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The Permeation of Ammonium through a Voltage-independent K^+ Channel in the Plasma Membrane of Rye Roots

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Abstract. Nitrogen is available to the plant in the form of NH_4^+ in the soil solution. Here it is shown that a voltage-independent K^+ channel in the plasma membrane of rye (*Secale cereale* L.) roots is permeable to NH_4^+. The channel was studied following its incorporation into planar 1-palmitoyl-2-oleoyl phosphatidyl ethanolamine bilayers. The unitary conductance of the channel was greater when assayed in the presence of 100 mM NH_4Cl than 100 mM KCl. However, the probability of finding the channel open (P_o) was lower in the presence of 100 mM NH_4Cl ($P_o = 0.63$) than in 100 mM KCl ($P_o = 0.8$), suggesting that P_o can be regulated by the (permeant) ions present in solution. When assayed in equimolar concentrations of NH_4Cl (*cis*) and KCl (*trans*), the zero-current (reversal) potential for the channel (E_{rev}) exhibited a complex concentration dependence. At low cation concentrations, the apparent permeability of NH_4^+ relative to K^+ (P_{NH4}/P_K) was greater than 1.0. However, as the cation concentration was increased, P_{NH4}/P_K initially decreased to a minimum of 0.95 at 3 mM before increasing again to a maximum of 1.89 at 300 mM. At cation concentrations above 300 mM, P_{NH4}/P_K decreased slightly. This implies that the pore of the channel can be occupied by more than one cation simultaneously. Ammonium permeation through the pore was simulated using a model which is composed of three energy barriers and two energy wells (the ion-binding sites). The model (3B2S) allowed for single-file permeation, double cation occupancy, ion-ion repulsion within the pore and surface potential effects. Results indicated that energy peaks and energy wells were situated asymmetrically within the electrical distance of the pore, that cations repel each other within the pore and that the vestibules to the pore contain negligible surface charge. The energy profile obtained for NH_4^+ is compared with ones obtained for K^+ and Na^+. This information allows the fluxes through the K^+ channel of the three major monovalent cations present in the soil solution to be predicted.

Key words: Ammonium (NH_4^+) — K^+-channel — Permeation — Planar Lipid Bilayer — Plasma membrane — Rye (*Secale cereale* L.)

Introduction

Ammonium is a major source of plant nitrogen. Knowledge of the mechanism(s) by which NH_4^+ is taken up by plant roots is therefore of basic importance in understanding the nitrogen nutrition of plants.

Unidirectional NH_4^+ influx into plant cells, measured over a period of less than 15 min using labeled ammonium ($[^{13}N]NH_4^+$ or $[^{15}N]NH_4^+$) or the ammonium analogue methylammonium ($[^{14}C]CH_3NH_3^+$), follows ''dual-isotherm'' kinetics (either approximating the sum of two Michaelis-Menten components or a Michaelis-Menten plus a linear component) as the NH_4^+ or $CH_3NH_3^+$ concentration in the extracellular medium is increased (Bertl, Felle & Bentrup, 1984; Deane-Drummond, 1986; Presland & McNaughton, 1986; Vale, Volk & Jackson, 1988; Wang et al., 1993*b*; Kosola & Bloom, 1994). This is thought to represent the sum of two transport mechanisms: (i) an ''active'' transport mechanism (Mechanism I), which operates at low NH_4^+ concentrations (<1 mM; K_m between 32 μM and 188 μM) and transports NH_4^+ into the cell against its electrochemical gradient with a capacity (V_{max}) that increases with decreasing external NH_4^+ supply and tissue NH_4^+ concentration (e.g., Wang et al., 1993*b*), and (ii) a passive, electrophoretic transport mechanism (Mechanism II), which facilitates NH_4^+ influx at higher NH_4^+ concentrations (> 1 mM).

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At present, interest is focused on the molecular identity of these NH_4^+-transport mechanisms. Recently, a gene (*AMT1*) encoding a high-affinity NH_4^+ uptake system was identified by functional complementation of a mutant yeast strain defective in NH_4^+ uptake (*Saccharomyces cerevisiae* strain 26972c) with cDNA from *Arabidopsis thaliana* (Ninnemann, Jauniaux & Frommer, 1994). Methylammonium uptake into mutants expressing AMT1 exhibited saturation kinetics with a K_m of 60 μM, was competitively inhibited by ammonium with a K_i of about 10 μM and was inhibited by protonophores (Ninnemann et al., 1994). The *AMT1* gene was highly related to an NH_4^+ transporter from yeast (Marini et al., 1994) and sequence homologies to genes of bacterial and animal origin indicate that this type of transporter is conserved over a broad range of organisms. The low affinity NH_4^+ uptake system is thought to be mediated by an ion channel.

It has often been suggested that NH_4^+ and K^+ might share the same transport mechanisms (Epstein, 1972; Marschner, 1995). This hypothesis was originally proposed on the basis of identical atomic radii and chemical similarity of K^+ and NH_4^+, and is supported by circumstantial evidence that K^+ and NH_4^+ exhibit mutual competition for transport and that the concentration-dependence of K^+ and NH_4^+ influx are similar.

Like NH_4^+ influx, K^+ influx into plant cells exhibits a biphasic response to increasing K^+ concentration (reviewed by Kochian & Lucas, 1988). Two high-affinity transport mechanisms mediating K^+ influx at the plasma membrane have been identified. Both are symporters, and K^+ influx is directly coupled to the influx of either H^+ or Na^+. Electrophysiological analysis of *Arabidopsis* root-cell protoplasts has demonstrated the presence of a H^+/K^+ symport (Maathuis & Sanders, 1994) and a gene (*HKT1*) encoding a Na^+/K^+ symport has been identified by functional complementation of a mutant yeast strain defective in K^+ uptake (*Sacharomyces cerevisiae* strain CY162) with cDNA from wheat roots (Schachtman & Schroeder, 1994; Rubio, Gassmann & Schroeder, 1995). When expressed in *Xenopus* oocytes and assayed at an extracellular cation concentration of 1 mM, HKT1 mediated NH_4^+ influx at approximately 15% the rate of K^+ influx (Schachtman & Schroeder, 1994). Thus, HKT1 may be responsible for a component of high-affinity NH_4^+ influx. Low-affinity, electrophoretic K^+ influx is likely to be mediated by an inwardly-rectifying or a voltage-independent K^+ channel (Maathuis & Sanders, 1993; Kochian & Lucas, 1993; Gassmann et al., 1993; Schroeder, Ward & Gassmann, 1994). Several inwardly-rectifying K^+ channels exhibit an appreciable permeability to NH_4^+. These include the inwardly-rectifying K^+ channel(s) in protoplasts from root hairs of wheat (Gassmann & Schroeder, 1994) and the inwardly-rectifying KAT1 K^+ channel (Schachtman et al., 1992; Bertl et al., 1995; Cao et al., 1995; Uozumi et al., 1995). It is not known whether voltage-independent K^+-channels are similarly permeable to NH_4^+.

Recently the gating kinetics and permeability of a voltage-independent K^+ channel in the plasma membrane of rye roots were documented (White & Tester, 1992; White & Lemtiri-Chlieh, 1995; White & Ridout, 1995). This channel is selective for monovalent cations and, when assayed at concentrations of 100 mM, the selectivity sequence determined from measurements of both unitary conductance and relative permeability was $Rb^+ \geqslant K^+ > Cs^+ > Na^+ > Li^+$ (White & Tester, 1992). The channel has a multiple ion residency pore, for which the spatial parameters and energy profiles for the permeation of both K^+ and Na^+ have been determined (White & Ridout, 1995). Here it is shown that NH_4^+ also permeates the channel and the energy profile for NH_4^+ permeation has been estimated. Thus, it is now possible to predict the fluxes of the three major monovalent cations present in the soil solution through this channel. Under physiological conditions, this channel might facilitate NH_4^+ influx across the plasma membrane of root cells in response to the electrical potential generated by the plasma-membrane H^+-ATPase.

Materials and Methods

PLANT MATERIAL AND PLASMA MEMBRANE ISOLATION

Rye (*Secale cereale* L. cv. Rheidol) was grown hydroponically in a complete nutrient medium containing 400 μM K^+ and 240 μM NH_4^+ as described by White (1993). Plants were harvested 14 days after sowing.

Plasma-membrane vesicles were obtained by aqueous-polymer two-phase partitioning of a microsomal fraction from rye roots. The procedure followed White & Tester (1992) except that the homogenization medium additionally included 2 mM phenylmethyl sulfonylfluoride, 4 mM dithioerythritol and 0.5% (w/v) polyvinylpyrrolidone as protectants. Plasma-membrane vesicles were stored at a concentration of 1 mg protein ml^{-1} at −20 °C in a medium containing 5 mM 2-{[2-hydroxy-1, 1-bis(hydroxymethyl)ethyl]-amino} ethanesulfonic acid (Tes), titrated to pH 7.5 using N-methyl-D-glucamine (NMDG).

ION CHANNEL RECORDINGS

Electrical recordings of ion channel activity were obtained following the incorporation of plasma-membrane vesicles into planar lipid bilayers (PLB) composed of 30 mM synthetic 1-palmitoyl-2-oleoyl phosphatidylethanolamine (PE) dispersed in n-decane, as described by White and Tester (1992). The bilayer (0.2 mm in diameter) separated solutions of 500 μl contained within a styrene copolymer cup (the *cis* chamber) and 5 ml contained within an outer Perspex container (the *trans* chamber). All aqueous solutions were filtered (pore diameter 0.2 μm) and buffered with 5 mM Tes, titrated to pH 7.5 using NMDG. Plasma-membrane vesicles were added to the *cis* chamber and fused to bilayers by stirring in the presence of a (*cis:trans*) 410:100 mM KCl or NH_4Cl gradient. When channel activity was detected, unfused vesicles were removed by perfusing the *cis* chamber with either 100 mM KCl or 100 mM NH_4Cl. Further changes in the identity or concentration of the permeant cation were effected either by perfusing a chamber with 30 volumes of the required concentration of cation chloride or by the addition of an appropriate volume of 3 M NH_4Cl or KCl. Experiments were performed at room temperature (20 to 24 °C).

Current was monitored under voltage-clamp conditions using a low noise operational amplifier with frequency compensation, connected to the bilayer chambers by calomel electrodes and 3 M KCl salt bridges. Data were stored unfiltered on Digital Audio Tape (DTC-1000ES; 44.1 kHz sampling; Sony, Japan) and simultaneously displayed on a digital storage oscilloscope (Gould 1602; Gould Electronics, Hainault, Essex, UK). Membrane potentials were recorded *cis* with respect to *trans*, which was held at ground. Since plasma-membrane ion channels become oriented with their cytoplasmic face exposed to the *trans* chamber (White & Tester, 1992), the sign of the membrane potential is opposite to that conventionally used in electrophysiological studies on plant cells in vivo. Movement of cations from the *cis* to the *trans* chamber is indicated by a positive current and appears as an upward deflection in current traces. The amplitude of single channel currents was determined directly from channel recordings filtered at 100 Hz using an 8-pole low pass Bessel filter (902LPF, Frequency Devices, Haverhill, MA).

The probability of the channel being open at any given voltage (the channel open probability; P_o) and the time constants of channel open- and closed-states at 60 mV were determined using a microcomputer-based system after appropriate digitization (Cambridge Electronic Design, Cambridge, UK). Recordings of channel activity were replayed, filtered using an 8-pole low pass Bessel filter (902LPF) and sampled by the computer. The P_o of the channel was calculated from current frequency distributions of electrical recordings filtered at 100 Hz and sampled at 500 Hz obtained over a period equivalent to recording a single channel for 25 to 40 min, as described by White and Tester (1992). Kinetic time constants were calculated as described by White and Tester (1992), using an electrical recording of an individual channel of 7.5 min duration obtained at 60 mV in symmetrical 100 mM NH_4Cl, which was filtered at 500 Hz and sampled at 2.5 kHz. To facilitate comparisons with previous studies (White & Tester, 1992) it was assumed that the channel exhibited at least two open states, with time constants τ_{o1} and τ_{o2}, and three closed states, with time constants τ_{c1}, τ_{c2} and τ_{c3}, subscripts being numbered successively from the shortest to the longest time constant. Duration frequency distributions were fitted by maximum likelihood and estimates of time constants are given as mean ± SE.

MATHEMATICAL MODELING

Estimates of energy profiles for permeant monovalent cations and channel spatial characteristics were obtained using the FORTRAN computer program AJUSTE (Alvarez, Villarroel & Eisenman, 1992) as described by White and Ridout (1995). Parameters were estimated by unweighted least squares. The model chosen (a 3B2S model) had energy profiles consisting of three energy barriers and two ion-binding sites (energy wells), and allowed for single-file permeation, double cation occupancy, ion-ion repulsion and surface potential effects. The energies of the unoccupied channel at zero voltage (in terms of multiples of the thermal energy, RT) were defined by three peaks G1, G2 and G3, and two wells, U1 and U2, with the postscript referring to their position relative to the *cis* compartment. The distances D1 to D5 refer to the position of successive peaks and wells in the electrical field relative to the *cis* compartment. The effects of ion-ion interactions (electrostatic and/or allosteric) were simulated by the addition of an energy factor to the peaks and wells adjacent to an occupied well. This varied inversely with the electrical distance (*d*) from the occupied well to mimic a coulombic interaction, and was calculated as A/d, where A is the ionic-repulsion energy parameter. Two parameters (R_{scis} and R_{strans}) were included in the model to describe surface charge effects. These parameters correspond to the radii of circles (expressed in angstrom, Å units) containing one electron charge in the *cis* and *trans* vestibules of the pore respectively, from which the fixed surface charge densities at the vestibule of the pore can be calculated ($\sigma = e/\pi R^2$). Since the PE bilayer is essentially uncharged under the experimental conditions, surface potentials are assumed to arise from charged amino acid side chains and/or nonprotein domains forming part of the molecular structure of the channel. They are calculated according to the Gouy-Chapman double-layer theory and therefore represent charged planar surfaces. The effect of applied voltage was modeled by addition of the zero-voltage energy to an electrical work term proportional to the valence of the ion and electrical distance. It was assumed that the electrical field, which is the algebraic sum of the electrical fields induced by the applied voltage and the asymmetry of the surface potential, dropped linearly through the energy profile and it was added point by point to compute the free energy of each peak and well. Rate constants for transitions between permissible states were formulated by the standard Eyring rate theory expression equal to the product of a pre-exponential term, kT/h, (where k/h is Boltzmann's constant divided by Planck's constant) and an exponential function of the energy difference, $\exp(\Delta G/RT)$. A similar expression was used for bimolecular rate constants describing the entry of ions from the internal or external solutions, except that the pre-exponential factor was also multiplied by the molar activity of the ion in solution divided by the molar concentration of water (55.5 M). Thus, the reference energy state of the model corresponds to 55.5 M solution. To compare the energy values reported here to models that use a 1 M reference state, 4.02 *RT* units must be added to the values in the Table.

RELATIVE IONIC PERMEABILITIES

Apparent relative ionic permeabilities were defined by the zero-current (reversal) potential (E_{rev}), determined by linear interpolation, when contrasting salt concentrations or ionic species were present in the *cis* and *trans* chambers, according to the conventional Goldman-Hodgkin-Katz (GHK) formulation (Hille & Schwarz, 1978). All ionic species were corrected for activities using published activity coefficients (Robinson & Stokes, 1959). Although ion activities are used exclusively for all calculations, for ease of discussion the concentrations given in the text and figures are absolute concentrations unless indicated. Data are from characteristic experiments and are representative of at least two experiments.

Results

CHANNEL-GATING KINETICS AND OPEN PROBABILITY

When assayed in the presence of symmetrical 100 mM NH_4Cl, channel gating (Fig. 1) resembled that observed in the presence of 100 mM KCl (White & Tester, 1992). However, the frequency of transitions between open- and closed-states of the channel was much greater in the presence of NH_4Cl than in the presence of KCl (226 events/min were detected in NH_4Cl compared to 35 events/min in KCl). In the presence of NH_4Cl, there was an apparent absence of brief openings (τ_{o1}) and τ_{o2} was

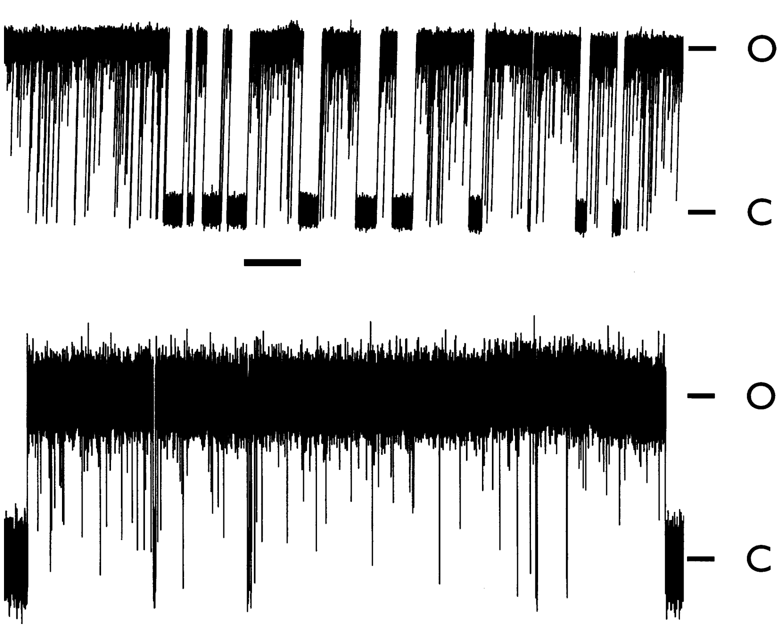

Fig. 1. Electrical recordings of a PE bilayer containing a single K$^+$ channel from the plasma membrane of rye roots assayed in symmetrical 100 mM NH_4Cl at a voltage of 60 mV. The top recording represents 6 min of data filtered at 100 Hz and illustrates primarily the long closures of the channel (τ_{c3}). The bottom recording represents 30 sec of data filtered at 500 Hz illustrating the brief closures (τ_{c1} and τ_{c2}) of the channel, which occurred during the burst of activity indicated by the bar beneath the top recording. The unitary current through the channel was 3.9 pA. *o,* channel open state; *c,* channel closed state.

shorter (420 ± 15.1 msec) than in the presence of KCl (5.7 sec; White & Tester, 1992). By contrast, the time constants of all three closed states were similar in the presence of NH_4Cl and KCl. The mean durations of the shorter closures (τ_{c1} and τ_{c2}) were 0.9 ± 0.04 ms and 30 ± 3.8 msec in the presence of NH_4Cl compared to 1.1 msec and 51 msec in the presence of KCl (White & Tester, 1992). The mean duration of the longest closures, τ_{c3}, was estimated at about 11 sec in the presence of NH_4Cl, a value similar to τ_{c3} obtained in the presence of KCl (10.6 sec; White & Tester, 1992).

When assayed in the presence of symmetrical 100 mM NH_4Cl the P_o of the channel was voltage-independent and approximated 0.63 (Fig. 2). This compares with a value of approximately 0.8 for P_o assayed in the presence of symmetrical 100 mM KCl (White & Tester, 1992) and is consistent with the longer τ_{o2} and shorter τ_{c2} observed in the presence of 100 mM KCl. These results suggest that channel gating and P_o may be regulated by the (permeant) ions present in solution, but this phenomenon was not investigated further.

Unitary Conductance

The unitary chord conductance (determined between ± 30 mV) in the presence of symmetrical 100 mM NH_4Cl was 61.3 ± 1.1 pS (mean ± SE, 15 experiments). This value is greater than that recorded in the presence of symmetrical 100 mM KCl (White & Tester, 1992; White, 1995; White & Ridout, 1995; White & Lemtiri-Chlieh, 1995). Previous estimates of unitary chord conductance in the presence of symmetrical 100 mM KCl vary between 38.5 pS and 54.4 pS, but the larger conductances obtained recently at HRI East Malling are a more appropriate comparison (*see* White & Ridout, 1995 for a discussion). When determined for individual channels in the present experiments, the ratio of unitary chord conductances in the presence of symmetrical 100 mM NH_4Cl relative to 100 mM KCl was 1.27. This value is consistent with the unitary chord conductances obtained in the presence of symmetrical 100 mM KCl in previous modeling studies at HRI East Malling (White & Ridout, 1995).

When assayed in the presence of symmetrical NH_4Cl solutions the unitary current through the channel exhibited complex relationships with voltage (Fig. 3). At low NH_4Cl concentrations (<10 mM) the unitary current was greater at negative voltages than at the corresponding positive voltages. However, at higher NH_4Cl concentrations (>10 mM) the converse was true. In addition, at low NH_4Cl concentrations the unitary current tended to saturate at extreme voltages, but at NH_4Cl concentrations above about 3 mM the unitary conductance increased with increasing voltage. The unitary chord conductance of the channel (determined between ± 30 mV) increased with increasing NH_4Cl concentrations (Fig. 3*I*). It did not follow Michaelis-Menten kinetics.

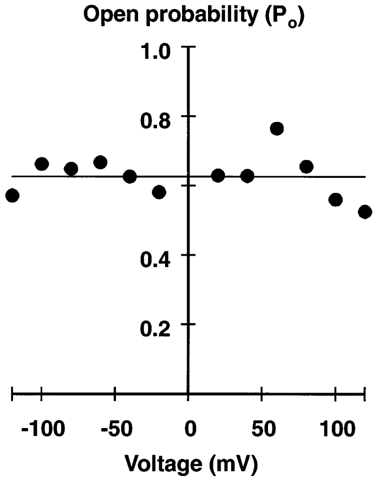

Fig. 2. The relationship between the probability of finding the K$^+$ channel from the plasma membrane of rye roots in an open state (P_o) and voltage, when channel activity was assayed in the presence of symmetrical 100 mM NH_4Cl. Data were obtained from electrical recordings of a single PE bilayer containing from 1 to 5 individual channels during a characteristic experiment performed over a period equivalent to recording a single channel for 25 to 40 min at each voltage. The line represents the mean P_o.

This has also been observed for both K$^+$ and Na$^+$ permeation of the channel (White & Ridout, 1995) and indicates that the channel has a multiple ion-residency pore structure (Hille, 1992).

RELATIVE IONIC PERMEABILITIES, P_{Cl}:P_{NH4} AND P_{NH4}:P_K

When the NH_4Cl concentration on the *trans* side of the channel was held constant whilst the NH_4Cl concentration on the *cis* side of the channel was varied between 1 mM and 1 M (Fig. 4), E_{rev} varied according to the Nernst equation (Fig. 4*I*). This indicates that the channel was ideally selective for NH_4^+ over Cl$^-$.

When assayed in equimolar concentrations of NH_4Cl (*cis*) and KCl (*trans*), E_{rev} exhibited a complex concentration-dependence (Fig. 5*I*). This phenomenon has also been observed in the presence of equimolar (*cis:trans*) NaCl:KCl (White & Ridout, 1995) and provides further evidence that the pore structure of the channel can be occupied simultaneously by more than one cation. When the cation concentration was 1 mM the apparent permeability of NH_4^+ relative to K$^+$ (P_{NH4}/P_K) was 1.25. As the cation concentration was increased, P_{NH4}/P_K initially decreased to 0.95 at 3 mM but then increased again to a maximum of 1.89 at 300 mM. At cation concentrations above 300 mM, P_{NH4}/P_K decreased slightly.

ENERGY-BARRIER MODELS FOR MONOVALENT CATION PERMEATION

The permeation of K$^+$ and Na$^+$ though the K$^+$ channel in the plasma membrane of rye roots has recently been modeled using an energy profile which is composed of three energy barriers and two ion binding sites (or energy wells), which allows for single-file permeation, double cation occupancy, ion-ion repulsion within the pore and surface potential effects (White & Ridout, 1995). This is called a 3B2S model. The 3B2S model was fitted to the data obtained under the contrasting ionic conditions employed in this paper (Table). Parameters were estimated using data either from experiments performed in the presence of NH_4Cl alone (Figs. 3 and 4) or from all experiments (Figs. 3–5). It was noted previously that at high concentrations E_{rev} in the presence of equimolar NH_4Cl (*cis*) and KCl (*trans*) deviated significantly from zero (Fig. 5*I*). This implies that the central energy peaks for K$^+$ and NH_4^+ differ (Hille & Schwarz, 1978; Hille, 1992). The difference in the central energy peaks for NH_4^+ and K$^+$ can be calculated from the P_{NH4}/P_K (1.58) obtained under bi-ionic 1 M NH_4^+ (*cis*):K$^+$ (*trans*), as described by Hille (1992). The central energy peak for NH_4^+ was 0.46 RT lower than for K$^+$ and, since the central energy peak for K$^+$ was fixed at −5.00 RT for the reasons given by White & Ridout (1995), the central energy peak for NH_4^+ was fixed at −5.46 RT.

Estimates of the energy profile for NH_4^+ permeation obtained from experiments in which NH_4^+ was the only cation present implied that the *cis* energy peak (G1) was lower than the *trans* energy peak (G3) and that the central energy peak (G2) was the lowest. The depths of both the *cis* energy well (U1) and the *trans* energy well (U2) for NH_4^+ permeation were similar, but U2 was slightly deeper than U1. The dissociation binding constants (K_d) for NH_4^+, referenced to the more conventional 1 M solution concentration, approximated 4.6 mM (U1) and 3.5 mM (U2) in an unoccupied channel at zero volts. Both the energy peaks and the energy wells for NH_4^+ permeation were situated asymmetrically within the electrical distance of the pore, as was observed previously for both K$^+$ and Na$^+$ permeation (Table; White & Ridout, 1995). Notably, the *trans* energy well was closer to the center of the pore than its *cis* counterpart. As was also observed previously, the vestibules to the pore contained negligible surface charge and cations repelled each other within the pore. A cation residing in an energy well would raise the energy of the adjacent, unoccupied well by 4.24 RT, thereby reducing the affinity for NH_4^+ binding by a factor of 69.4. This generates a characteristic relationship between unitary conductance and cation concentration (Hille & Schwarz, 1978; Hille, 1992). The predicted unitary conductance of the channel (determined between ± 30 mV; Fig. 3*I*) initially increased as the NH_4^+ activity was increased, reaching a maximum of 286 pS at (unphysiological) NH_4^+ activities between 12 and 35 M, before declining as the NH_4^+ activity was increased further.

Estimates of parameters for NH_4^+ permeation did not change when experiments in the presence of NH_4Cl

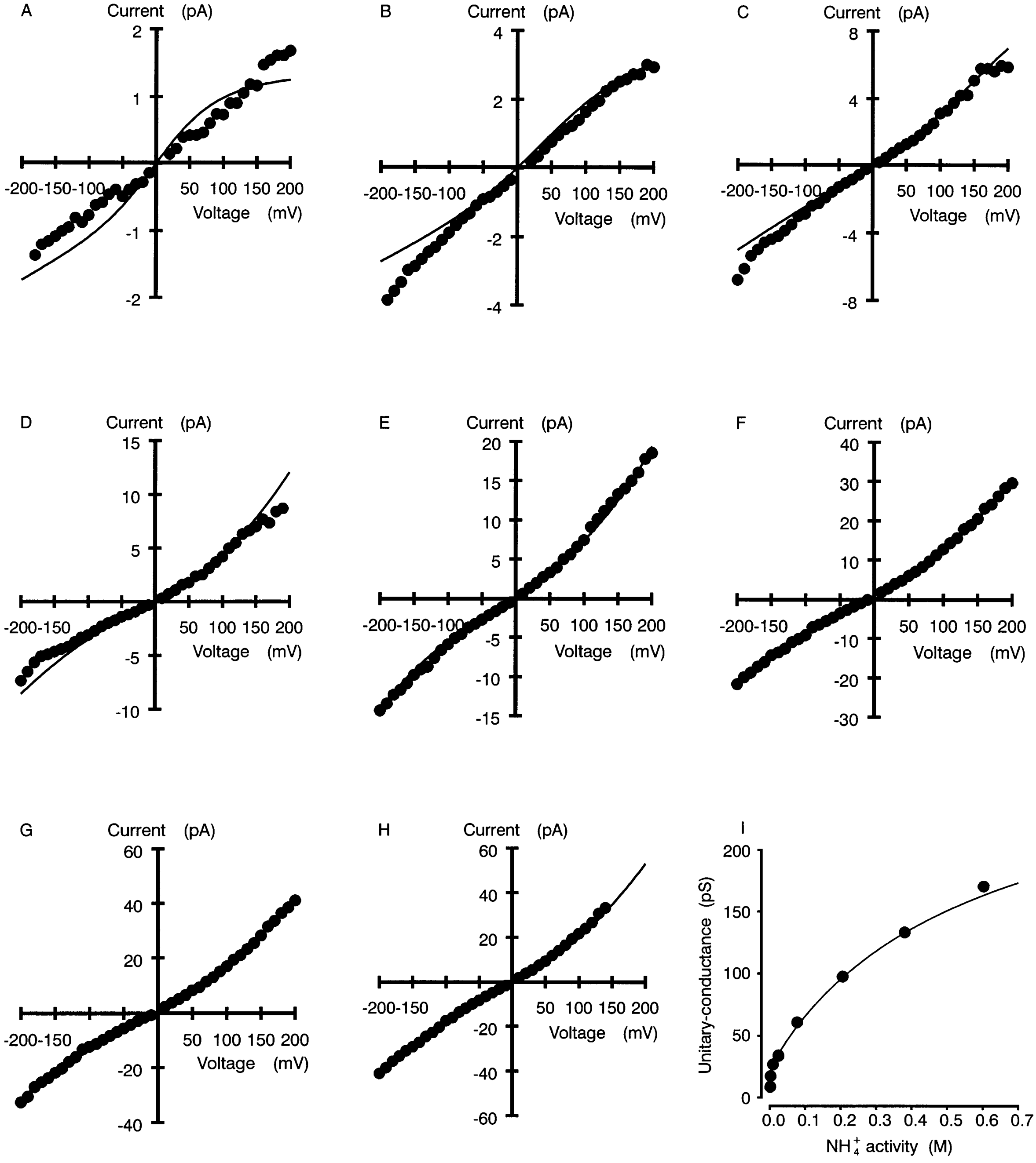

Fig. 3. (*A-H*) Unitary current vs. voltage relationships for the K$^+$ channel in the plasma membrane of rye roots incorporated into a PE bilayer. Solutions contained (*cis:trans*) (*A*) 1:1, (*B*) 3:3, (*C*) 10:10, (*D*) 30:30, (*E*) 100:100, (*F*) 300:300, (*G*) 600:600 and (*H*) 1000:1000 mM NH$_4$Cl. (*I*) The relationship between unitary chord conductance (determined between ± 30 mV) and NH$_4^+$ activity. The curves are derived from a theoretical 3B2S model using the parameters for NH$_4^+$ shown in the Table under 'multiple cationic species.'

alone were combined with experiments performed under bi-ionic equimolar NH$_4$Cl (*cis*):KCl (*trans*). Furthermore, estimates of parameters for K$^+$ permeation in the latter experiments were similar to those obtained previously (Table; White & Ridout, 1995). Comparing the energy profile for NH$_4^+$ permeation with that for K$^+$ permeation suggests that both the *cis* and *trans* energy peaks are similar for NH$_4^+$ and K$^+$, that the central energy peak is lower for NH$_4^+$ than for K$^+$, that the *cis* energy well for NH$_4^+$ is equal to or shallower than that for K$^+$ and that the *trans* energy well is deeper for NH$_4^+$ than K$^+$. Comparing the energy profile for NH$_4^+$ permeation with that for Na$^+$ permeation suggests that both the *cis* and central energy peaks are lower for NH$_4^+$ than for Na$^+$, that the *trans*

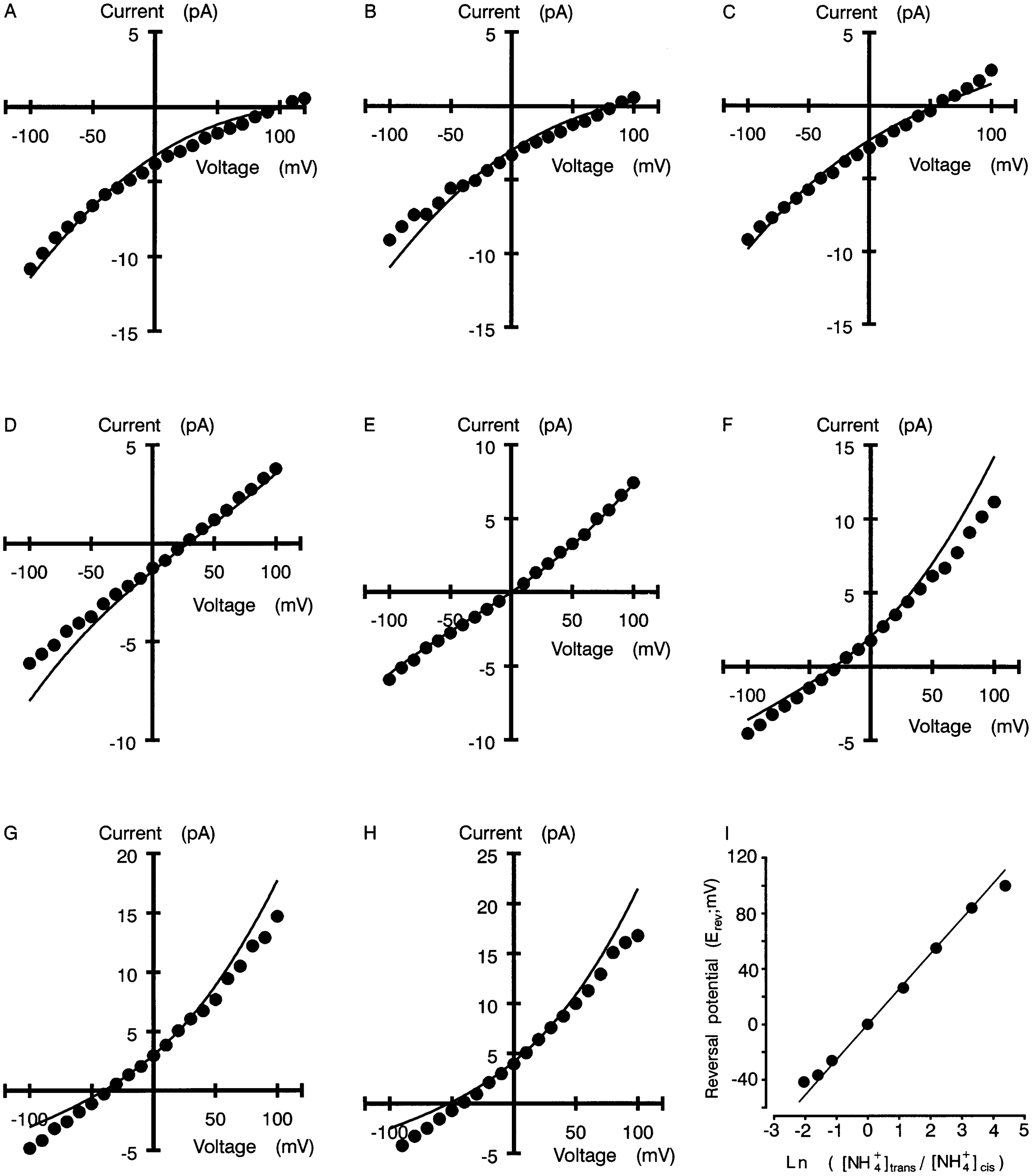

Fig. 4. (*A-H*) Unitary current vs. voltage relationships for the K$^+$ channel in the plasma membrane of rye roots incorporated into a PE bilayer. Solutions contained (*cis:trans*) (*A*) 1:100, (*B*) 3:100, (*C*) 10:100, (*D*) 30:100, (*E*) 100:100, (*F*) 364:100, (*G*) 600:100 and (*H*) 1000:100 mM NH_4Cl. The curves are derived from a theoretical 3B2S model using the parameters for NH_4^+ shown in the Table under 'multiple cationic species.' (*I*) The relationship between the measured reversal potential (E_{rev}) and the natural logarithm of the NH_4^+ gradient imposed ($[NH_4^+]_{trans}/[NH_4^+]_{cis}$, expressed in terms of activities). The line represents the Nernst potential for ideal NH_4^+ selectivity.

energy peak is similar for NH_4^+ and Na$^+$, and that both the *cis* and *trans* energy wells are deeper for NH_4^+ than for Na$^+$.

The apparent selectivity of the channel is determined by differences in the energy-barrier profiles for monovalent cations (Hille & Schwarz, 1978). Such differences account for the complex relationships between E_{rev} and monovalent cation concentration when assayed in the presence of equimolar (*cis:trans*) NaCl:KCl (*see* White & Ridout, 1995) and NH_4Cl:KCl (Fig. 5*I*). This can be

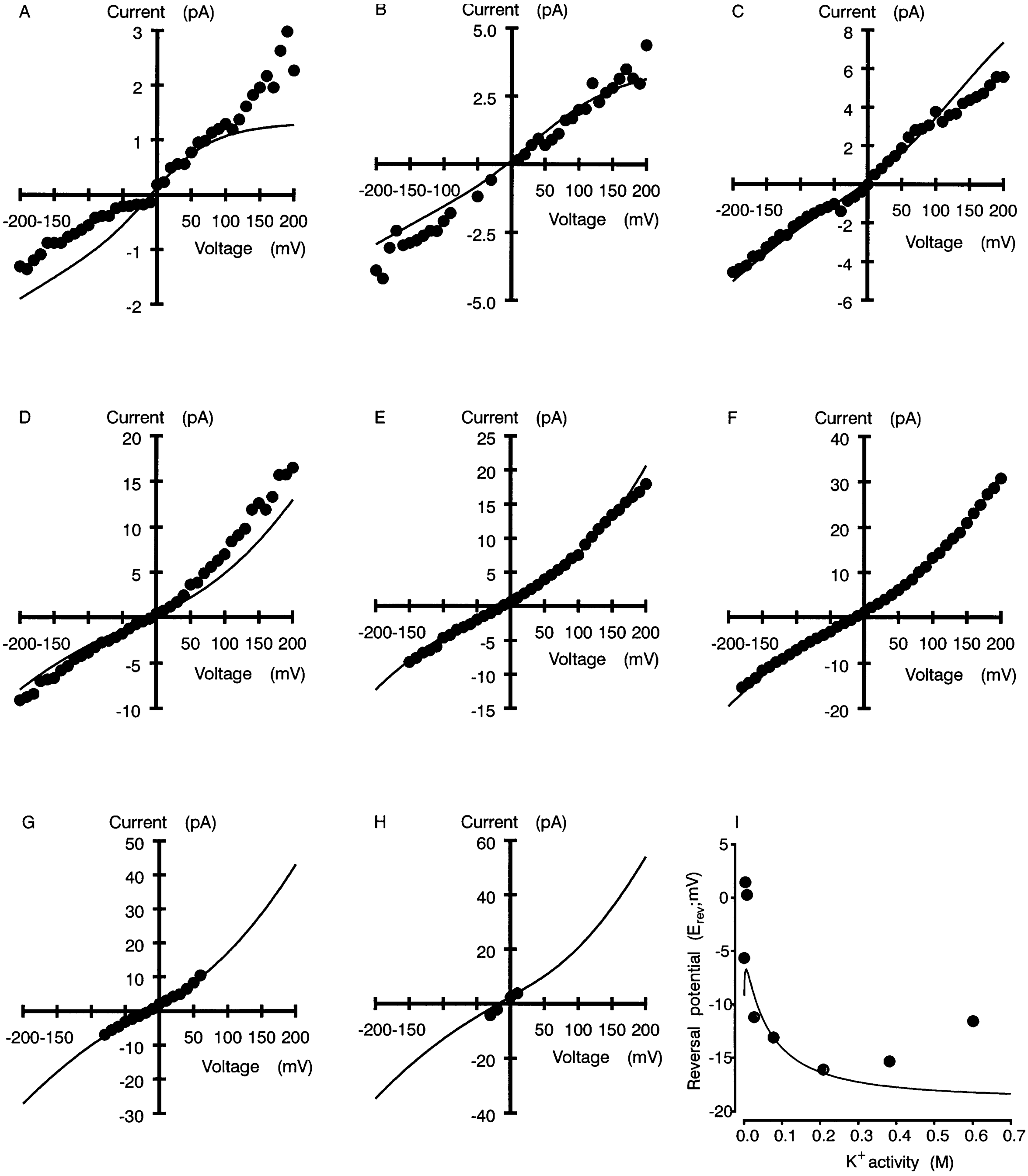

Fig. 5. (*A-H*) Unitary current vs. voltage relationships for the K$^+$ channel in the plasma membrane of rye roots incorporated into a PE bilayer. Solutions contained equimolar NH_4Cl (*cis*) and KCl (*trans*) at concentrations of (*A*) 1, (*B*) 3, (*C*) 10, (*D*) 30, (*E*) 100, (*F*) 300, (*G*) 600 and (*H*) 1,000 mM. (*I*) The relationship between reversal potential (E_{rev}) and the K$^+$ activity in the *trans* chamber. The curves are derived from a theoretical 3B2S model using the parameters shown in the Table under 'multiple cationic species' (NH_4^+ with K$^+$).

illustrated by reference to the relationship between E_{rev} and cation concentration in the presence of equimolar (*cis:trans*) NH_4Cl:KCl (Fig. 5*I*). Theoretically, at low cation concentrations P_{NH4}:P_K depends only on the heights of the highest energy peaks. Since the highest energy peaks are similar for NH_4^+ and K$^+$, P_{NH4}:P_K approximates unity under these conditions (Fig. 5*I*). As the cation concentration is increased, the heights of all energy peaks and the depths of all energy wells influence E_{rev}. In this region, differences in the depths of the *trans* energy well and the heights of the central energy peak between NH_4^+ and K$^+$ will determine E_{rev}. Thus P_{NH4}:P_K alters. Finally, at high cation concentrations, E_{rev} is determined solely by the relative heights of the central

Table. Estimated parameters for the 3B2S double ion-residency model for NH_4^+, K^+ and Na^+ permeation of the K^+ channel in the plasma membrane of rye roots

	Single species	Multiple cationic species			
	NH_4^+	NH_4^+	K^+	K^+	Na^+
G1	2.51 ± 0.08	2.69 ± 0.10	3.56 ± 0.59	1.36 ± 0.16	3.55 ± 0.09
G2	−5.46	−5.46	−5.00	−5.00	−5.00
G3	4.13 ± 0.10	4.16 ± 0.10	3.96 ± 0.24	4.42 ± 0.12	4.47 ± 0.19
U1	−9.40 ± 0.10	−9.14 ± 0.11	−9.40 ± 0.32	−11.48 ± 0.23	−7.99 ± 0.39
U2	−9.68 ± 0.15	−9.49 ± 0.14	−6.93 ± 0.49	−6.86 ± 0.15	−8.39 ± 0.29
A	1.40 ± 0.05	1.39 ± 0.05		1.85 ± 0.09	
D1	0.00				
D2	0.25				
D3	0.50				
D4	0.58 ± 0.01	0.59 ± 0.01		0.60 ± 0.02	
D5	0.92 ± 0.01	0.94 ± 0.01		0.93 ± 0.03	
R_{scis}	1,000[a]	176.9 ± 156.0		1,000[a]	
R_{strans}	43.3 ± 2.8	40.9 ± 2.5		68.6 ± 15.4	
N	477	729		264	
SUMSQ	268.4	522.4		7.5	

Parameters were estimated from unitary current *vs.* voltage relationships obtained in the presence of either a single permeant cationic species (Figs. 3 and 4 for NH_4^+) or up to two cationic species (Figs. 3,4,5 for NH_4^+ and K^+; Fig. 7 in White and Ridout (1995) for K^+ and Na^+). Standard errors are given for parameters that were free to vary. SUMSQ is the residual sum of squares and N is the number of experimental points. The solution reference state is 55.5 M. Parameters for bi-ionic K^+ and Na^+ are taken from White and Ridout (1995).

[a] Parameter estimates are on the boundary of the parameter space.

energy peak and, since this is lower for NH_4^+ than for K^+, P_{NH4}:P_K is greater than unity at extreme concentrations. Similarly, when determined in the presence of equimolar (*cis:trans*) NH_4Cl:NaCl, P_{NH4}:P_{Na} is expected to be greater than unity at low cation concentrations since the extreme energy barriers are lower for NH_4^+ than for Na^+, at medium cation concentrations P_{NH4}:P_{Na} will decrease but remain greater than unity, and at high cation concentrations P_{NH4}:P_{Na} will approximate 1.6 since it is determined by the difference in heights of the central energy peak.

Discussion

ENERGY-BARRIER MODELS FOR MONOVALENT CATION PERMEATION

A 3B2S model has been used to describe the permeation of NH_4^+, K^+ and Na^+ through the pore of a K^+ channel in the plasma membrane of rye roots. Parameters for spatial characteristics of the channel, ionic interactions within the pore and energy profiles for individual cations are sufficient to simulate the unitary current vs. voltage (*I/V*) relationships obtained under a wide variety of ionic conditions (Figs 3–5; White & Ridout, 1995). However, some *I/V* relationships observed in the present paper did not correspond exactly with the model predictions. For example, the model predicts current saturation at high voltages at cation concentrations below 3 mM, which we did not observe in equimolar (*cis:trans*) NH_4^+:K^+ (Fig. 5), and a greater curvature to *I/V* relationships obtained when contrasting NH_4Cl concentrations are present on either side of the channel (Fig. 4). In addition, the 3B2S model was unable to simulate precisely the complex relationship between E_{rev} and cation concentration when assayed in equimolar (*cis:trans*) NH_4^+:K^+ (Fig. 5*I*). In particular, it was unable to simulate the trend for E_{rev} to become less negative at cation concentrations between 300 mM and 1 M. It is possible that a more complex energy-barrier model, for example, one composed of four energy barriers and three energy wells (a 4B3S model), could provide a better fit to both *I/V* relationships and the complex relationship between E_{rev} and cation concentration when assayed in equimolar NH_4^+ (*cis*):K^+ (*trans*). However, for the most part, the simple 3B2S model simulates the data well.

Physiological Role(s) of the Channel

The physiological role of the channel studied in this paper will be determined by its tissue location, selectivity and regulation by voltage and/or chemical effectors. Since we have used whole roots for the preparation of plasma-membrane vesicles, the tissue location of the channel is unclear. However, a K^+ current with similar properties to the voltage-independent K^+ channel studied in PLB is present in protoplasts of epidermal cells from rye roots (White & Lemtiri-Chlieh, 1995). This 'instantaneous' K^+ current has been associated with the voltage-independent K^+ channel on the combined bases of: (i) its insensitivity to voltage, (ii) its low selectivity between monovalent cations and (iii) its unique pharmacology (White & Lemtiri-Chlieh, 1995).

The voltage-independent K^+ channel is relatively nonselective among monovalent cations, especially when these cations are present at high concentrations (Fig. 5*I*; White & Tester, 1992; White & Lemtiri-Chlieh, 1995; White & Ridout, 1995). In PLB, it shows neither inactivation nor voltage-dependent P_o (Fig. 2; White & Tester, 1992), although some control of P_o by (permeant) ions is indicated by differences in P_o when assayed in the presence of symmetrical 100 mM KCl ($P_o = 0.8$) and 100 mM NH_4Cl ($P_o = 0.63$). These properties allow the channel to facilitate both the influx and efflux of monovalent cations, the direction of transport being dependent upon the electrochemical gradient on a particular cation. This is determined both by the trans-plasma membrane voltage difference (E_M) and the concentration gradient of the cation. In nonsaline environments, the monovalent cation with the highest concentration in both the soil solution and the cytoplasm of the cell is K^+ (Marschner, 1995). This fact, together with the improved selectivity for K^+ against Na^+ at low cation concentrations (White & Ridout, 1995), suggest that the K^+ flux will dominate the current through this channel. For this reason, it has been suggested that the voltage-independent K^+ channel may function to stabilize E_M at a negative value close to the Nernst (equilibrium) potential for potassium (E_K; White & Lemtiri-Chlieh, 1995) in a manner analogous to its counterparts in animal cells (Hille, 1992). This function is vital if the root cell is to compensate electrically for the many electrogenic transport processes occurring at the plasma membrane, not only as roots explore the soil and take up mineral nutrients but also as they respond to stimuli triggering the influx of signaling ions such as Ca^{2+} (Tyerman & Schachtman, 1992; White & Lemtiri-Chlieh, 1995).

The channel may also have a role in the mineral nutrition of the plant since it can potentially mediate the influx and accumulation of essential monovalent cations or nitrogen in the form of NH_4^+. The annual average NH_4^+ concentration in the soil solution of an arable soil approximates 50 μM (Marschner, 1995). But the instantaneous NH_4^+ concentration in the soil solution may vary substantially, from submicromolar to millimolar concentrations, depending upon fertilizer application (Marschner, 1995). The membrane potential required for channel-mediated NH_4^+ uptake (a voltage more negative than E_{NH4}, at a given extracellular NH_4^+ concentration can be estimated if cytoplasmic NH_4^+ concentration is known. For example, assuming the ionic strength of the cytoplasm to be 200 mM E_{NH4} of −186, −130, and −83 mV can be calculated for root cells of rice plants with cytoplasmic concentrations of 3.72, 20.55 and 30.08 mM NH_4^+ grown in nutrient solutions containing 2, 100 and 1000 μM NH_4^+, respectively (Wang et al., 1993*a*). These values compare with measurements of membrane potential of −116 and −89 mV in identical root cells of rice plants grown at 2 and 100 μM NH_4^+ (Wang et al., 1994) and an estimated membrane potential of between −80 and −100 mV for plants grown at 1000 μM NH_4^+ based on the apparent relationships between NH_4^+ influx (Wang et al., 1993*b*) and membrane depolarization (Wang et al., 1994) with increasing extracellular NH_4^+. This implies that, since the apparent potentials measured by impalement electrodes may be 20 to 50 mV more positive than actual plant cell resting potentials (*for discussion consult* Gassmann, Ward & Schroeder, 1993; Gassmann & Schroeder, 1994; Schroeder et al., 1994), channel-mediated NH_4^+ uptake is feasible at extracellular NH_4^+ concentrations above about 100 μM and that the voltage-independent K^+-channel in the plasma membrane of rye root cells can mediate NH_4^+ uptake in well fertilized soils. Furthermore, under these conditions net NH_4^+ uptake can be maintained by the rapid assimilation of NH_4^+ into amides and amino acids (Lee & Ratcliffe, 1991). Similarly, the removal of other monovalent cations from the cytoplasm will enable their net uptake and accumulation. Unfortunately, the ability to accumulate a variety of monovalent cations via this channel is not without its drawbacks: the accumulation of Na^+ in saline environments or a toxic monovalent cation such as Cs^+ in a polluted soil could be harmful to the plant.

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