may reduce the pressure within the channels according to Bernoulli’s principle. This may result in a mechanical distortion or even collapse (see below). Alternatively, the energy (volume work) imposed at the constriction of the water channels may cause a conformational change of the transport protein resulting in channel closure. In this second scenario, the kinetic energy of water molecules would be transferred to the NPA motif which causes the major constraint in water channels and controls its selectivity pattern. There are no data for the hydraulic conductivity of individual water channels of cortical cells of corn roots, but there is an estimate for AQP1 (CHIP28) of red blood cells for the osmotic permeability of individual water channels (pₚ) which is pₚ=5.43×10⁻¹⁴ cm³ (s-subunit)⁻¹ (purified AQP1 from human red blood cells and reconstituted in vesicles from E. coli lipids; Walz et al., 1994). Recalculating this into a hydraulic conductivity of an individual channel (lp=Vₛ/RT×pₛ; Vₛ is the molar volume of water) yields lp=4.01×10⁻²⁸ m³ H₂O (s-Pa-subunit)⁻¹ or 1.34×10⁶ water molecules (s-bar-subunit)⁻¹. This is similar to the value of 1.5×10⁶ water molecules (s-bar-subunit)⁻¹ estimated by Tyerman et al. (1999). Taking this figure and assuming a diameter of water molecules of 0.3 nm (3 Å), the speed of water within the single-file (no-pass) part of the pore would be: v=0.3×1.34×10⁶×10⁻⁹=4.0×10⁻⁴ m s⁻¹ or 400 μm s⁻¹ at a driving force of 0.1 MPa (1 bar). In view of the diffusional mobility of water, this value is reasonable. In bulk water, the self diffusion coefficient is D=2.44×10⁻⁹ m² s⁻¹ (Wang et al., 1953). Hence, a water molecule will cover a distance of √2×10⁻⁵×√24.4=9.9×10⁻⁹ m=70 μm (Fick’s second law). In a single-file pore, diffusion is restricted, but the hydraulic water permeability should be substantially larger than the diffusional, depending on the number of water molecules in the pore (Levitt, 1974). Hence, the figure of 400 μm (bar-s)⁻¹ may be accepted as reasonable.

How fast are water molecules when passing an aquaporin?

The figure of 1.34×10⁶ molecules of water passing an AQP1 water channel per second at a driving force of 0.1 MPa (1 bar) is at variance to the huge figure of 3×10⁹ water molecules per second which has been referred to AQP1 (Zeidel et al., 1992). The discrepancy is due to the fact that these authors did not (i) convert the per channel osmotic permeability coefficient of water (pₛ) into a per channel hydraulic conductivity (in m² H₂O subunit⁻¹ s⁻¹ MPa⁻¹) before calculating the rate of water molecules moving through (see above). (ii) More serious is the fact that the pₛ in cm³ (subunit s⁻¹) has to refer to unit osmolality which would be as large as 1 mol cm⁻³ in this case. This is out of any practical or physiological range. Hence, the driving force to which the figure of 3×10⁹ water molecules per second refers, is not realistic. Using an osmotic driving force of 1 mol l⁻¹ (still quite high), the figure reduces by three orders of magnitude. The mistake is evident even without any calculation. At a diameter of the water molecule of 0.3 nm, a rate of 3×10⁹ molecules s⁻¹ subunit⁻¹ would result in a speed of water through the single-file part of the pore of v=0.3×3×10⁹×10⁻⁹=0.9 m s⁻¹ which is not possible at usual differences of osmotic or hydrostatic pressure. Nevertheless, the high rate is often cited in original and review papers, for example, when the efficiency of water channels is compared with those of ion channels (Kozono et al., 2002; Engel and Stahlberg, 2002).

Bernoulli’s principle states that in a liquid which is in motion (such as water passing an aquaporin), the hydrostatic pressure should be reduced. According to Bernoulli, the sum of hydrostatic pressure (P) and of the
 energies of proteins embedded in membranes are some-
bar (10^5 Pa as applied during the pulsing experiments),
Bernoulli’s principle. At a static pressure difference of
speed of water molecules may be calculated according to
absence of frictional losses within the pore, the speed of
water molecules within the pore should be as large as 14 m s\(^{-1}\), in the absence of any friction. This
maximum rate is much larger than the figure of 400 \(\mu\)m s\(^{-1}\) estimated in the previous section. The difference is due to
the fact that most of the energy injected into the pore is
used up by friction. Hence, there should be no reasonable
reduction in pressure which could lead to a tension within
the pores. It is concluded that Bernoulli’s principle does
not play a role during the mechanical inhibition observed.

Frictional losses of kinetic energy may cause
conformational changes

Most of the energy put in during a pressure pulse would be
used up by friction within the pore (and transferred to the
protein), most likely at the NPA constriction along a
single-file distance of \(1.8\) nm (Ren et al., 2001). The per
second energy input caused by \(1.34 \times 10^6\) molecules s\(^{-1}\) at
a pressure difference of 1 bar would be
\[1.34 \times 10^6 \times 18 \times 10^{-6} \times 10^{-3} \times (6.022 \times 10^{23})^{-1} \approx 4.01 \times 10^{-18}\]
Nm s\(^{-1}\) or \(990\) k\(_B\)T s\(^{-1}\) (\(k_B=\)Boltzmann constant; 6.022 \(\times\) 10\(^{23}\)=Avogadro number). With a typical half
time, \(T_p\), of \(0.4\) s, the effective pulse length at a pressure
of 1 bar may be \(0.2\) s. This results in an energy input of \(198\)
k\(_B\)T. This energy input is quite large compared to the
thermal energy of aquaporins. It would still be quite large,
even when part of it is used by friction between water
molecules and between water and other parts of the
membrane. When concentrated around the NPA motif, the
energy input may result in changes in the conformation of
the protein and in an inhibition of water channel activity. In
aqueous solutions of proteins, typical values of activation
energies (free energies) for the transition to locally
unfavourable conformations (energy landscape theory;
Wolynes et al., 1995) of proteins during sequential
unfolding are of an order of \(9-22\) k\(_B\) T (Bieri and Kiefhaber, 2000). Even when considering that activation
ergies of proteins embedded in membranes are some-
what larger (Booth et al., 2001), energy inputs of
\(200\) k\(_B\)T should be sufficient to cause local unfolding
(gating). Depending on the energy input, this should result
in an ensemble of different conformational states of the
transport protein which may differ in the rate at which they
relax back to the native (conductive) protein. Hence, the
differences in the times required for relaxation back to the
ground state would be understandable.

The energy input would increase in proportion to
\(\Delta P(I_v)\). The above calculation demonstrates that an
energy-input model of a transfer of kinetic energy to the
aquaporin may be reasonable. Estimated rates of water
transport across different AQPs range over more than one
order of magnitude (Engel and Stahlberg, 2002). Those of
corn may differ somewhat from that used in the calculation
given here. The fact that a mechanical inhibition was not
yet observed during osmotic experiments with red cells or
vesicles containing AQP1 may be due to the fact that short
pulses of a duration of a few milliseconds have been
applied, when measuring \(P_e\), i.e. a relatively low energy
input. On the other hand, the mechanical stability of AQP1
may be rather high. Depending on the amount of the
energy transferred and on the mechanical rigidity of corn
aquaporins, conformational changes may be more or less
severe or may even be missing. Depending on the
distortion of the protein, the rate (overall activation
energy) of the switching back from a closed to the open
(native) state may differ considerably, as found. Responses
suggest that there is more than just one closed state of corn
aquaporins.

Cohesion±tension models may represent an
alternative to energy-input

Although a cohesion±tension mechanism according to
Bernoulli may be excluded during pressure pulsing, a
cohesion±tension mechanism may operate under static
conditions in the presence of osmotic solutes which are
excluded from the pores. Such solutes should induce
tensions within the pores which may result in a mechanical
collapse as demonstrated for ion channels by Zimmerberg
and Parsegian (1986). For corn root cells, external osmotic
pressures of about 5 bar (100 mM NaCl) caused a
reduction of cell \(L_p\) by a factor of 3–6 (Azaizeh et al.,
1992). However, for internodes of \textit{Chara} much higher
concentrations of up to 800 mM (20 bar of osmotic
pressure) were required to produce similar effects (Ye et
al., 2004). Most interestingly, it was not possible to reduce
\(L_p\) in \textit{Chara} by pressure pulsing, even when
employing pulses of >0.3 MPa at half-times, which were
longer than those of corn (Q Ye et al., unpublished results).
The comparison may indicate that the mechanical stability
of water channels of different species is different and that
water channels of corn roots are rather sensitive to
mechanical perturbations either applied as tensions within
the channels or by an input of kinetic energy.

The application of macroscopic models such as tensions
or Bernoulli’s principle for pipes of molecular dimensions
may be questioned. For example, Bernoulli’s equation
assumes laminar flow of a non-viscous fluid across
macroscopic pipes in the absence of turbulences, i.e.
frictional or other losses of energy are excluded. For
single-file pores, the condition of the absence of turbu-
rences should hold, but there should be considerable
frictional interactions between water and the walls of pores
as hydrogen bonds are created or severed, when the water
is dancing through the pipe (see above). In the mouth of pores, there should also be viscous interactions between water molecules, which may use up part of the energy put in. In a single-file pore, tensions should not develop as in a macroscopic pipe in the presence of high rates of water flow. Rather, forces should be directly transmitted to the protein, most likely via hydrogen bonds. However, this is what is meant when using macroscopic pictures: a mechanical stimulus is transferred together with the water moving through single-file pores, in one way or the other.

Both the energy-input and the cohesion–tension model may be tested further for corn roots. Using pressure clamp (volume relaxations) besides the pressure relaxations, the energy input may be increased in the presence of small changes of pressure, thus increasing the length of the pulse to compensate for a small $\Delta P$ at the same energy dose. Using osmolytes of different molecular size it may be worked out whether or not the cohesion–tension mechanism works for cortex cells as shown for Chara internodes (Ye et al., 2004). Combinations of measurements of this kind are possible as well, to characterize the mechanosensitivity of the water permeability of corn root cells in more detail. Step changes of pressure (short and long-term signals) may be a way for the plant to sense changes in water supply (water potential) by measuring cell turgor and adjust the water permeability of cells (roots) accordingly. Short-term changes (causing step changes in water flow) may be due to rapid changes in transpiration. Long-term changes (causing tensions within pores) could be due to changes in the water supply as the soil dries or gets more saline. Both types of stresses could result in ABA-dependent changes in water transport.

**Role of ABA**

ABA had an ameliorative effect on the readjustment of the original structure of channels. At present, there is no detailed picture of the mechanism(s) of the action of ABA, but it may be speculated that ABA binds to aquaporins thus reducing the activation energy for a change from closed states to the open. However, a binding of ABA to the aquaporin from the apoplastic side may not be too important. Binding to the water channel from outside may impede the pore and reduce the water conductivity rather than stimulate it. Hose et al. (2000) have shown that the effect of ABA only developed under acidic conditions when a high proportion of ABA is present in the lipophilic protonated form. They concluded that ABA had to penetrate the lipid bilayer before stimulating the hydraulic conductivity at the cell and root level. Using a Langmuir trough, Bürner et al. (1993) have studied the effect of surface pressure on the incorporation of ABA into phospholipid monolayers. They found an enhancement of ABA incorporation by monolayers at low surface pressures only under acid conditions. In plasma membranes of maize cortical root cells, ABA may be incorporated by the phospholipid bilayer and attached to the outer parts of the aquaporin. This may stabilize the open state of water channels, thus preventing mechanical collapses, either at short-term values of high water transport velocities when the pressure in the pore decreases or during the development of long-term tensions within the pores. ABA has been reported to alter the microheterogeneity of phospholipid bilayers (Parasassi et al., 1990). In $\text{Ca}^{2+}$ and $\text{K}^+$ channels, it has been speculated that the binding of ABA to specific receptors (as yet not identified) favours the open state of channels (Blatt, 2000). When similar things happen in the vicinity of water channels, this may alter the conformation of the protein with consequences for water permeability. In order to test these ideas further it will be important to check the specificity of the effect of (+) cis-trans ABA in relation to its isomers and to effects of pH. In earlier experiments, Hose et al. (2000) showed that the effects of ABA were quite specific.

**Conclusions**

The results indicate a new type of a mechanical gating of water channels. Rather than by step changes in turgor pressure, gating was perceived by changes in water flow across the channels, which increased in proportion to pressure changes. The absolute value rather than the direction of water flow was important. Small stimuli caused an inhibition which was spontaneously reversible. In the presence of large stimuli, inhibition was reversed in the presence of small concentrations of ABA which is known to stimulate water uptake by plant roots. Two possible mechanisms have been considered, i.e. either the transfer of kinetic energy from the water to the aquaporin during the mechanical perturbation (energy-input model), or a cohesion–tension mechanism according to Bernoulli. Evidence is presented that the energy-input model is likely to explain the effects. On longer terms such as during osmoregulation, an osmotic cohesion–tension mechanism may apply as well. The input of kinetic energy increases in proportion to the size of pulses and may cause changes in the conformation of the transport protein which is then readjusted in the presence of ABA. Details of the mechanism(s) are not yet clear but will be worked out in the future by studying the kinetics of the effects of pressure pulses of different size and duration in more detail, including osmotic and pH experiments, in the presence or absence of substances which may affect the readjustment of channel structure such as the stress hormone ABA.

**Acknowledgements**

We thank Professors David Clarkson, University of Bristol, UK, Franz X Schmid, University of Bayreuth, and Alaina Garthwaite, University of Western Australia, Nedlands, for reading and
discussing the manuscript and Burkhard Stumpf, Lehrstuhl Pflanzenökologie, Universität Bayreuth, for expert technical assistance. This research was supported by a grant from the Deutsche Forschungsgemeinschaft (Ste 319/6-1) to ES.

References


Tyerman SD, Bohnert HJ, Maurel C, Steudle E, Smith JAC. 1999. Plant aquaporins: their molecular biology, biophysics and


