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Pump and K^+ Inward Rectifiers in the Plasmalemma of Wheat Root Protoplasts

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Abstract. An electrogenic pump, a slowly activating K^+ inward rectifier and an intermittent, "spiky," K^+ inward rectifier, have been identified in the plasmalemma of whole protoplasts from root cortical cells of wheat (*Triticum*) by the use of patch clamping techniques. Even with high external concentrations of K^+ of 100 mM, the pump can maintain the membrane potential difference (PD) down to −180 mV, more negative than the electrochemical equilibrium potentials of the various ions in the system. The slowly activating K^+ inward rectifier, apparent in about 23% of protoplasts, allows inward current flow when the membrane PD becomes more negative than the electrochemical equilibrium potential for K^+ by about 50 mV. The current usually consists of two exponentially rising components, the time constant of one about 10 times greater than the other. The longer time constant is voltage dependent, while the smaller time constant shows little voltage dependence. The rectifier deactivates, on return of the PD to less negative levels, with a single exponential time course, whose time constant is strongly voltage dependent. The spiky K^+ inward rectifier, present in about 68% of protoplasts, allows intermittent current, of considerable magnitude, through the plasmalemma at PDs usually more negative than about −140 mV. Patch clamp experiments on detached outside-out patches show that a possibly multi-state K^+ channel, with maximum conductance greater than 400 pS, may constitute this rectifier. The paper also considers the role of the pump and the K^+ inward rectifiers in physiological processes in the cell.

Key words: Pump — Inward rectifiers — Wheat — K^+ channels — Plasma membrane

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Introduction

The cortical cells of roots are an important component of the route of uptake of nutrients from the soil to the plant and show uptake patterns similar to intact roots (Cram, 1973). Some ions, if not the majority, on their way from the soil to the xylem, must pass through the symplasm of the cortical cells, and thus any controls on the entry of ions to the symplasm, exercised by the surrounding plasma membrane and tonoplast, will affect eventual uptake of these ions to the shoots of the plant (*see review by* Pitman, 1982). There are, of course, other points of regulation of uptake in the transport route. Fairly certain, the loading of ions from the xylem parenchyma to the xylem is a crucial step in the transport process (*see review by* Pitman, 1982), but the plasmalemma of the endodermal cells might also be a point of control of ion transport through the root (*see review by* Kochian & Lucas, 1988). Any investigations of the role of membrane channels in the transport of ions through the root require experimental access to the cellular membranes, and in particular to the plasmalemma. The extent of our characterization of the electrophysiology of ion transport systems in the plasma membrane of root cells, however, has been severely limited, until recently. This limitation exists because the standard technique of voltage clamping, necessary for investigations of membrane conductance, when applied to intact cells, is hindered by electrical coupling of the cortical cells into one large syncytium (Spanswick, 1972; Lew 1991). Electrophysiological studies on intact roots, as a consequence, have been confined to the measurement of membrane potential and membrane resistance. These studies have revealed that cortical cells of roots have electrogenic pumps and that the membrane is permeable mainly to K^+ (Reid, Dejaegere & Pitman, 1985). A high chloride permeability has also been shown at high external chloride concentrations (Cram, 1973).

Potassium uptake by passive flow through the plasmalemma of root cortical cells occurs for external K^+ concentrations greater than about 1 mM by the so-called mechanism II (Epstein & Hagen, 1952). High concentrations are required to saturate this component of uptake, and TEA^+, a K^+ channel blocker, inhibits the influx (Kochian, Xin-Zhi & Lucas, 1985). At high external K^+ concentrations, the electrochemical equilibrium potential for potassium, E_K, is usually more positive than the potential difference that the proton pump can generate, and thus an inwardly directed electrochemical gradient will exist that can produce a K^+ influx (Reid et al., 1985). There is also evidence for secondary active transport of various anions, such as nitrate, through coupling to proton influx (McClure et al., 1990; Glass, Shaff & Kochian, 1992). However, since most ion transport systems (pumps and channels) are strongly voltage dependent, ambiguity occurs in the interpretation of results when the membrane potential is allowed to vary.

Patch clamp techniques applied to protoplasts isolated from roots should enable identification and characterization of voltage-dependent ion transport in the plasma membrane. The information so gained can then be used to test hypotheses on the function of these transport systems in intact root cells. To date, two types of voltage-dependent outward rectifiers have been identified in wheat roots. Schachtman, Tyerman and Terry (1991) have described a K^+-selective outward current with slowly activating sigmoidal time course, which flows when the membrane PD is made positive of E_K. The current deactivates with an approximately exponential time course. This current is similar to that found in a number of types of plant plasma membranes such as in the trap-lobe cells of *Dionaea* (Iijima & Hagiwara, 1987), guard cells (Schroeder, 1989), suspension cells of corn root (Ketchum, Shrier & Poole, 1989), cotyledon and hypocotyl cells of *Amaranthus* (Terry, Tyerman & Findlay, 1991; Terry, Findlay & Tyerman, 1992), excitable pulvinar motor cells of *Mimosa* (Stoeckel & Takeda, 1993) and tobacco (Van Duijn, 1993). Skerrett and Tyerman (1993) have recently described a very fast activating Cl^--selective outward current in wheat root cells. This current, probably the same current as described, but not identified, by Schachtman et al. (1991), may occur separately or together with the K^+-selective outward current. One function of these two types of rectifying currents may be to keep the membrane potential within a tolerable negative range by clamping the potential to the equilibrium potential for either potassium or chloride under conditions that depolarize the cell (Walker, 1980).

In this paper, we describe patch clamp experiments both on whole cells and detached patches of the plasmalemma of protoplasts of wheat. These experiments have enabled us to characterize a further three types of membrane conductance, an electrogenic pump, a slowly activating potassium inward rectifier and an intermittent or "spiky" conductance apparent at PDs more negative than about −140 mV. The pump functions to maintain the membrane PD more negative than the electrochemical equilibrium potentials of the ions in the system, while the K^+ and spiky conductances allow the passage of inward current when the membrane is hyperpolarized. The K^+ inward rectifier is similar to that described in guard cells of *Vicia* (Schroeder & Fang, 1991; Blatt, 1992) and mesophyll cells of *Avena* (Kourie & Goldsmith, 1992). The spiky conductance does not seem to have been described previously.

Materials and Methods

Protoplasts

Protoplasts were prepared from 8- to 10-day-old roots of three varieties of wheat: Karchia (*Triticum aestivum*), Modoc (*Triticum turgidum*) and Machete (*Triticum aestivum*). Plants were grown in either perlite or vermiculite kept moist with one-half strength Hoaglands solution. After germination in the dark (3–4 days), plants were transferred to a growth cabinet (12 hr, 20°C, light/12 hr, 22°C, dark) for 5–8 days.

The technique of protoplast isolation has been described previously (Schachtman et al., 1991). Root tissue, after removal of tips, was enzymatically digested for 2 hr in 0.8% cellulase (Onozuka RS, Yakult Honsha, Tokyo) plus 0.08% pectolyase (Sigma Chemical) and for a further 2 hr in the same solution containing extra fresh 0.8% cellulase with pectolyase diluted to 0.04%. A sucrose density step gradient, as described by Schachtman et al. (1991), was then used to collect clean protoplasts. The protoplasts could be maintained in ice-cold solution for up to 4 hr before patching experiments.

In preparing protoplasts, whole segments of roots without the tips were used, and no attempt was made to separate various parts of the root tissue, largely because of the small size of the roots. While we were unable to distinguish between protoplasts from cortical cells and those from xylem parenchyma, we assumed that, because in any root the number of cortical cells is very much greater than the number in the xylem parenchyma, we were more likely to have been using protoplasts from the cortex.

The experimental chamber had a volume of approximately 0.5 ml, with its base constructed from a thin glass coverslip. A coverslip was also placed on top of the chamber to prevent evaporation. After the protoplasts added to the chamber had settled, about 5 ml of "sealing solution" (in mM: 100 KCl, 10 $CaCl_2$, 5 MES, 2–3 KOH, pH 6, with osmolality adjusted to 700 mmol/kg with sorbitol) was perfused through the chamber to remove protoplasts which had not stuck to the base of the chamber. Only phase bright, cytoplasmically streaming protoplasts, with diameters in the range 25 to 45 μm (1.96×10^{-9} m^2 to 6.36×10^{-9} m^2, assuming spherical shape), were patched. With protoplasts in the sealing solution, gigaohm seals could be obtained without much difficulty.

Electrophysiology

Patch pipettes were pulled from borosilicate glass blanks (Clark Electrochemical, Reading, UK), coated with Sylgard® (Dow Corning), and fire-polished to a tip resistance between 10 and 20 MΩ. The voltage across the patch was controlled and the current amplified by the use

of either List EPC7 or Dagan 3900A patch clamp amplifiers. The patch clamp technique (Hamill et al., 1981) was applied to whole cell and detached outside-out membrane patches. The whole cell configuration was obtained by first forming a gigaseal (resistance >5 GΩ) in the cell-attached mode, and then applying extra suction to rupture the membrane patch. The criterion for attainment of the whole cell mode was a substantial increase in capacitance. The capacitance was proportional to the surface area of the protoplast (*data not shown*). The outside-out configuration could be obtained from the whole cell configuration by separating the protoplast from the pipette.

Current and voltage data were saved directed to computer either by using a STROBES acquisition unit (Strobes Engineering, Wellington, New Zealand) that digitized the analog data, with pulse commands generated by an in-house analog pulse generator and triggered by the acquisition unit, or by using the pCLAMP suite of programs. For single channel recordings from detached outside-out patches, the amplifier output was digitized by a Sony PCM 701 pulse code modulator and recorded on video tape. For analysis, the recorded data were filtered at 1 kHz with a 6-pole Bessel filter and digitized at 6.67 kHz using Adcin (Dr. J. Pumplin, Dept. of Physics, Michigan State University). Single channel events were analyzed using the programs TRAMP (Tyerman, Terry & Findlay, 1991) and IPROC (F. Sachs, J. Neil, State University of New York at Buffalo). Further details have been given by Skerrett and Tyerman (1993).

All recordings were made at 22 to 25°C. Electrode tip potentials nulled during the patch clamp procedure (junction potentials) and those due to subsequent bath solution changes were calculated and corrected for, using the program JPCalc (P.H. Barry, University of New South Wales, Sydney, Australia).

We have used the standard convention that outward current is positive. For whole cell preparations current density (mA m^{-2}) was calculated on the basis that the protoplasts were spherical. For detached patches, the actual current flow through the patch is indicated.

Solutions

Intracellular Solutions

A variety of pipette solutions were used in the experiments. The basic solution consisted of the following (in mM): K glutamate 90, KCl 10, K_2ATP 2, $MgCl_2$ 2, HEPES[1] 10, $CaCl_2$ 2.3, EGTA[2] 10, KOH 35. The solutions were adjusted to pH 7.2 with KOH and an osmolality of 720 mmol/kg with sorbitol. The free calcium concentration, calculated with the program Buffa (Dr. R.G. Ryall, Flinders Medical Centre, South Australia), was 50 nM. Variants of this pipette solution are described, as appropriate, within the text or in the figure legends by showing those components that are different from those in the standard solution.

Extracellular Solutions

These solutions, while varying in ionic concentrations, were all adjusted to pH 6 with 5 mM MES[3] and an osmolality of 700 mmol/kg with sorbitol. The details of the solutions used are given in the text and in the figure legends, where appropriate. All of the solutions were filtered with a 0.22 μm Millipore filter before use.

[1] HEPES: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

[2] EGTA: ethyleneglycol-bis-(2-aminoethylether),*N,N,N',N'*-tetraacetic acid.

[3] MES: (2-*N*-Morpholino)ethane-sulfonic acid.

Results

Evidence for the Operation of a Proton Extrusion Pump

We usually found that immediately after the change from the cell-attached configuration to the whole cell configuration, the membrane PD was either positive or near zero, but in more than 50% of protoplasts became increasingly negative with time, and more negative than any ionic diffusion potentials.

In the steady-state, the PD showed a wide range of values from +40 to −180 mV. For a group of 39 protoplasts, the overall conductance of the membrane (calculated as the slope of the current-voltage curve at the resting PD) as a function of PD in different cells is shown in Fig. 1, and shows a decrease of about two orders of magnitude over the range of PD from +20 to −180 mV. As values of V_m became more negative than any diffusion PD, there was always increased noise in the PD (Fig. 2*a*) that we believe is due to the opening of channels of the K^+ inward rectifiers described in this paper, but *see* Discussion later.

Treatment of protoplasts in the hyperpolarized state (where the membrane PD was more negative than any of the ion diffusion potentials; *see* Fig. 1) with DCCD (*N,N'*-Dicyclohexylcarbodiimide), an inhibitor of the H^+ efflux pump, caused a depolarization of the membrane, as shown in Fig. 2*b*. However, there could be a considerable delay, up to 200 sec, for the effect of the DCCD to become apparent, and we are uncertain as to the cause of this delay. Steady-state current-voltage curves obtained with and without externally applied DCCD are shown in Fig. 3, for a protoplast in which the resting membrane PD was about +25 mV. The curve obtained with DCCD did not show inward or outward time-dependent currents, and this was probably due to low KCl concentrations outside the protoplast and in the pipette. These conditions were used to reveal more clearly the pump current. The difference curve (dashed line in the figure) is attributed to the pump, and shows a region of relatively constant current between −150 and −50 mV. Similarly shaped curves have been obtained from other protoplasts, but with variable reversal potentials and current magnitudes. The very negative reversal PD of the pump and its variable nature can probably be attributed to variation in the cytoplasmic ADP and P_i concentrations, since we supplied only ATP, through the pipette, to the interior of the protoplasts.

Figure 4*a,b* shows current-voltage curves from a protoplast in the hyperpolarized state for two levels of external K^+ concentration, 103 and 4 mM. These curves were obtained from a series of depolarizing voltage steps from holding PDs of −127 and −177 mV, re-

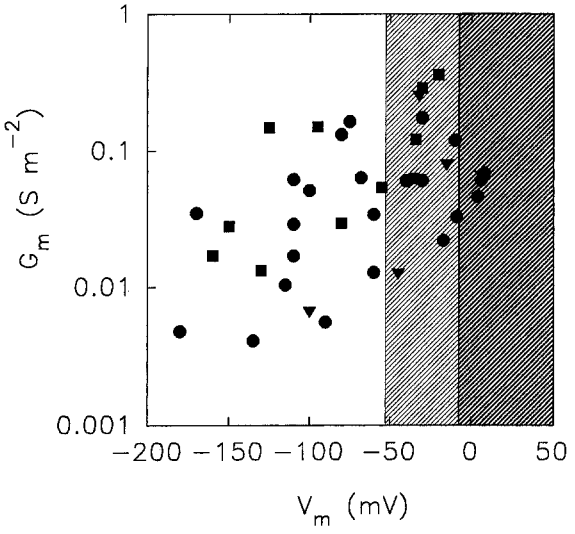


Fig. 1. The membrane conductance in whole protoplasts, measured as the slope of the current-voltage curve at the resting membrane PD (determined under zero current clamp mode), as a function of the membrane PD in different protoplasts. Data from 23 protoplasts of Kharchia (●), 11 of Machete (■) and 5 of Modoc (▼). Various bathing and pipette solutions were used. The unshaded area of the graph shows membrane PDs more negative than the Nernst potentials of the various ions present in the system. The left boundary of the lightly shaded area shows the most negative value of E_{Cl} and the left boundary of the more heavily shaded area the most negative value of E_K. Solutions: bath = (in mM) K^+ in the range 3 to 103, Cl^- in the range 3 to 120, $CaCl_2$ less than or equal to 10, MES 5, KOH 3, pH 6; pipette = (in mM) K^+ 14–39, Cl^- 14–114, K_2ATP 2, MgCl 2, HEPES 10, $CaCl_2$ 2.3, EGTA 10, pH 7.2.

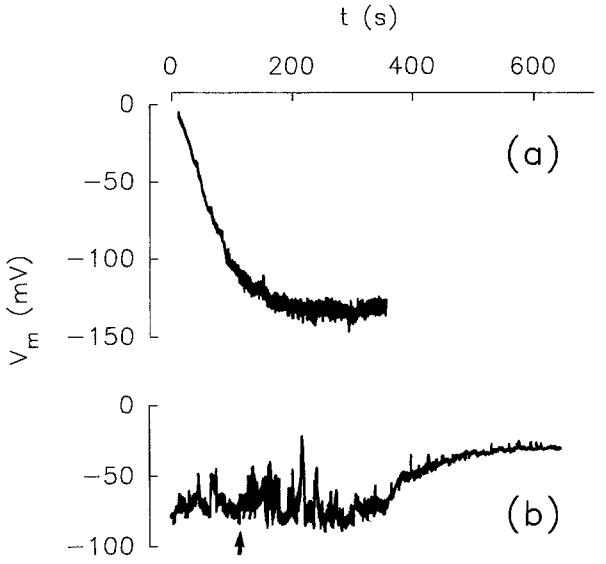


Fig. 2. (*a*) The time course of the membrane PD in a protoplast of Kharchia after the whole cell mode was attained at approximately time $t = 0$. Solutions: Ext = (in mM) KCl 100, $CaCl_2$ 1, MES 5, KOH 3, pH 6; Cyt = (in mM) K glutamate 10, remainder as for standard solution. (*b*) The effect of the addition of 100 μM DCCD to the external solution on the membrane PD of a protoplast of Machete. Note the considerable delay before the decline in the PD. Solutions: bath = (in mM) KCl 100, $CaCl_2$ 10, MES 5, KOH 3, pH 6; pipette = (in mM) K glutamate 10, remainder as for standard solution.

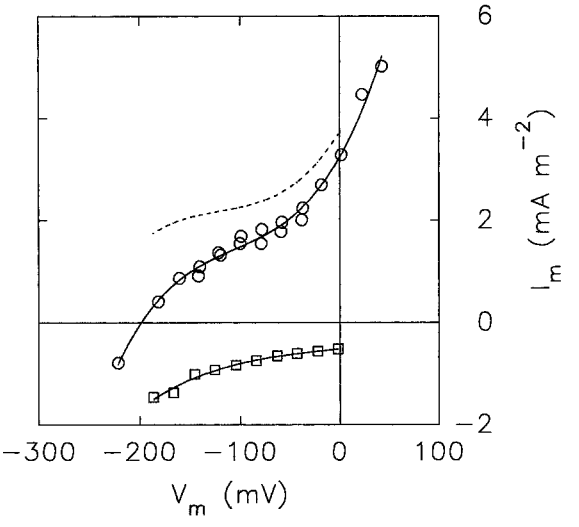


Fig. 3. *I-V* curves for a protoplast of Kharchia before (○) and after (□) the addition of 25 μM DCCD to the external solution. Before the addition of the DCCD, the membrane PD was about −200 mV. The dotted line shows the difference between the two curves and represents the *I-V* curve for the pump. Solutions: bath = (in mM) KCl 1, $CaCl_2$ 1, MES 5, KOH 3, pH 6; pipette = (in mM) K glutamate 0, sorbitol 631, TRIS 35, KOH 0, pH 7.2, remainder as for standard solution.

spectively. The instantaneous current-voltage curves shown by the open symbols and obtained at about 100 msec after the start of the voltage clamp steps are different from the steady-state curves shown by the filled symbols. During the depolarizing pulses, the current increased with time due to the deactivation of the inward K^+ rectifier at PDs negative of E_K and activation of the K^+ outward rectifier at PDs positive of E_K (Schachtman et al., 1991; Skerrett & Tyerman, 1993). This and other results indicated that there was a component of time-dependent inward K^+ current flowing at the resting PD. There is also the possibility that the activity of the pump could be a function of the external K^+ concentration.

Slowly Activating K^+ Inward Rectifier

Current Activation

In about 23% of protoplasts (*see* the Table), we have observed a smoothly activating inward current in response to negative steps in V_m. Figure 5*a–d* shows results from a whole protoplast of Machete, for four levels of external K^+ concentration. It is clear that when the external K^+ concentration is low, the inward currents are small.

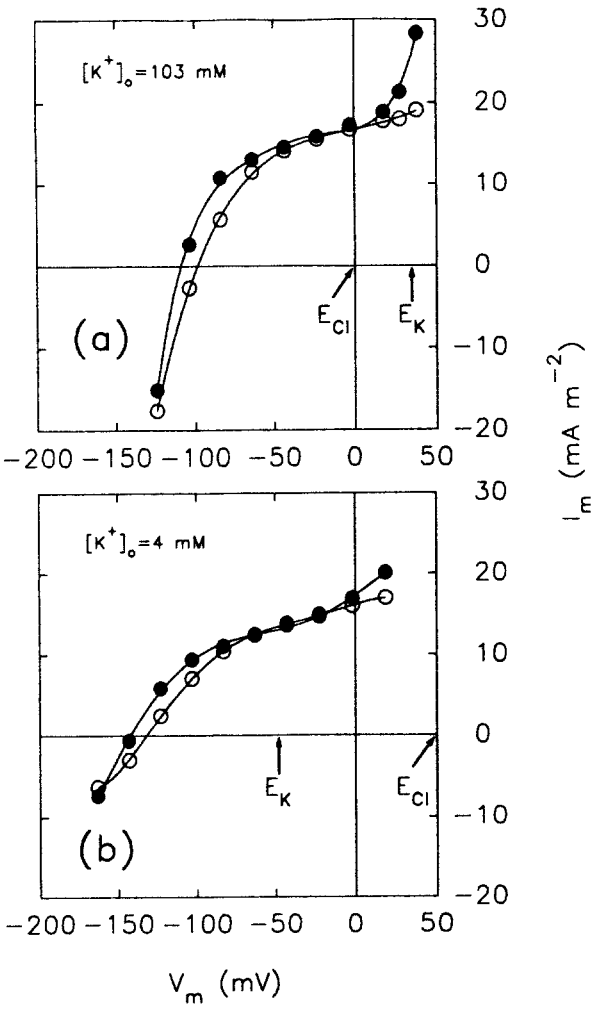


Fig. 4. *I-V* curves for a protoplast of Kharchia in which the pump was operating, for two different levels of external K^+. (*a*) 103 mM (*b*) 4 mM. The open symbols are initial currents at about 100 msec after the start of the voltage pulses and the filled symbols the steady-state currents at the end of voltage pulses of 8,000 msec duration, commencing from holding values at the most negative data points. The equilibrium potentials for K^+ and Cl^- are shown by the arrows. Note that the reversal PDs for the curves are considerably more negative than that of either E_K or E_{Cl}. Solutions: bath = (*a*) (in mM) KCl 10, K glutamate 90, $CaCl_2$ 2, KOH 3, pH 6. (*b*) (in mM) KCl 1, $CaCl_2$ 1, KOH 3, pH 6; pipette = No K glutamate, remainder as for standard solution.

Figure 5*e* shows *I-V* curves for the total steady-state current. There is inward rectification of the current and although the reversal PD for each of the curves cannot be determined accurately, its value, particularly for the lower concentrations of K^+, is reasonably close to the corresponding values of E_K, suggesting that these currents are carried predominantly by K^+. In principle, it would be better to construct current-voltage curves using the time-dependent component of the current, measured directly from the total currents or from fitted curves, as described in the next paragraph. However, such curves, of course, do not intersect the voltage axis, and estimation of E_r depends on extrapolation. A stronger confirmation that the inward rectifier is a K^+ rectifier comes from consideration of current-voltage curves obtained from the deactivation curves, as described later.

We have fitted an equation of the form

$$I_m = I_o + I_{K1}(1\text{-}\exp(t/T_1)) + I_{K2}(1\text{-}\exp(-t/T_2)), \quad (1)$$

where I_o is a constant leak and I_{K1} and I_{K2} are the maximum amplitudes of the two time-dependent components of the current, to the curves shown in Fig. 5. Figure 5*f* shows the two time constants as functions of V_m. For each K^+ concentration, the longer time constants appear to show some pattern of voltage dependence, but given the variability in the data, as discussed below, this may not have much significance, although overall, the time constant decreases as V_m becomes less negative. Except for the extreme point at about −100 mV, the shorter time constant appears to be both voltage and K^+ independent.

The noisy nature of the currents flowing during voltage clamp steps is a feature of current data from wheat protoplasts, and indeed also from other protoplasts, e.g., those of the guard cells of *Vicia* (Schroeder & Fang, 1991) and presumably arises from the relatively small number of channels in the whole protoplast membrane. In some instances, steps in the current arising from single channel openings are visible; *see* Fig. 7*d*. The fluctuations in current may well introduce considerable variability in any measures of kinetics of activation or deactivation of the time-dependent currents. In an attempt to overcome this problem, we first examined the kinetics of activation of the K^+ rectifier by taking the mean of eight to ten identical steps in PD, usually to about −150 mV, and then fitting curves to this mean current. Figure 6*a* shows currents for ten identical voltage clamp steps, and Fig. 6*b* shows the mean current, fitted by a constant leak and two exponentially rising components, from a protoplast of Kharchia. In this example, the two fitted time constants were 694 and 92 msec. While a slight improvement in the fit could have been achieved by the addition of a third exponential component, most of our data could be fitted adequately with two exponential components.

The voltage dependence of the time constants of activation were estimated for one protoplast in detail, in a similar way, by repeating a number of times a protocol of voltage clamp steps from a holding PD to progressively increasing negative PDs, and then fitting curves to the set of mean currents. Figure 6*c* shows mean currents for 10 repeats of a protocol in which a series of negative going steps were applied to the membrane PD in a protoplast of Kharchia with [K] = 103

Table. Frequency of occurrence of various types of plasmalemma conductance, either singly or in combination with others

Type	Kharchia % (*n*)	Modoc % (*n*)	Machete % (*n*)	All % (*n*)
Cl^- OR	86 (143)	95 (37)	88 (27)	86 (207)
K^+ OR	70 (96)	58 (24)	56 (23)	66 (143)
K^+ IR	25 (126)	19 (31)	15 (27)	23 (184)
K^+ Spiky IR	73 (96)	71 (34)	48 (27)	68 (157)
Cl^- OR and K^+ OR	62 (95)	57 (21)	55 (22)	60 (138)
(Cl^- and/or K^+ OR) and K^+ IR	26 (90)	17 (29)	8 (26)	18 (145)
K^+ IR and K^+ Spiky IR	10 (91)	3 (31)	4 (26)	9 (148)
K^+ OR and K^+ IR	29 (69)	25 (16)	22 (23)	19 (108)
Cl^- and K^+ OR & K^+ IR and K^+ Spiky IR	5 (62)	0 (13)	5 (22)	4 (97)

Inward rectifiers are designated IR, and outward rectifiers, OR. Data for the three varieties of wheat protoplasts separately and for all three combined, are shown as a percentage, with the sample size representing the number of protoplasts in which the particular rectifier was actually tested for in parentheses. The size of the total data sets were: Kharchia, 145; Modoc, 39; Marchette, 28.

mM. Current flow during a second identical series of pulses on the same cell at a later time is shown in Fig. 6*d*. It is clear that the conductance of the rectifier had decreased in the period between the acquisition of the two sets of data. It is possible that this decrease in the conductance could arise from activation of G proteins in the protoplast (Fairley-Grenot & Assmann, 1991). Two-component exponential curves have been fitted to the data. Figure 6*e* shows the two time constants of activation for the data shown in Fig. 6*c* and *d*, as a function of membrane PD. The slower of the time constants shows some voltage dependence, but even with this averaged data, there seems to be considerable variability. The regression line fitted to the slow component is for the equation

$$T_I = \exp(-K_I(V_m - V_0)), \quad (2)$$

where V_0 is the PD for $T_I = 1$, with $V_0 = 452$ mV, $V_{1,000}$ (PD for $T_I = 1{,}000$) $= -80$ mV and $k_I = 0.013$ mV^{-1}.

In most of the protoplasts from the three varieties of wheat, the K^+ inward rectifier could be fitted by a leak and two exponentials with the slower time constants of activation showing voltage dependence similar to that in Fig. 6*e*. Combined data from the three varieties, including the data shown in Fig. 6, gave for the long time constant component $V_{1,000} = -98 \pm 6$ (SE, $n = 7$) mV and $k_I = 0.0172 \pm 0.007$ (SE, $n = 7$) mV^{-1}, and for the short time constant $T = 138 \pm 7$ (SE, $n = 7$) msec.

Deactivation of the Slowly Activating Inward Rectifier

Figure 7*a–d* shows data from an experiment in a whole protoplast of Machete (which also yielded the data in Fig. 5), in which V_m was stepped negatively for 6 sec to activate the inward rectifier, and then stepped back to a series of less negative values. This protocol was repeated for a range of external K^+ concentrations. These deactivation curves were best fitted with a single decaying exponential and a leak, of the form $I_m = I_0 + I_{K1}\exp(-t/T_{K1})$ and such fits to the data are shown in the figure. The time-dependent inward current is almost absent when $[K]_o = 4$ mM, Fig. 7*d*, but the data are interesting in that they show individual channel openings in the outward currents, most likely of the K^+ outward rectifier (Schachtman et al., 1991). Current-voltage curves of the time-dependent current, I_{K1}, determined from the fitted curves, are shown in Fig. 7*e*. These curves also indicate that the current is carried predominantly by K^+, although in this protoplast, as in some others, V_r, the reversal PD, for the external K^+ concentration of 100 mM, is more negative than the corresponding E_K. Given the location of E_{Cl} (-51 mV), negative to E_r for $[K]_o = 103$ mM, a likely explanation is that when the PD is stepped in the positive direction after the activating pulse, an outward Cl^- current rapidly activates, with the effect that the current-voltage curve is raised upwards, with the consequent displacement in E_r (Skerrett & Tyerman, 1993).

The time constant of decay of the time-dependent current was clearly voltage dependent and decreased markedly as V_m was stepped in the positive direction. The time constants of decay, as a function of V_m, for the four levels of $[K]_o$, are plotted on one graph in Fig. 7*f*. While three of the curves show a possible peak, the overall result appears to be that the relationship between T and V_m is little affected by external K^+ concentration, and a regression line has been fitted to the combined data. This regression line is for the equation

$$T = \exp(-k(V_m - V_0)), \quad (3)$$

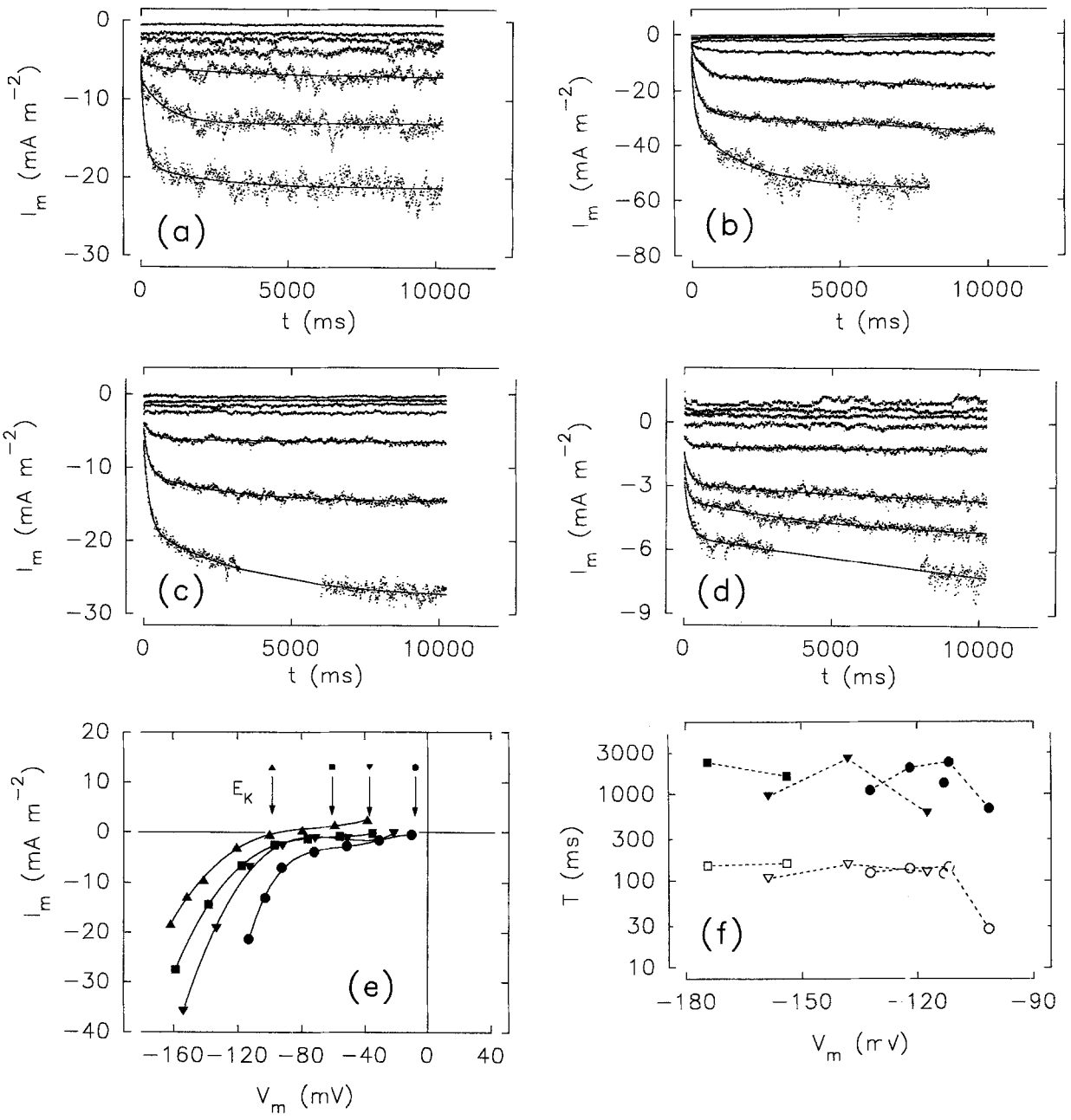


Fig. 5. Activation of the K^+ inward rectifier. Data from a whole protoplast of Machete. Membrane currents flowing as a result of a series of equally spaced negative-going voltage clamped steps in the membrane PD from a holding PD (V_h) to the maximum level (V_{max}), for four levels of external K^+ concentration. Gaps in the data indicate the time of occurrence of spiky current. These currents are not included in the record. In the following, the PDs are shown in the order V_h, and V_{max}. (*a*) 103 mM, −10, −113 mV. (*b*) 33 mM, −22, −174 mV. (*c*) 13 mM, −35, −181 mV. (*d*) 4 mM, −38, −162 mV. The data have been fitted with an equation of the form $I_m = I_0 + I_{K1}(1\text{-exp}(-t/T_{K1})) + I_{K2}(1\text{-exp}(-t/T_{K2}))$. (*e*) Current-voltage curves for the total time-dependent steady-state currents, i.e., $I_{K1} + I_{K2}$, calculated from the fitted curves: (▲) [K] = 4 mM, (■) [K] = 13 mM, (▼) [K] = 33 mM, (●) [K] = 103 mM. The K^+ equilibrium potentials are indicated by the arrows. (*f*) Voltage dependence of the time constants of activation of the inward rectifier as a function of external K^+ concentration; filled symbols show short time constants. Solutions: bath = (in mM) $CaCl_2$ 10, MES 5, KOH 3, pH 6; with KCl 100, 30, 10, 1; pipette = standard.

where V_0 is the PD for $T = 1$, with $V_0 = 75$ mV, V_{100} (PD at which $T = 100$) = −97 mV and $k = 0.027$ mV^{-1}.

We have fitted single exponential decay curves to other data from the three varieties of wheat and the results from the combined data were, for $[K]_o$ = 103 mM, $V_{100} = 83 \pm 14$ (SE, $n = 5$) mV and $k = 0.016 \pm 0.002$ (SE, $n = 5$) mV^{-1}, and for $[K]_o$ = 1 mM, $V_{100} = -87 \pm 18$ (SE, $n = 4$) mV and $k = 0.011 \pm 0.0009$ (SE, $n = 4$). In some protoplasts, particularly in Kharchia, the de-

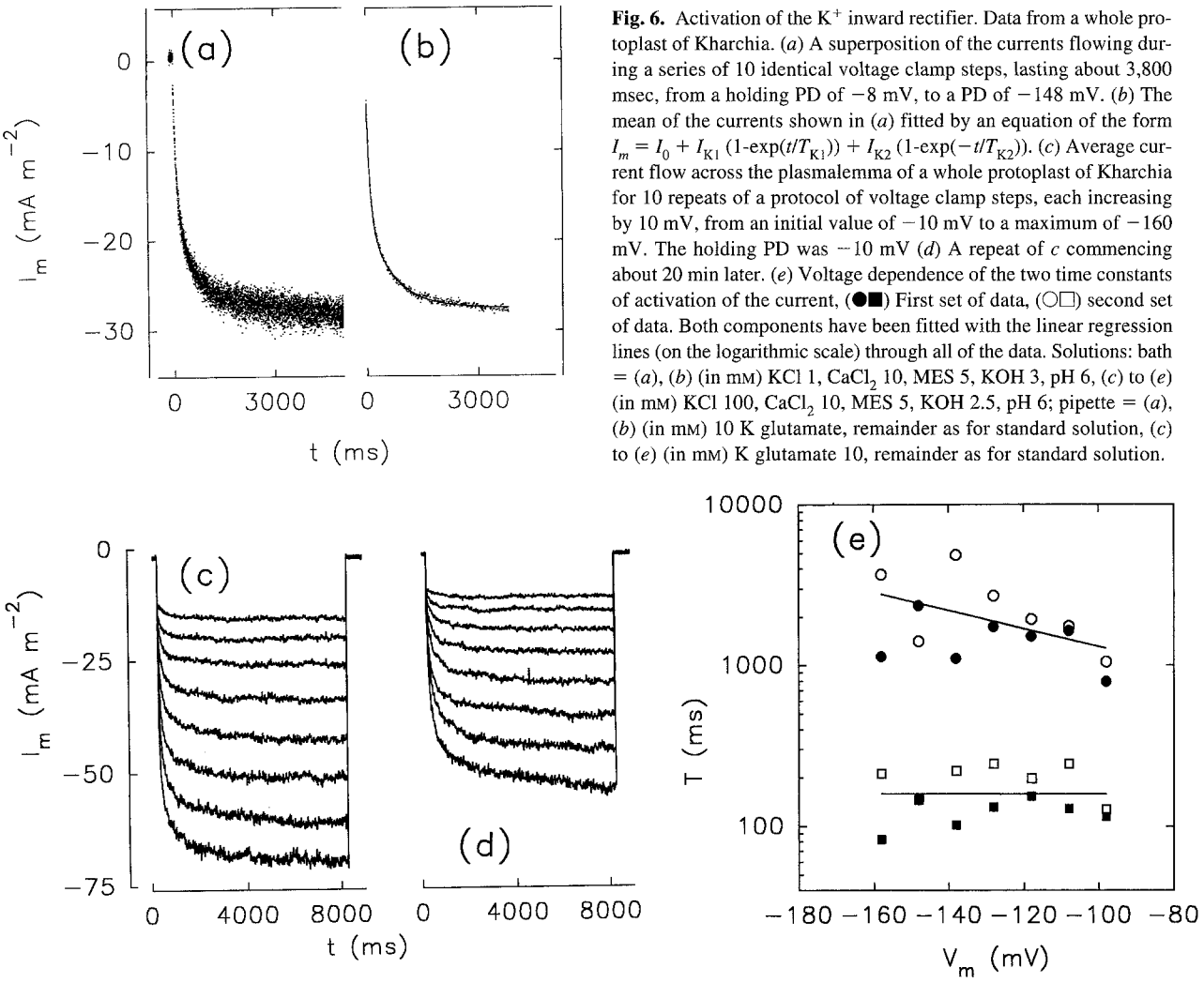


Fig. 6. Activation of the K^+ inward rectifier. Data from a whole protoplast of Kharchia. (*a*) A superposition of the currents flowing during a series of 10 identical voltage clamp steps, lasting about 3,800 msec, from a holding PD of −8 mV, to a PD of −148 mV. (*b*) The mean of the currents shown in (*a*) fitted by an equation of the form $I_m = I_0 + I_{K1}(1\text{-exp}(t/T_{K1})) + I_{K2}(1\text{-exp}(-t/T_{K2}))$. (*c*) Average current flow across the plasmalemma of a whole protoplast of Kharchia for 10 repeats of a protocol of voltage clamp steps, each increasing by 10 mV, from an initial value of −10 mV to a maximum of −160 mV. The holding PD was −10 mV (*d*) A repeat of *c* commencing about 20 min later. (*e*) Voltage dependence of the two time constants of activation of the current, (●■) First set of data, (○□) second set of data. Both components have been fitted with the linear regression lines (on the logarithmic scale) through all of the data. Solutions: bath = (*a*), (*b*) (in mM) KCl 1, $CaCl_2$ 10, MES 5, KOH 3, pH 6, (*c*) to (*e*) (in mM) KCl 100, $CaCl_2$ 10, MES 5, KOH 2.5, pH 6; pipette = (*a*), (*b*) (in mM) 10 K glutamate, remainder as for standard solution, (*c*) to (*e*) (in mM) K glutamate 10, remainder as for standard solution.

activation of the K^+ inward rectifier showed a small extra exponential component, with a long time constant, up to 20 sec.

Spiky K^+ Inward Rectifier

Whole Cell Data

In many protoplasts (68%), there was a rather noisy or "spiky" inward current first apparent at PDs of about −140 mV, but occasionally as low as −100 mV, and becoming greater in magnitude and frequency of occurrence as the PD becomes more negative; (Fig. 8*a*). In the protoplast from which these data were obtained, the membrane PD was −180 mV, measured under zero current clamp, but had superimposed on it transient depolarizations, as shown in Fig. 8*b*. These changes in PD most probably arise as a result of the conductance changes that produce the spiky currents when the membrane PD is held constant during a voltage clamp.

There is some evidence that the threshold level of PD at which the spiky current appears is a function of the external KCl concentration. In one protoplast, with 1 mM K^+, there were no spikes when the PD was set at −200 mV, but at the membrane PD of −225 mV, as measured under current clamp, spiky current was present. For 10 mM K^+, spikes occurred at a PD of −180 mV, and were also present at the free running membrane PD of −180 to −200 mV. For 100 mM K^+, spikes occurred at −140 mV, and the free running PD was −160 mV.

Single Ion Channels Constituting the Spiky Inward Rectifier

In several outside-out detached patches, we have recorded what appears to be activity of channels of large conductance at membrane PDs more negative than about −100 mV. Figure 9*a* shows current through an outside-out patch held at −168 mV. Two types of channel cur-

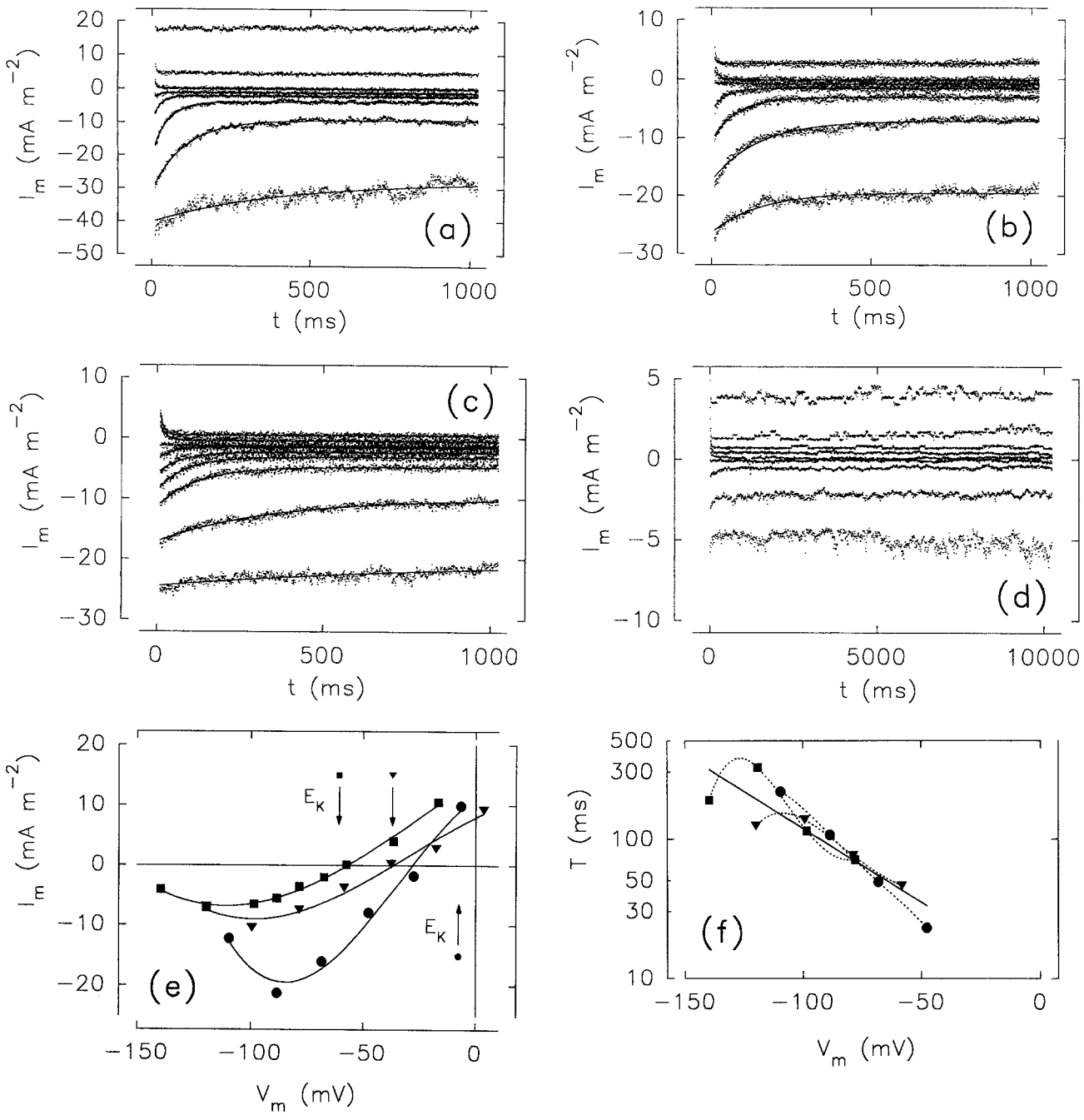


Fig. 7. Deactivation of the K^+ inward rectifier. Data from a whole protoplast of Machete. Membrane current flowing as a result of a series of equally spaced positive-going voltage clamped steps in membrane PD, from a holding potential (V_h), to levels between an initial level (V_i) and a maximum level (V_{max}), for four levels of external K^+ concentration. In the following, the external K^+ concentration is shown first followed by the PDs in the order V_h, V_i and V_{max}. (*a*) 103 mM; −130, −109, 35 mV (*b*) 33 mM; −140, −120, 4 mV (*c*) 13 mM; −160, −140, −16 mV (*d*) 4 mM; −140, −120, 24 mV. The data in *a–c* have been fitted by single exponential decays of the form $I_m = I_0 + I_K \exp(-t/T_n)$. (*e*) The instantaneous K^+ current, I_K in the equation, obtained from the fitted curves, as a function of V_m: (■) [K] = 13 mM, (▼) [K] = 33 mM, (●) [K] = 103 mM. The K^+ equilibrium potentials are shown by the arrows. (*f*) Voltage dependence of the time constants of activation. The dashed lines are through points for the same K^+ concentration, and the unbroken line is a linear regression line fitted to all the points. Solutions: bath = (in mM) $CaCl_2$ 10, MES 5, KOH 3, pH 6; with KCl 100, 30, 10, 1; pipette = standard.

rent are apparent, the first type an infrequently occurring and rather short-lived current with variable and large amplitude, and the other of more conventional kind, with magnitude about 4 pA. The identity of this latter channel is not known. Current-voltage curves for the other channel were determined in another patch that showed considerable activity of spiky current, even at −90 mV, by applying ramps in PD and recording the

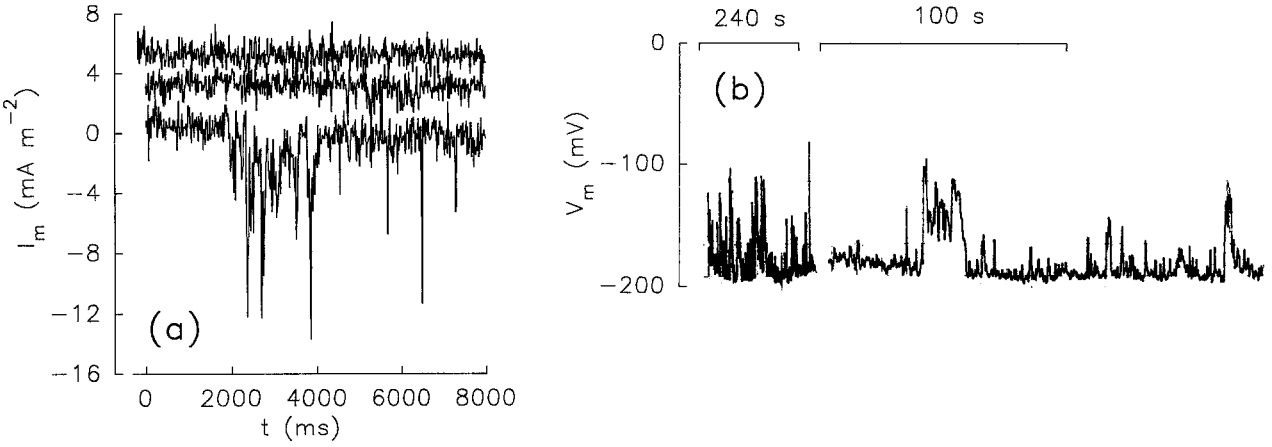


Fig. 8. Activation of the inward "spiky" current. (*a*) Current flowing across the plasmalemma of a whole protoplast of Kharchia during three voltage clamp steps in V_m, from a holding PD of −90 mV, to −110, −150 and −190 mV. The spiky current appears during the step to −190 mV. (*b*) Part of the time course of V_m, measured under current clamp conditions with current set at zero. The PD during the record is close to −190 mV, and depolarizing spikes are apparent. Solutions: bath = (in mM) KCl 100, $CaCl_2$ 10, MES 5, KOH 2.5, pH 6; pipette = (in mM) K glutamate 10, remainder as for standard solution.

current flow. Figure 9*b* shows current flow during 10 repeats of a protocol of pairs of 25 msec ramps. There are several discrete levels of conductance, and furthermore, during the holding level of −90 mV between the two ramps, there is a transition in current to a lower conductance level for about 5 msec. Current-voltage curves of the highest conductance substate, and one with a lesser conductance, obtained (as described by Tyerman & Findlay, 1989) by subtracting the current voltage curve for the lowest conductance state (most likely the closed state) from the curves for the higher conductance states are shown in Fig. 9*c*. The lower curve has a slope of 486 pS and the upper curve of 115 pS; both curves have reversal PDs very close to E_K, indicating that the channels are permeable mainly to K^+.

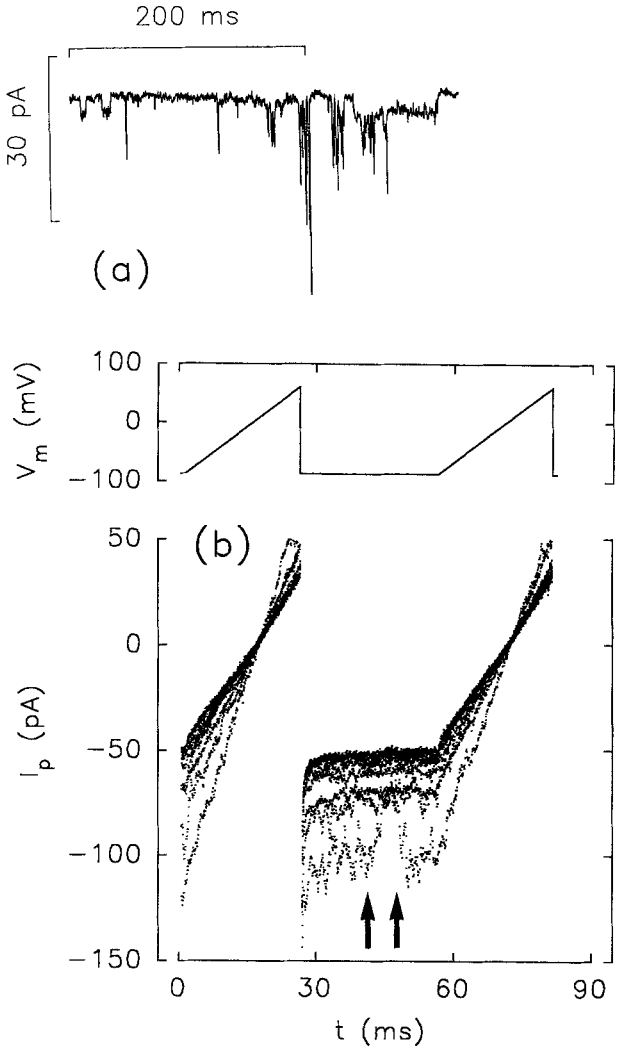
Frequency of Occurrence of Conductance Components in the Protoplast Plasmalemma

In the course of our experiments we have observed five types of conductance in the plasmalemma of wheat protoplasts, a pump conductance and four passive conductances. The passive conductances are a slowly activating K^+ outward rectifier (described by Schachtman et al., 1991), a fast activating Cl^- outward rectifier (described by Skerrett & Tyerman, 1993), a slowly activating K^+ inward rectifier and a probable K^+ spiky conductance. The Table shows the frequency of distribution of the various types of passive conductances. The sample size in each part of the Table represents the number of protoplasts which were actually tested for the presence or absence of the various conductances, and all of these sample sizes were less than the total data set of 217 protoplasts. Of the passive conductances, the most commonly occurring was the fast activating Cl^- outward rectifier (86%). The slowly activating inward K^+ rectifier occurred least (23%). An interesting feature of the data is the rather low occurrence of the inward rectifiers together (9%). An example of the two occurring together is shown in Fig. 8. The four conductances almost never occurred together (4%).

Discussion

The plasma membrane of root cortical cells are primarily permeable to K^+ (Pitman, 1982), and in this paper we have investigated the basis of this permeability by examining ion channels in protoplasts that are selective for K^+. We have focused on the concentration range that corresponds to mechanism II uptake (Epstein & Hagen, 1952), that is, concentrations between about 1 and 100 mM K^+ where the influx of K^+ is predominantly passive and flux kinetics show first order behavior. It has previously been proposed on the basis of the kinetics and responses to inhibitors and channel blockers (Kochian et al., 1985) that K^+ influx in this range is mediated by ion channels (Kochian & Lucas, 1982).

In experiments described in this paper we have identified two types of inward rectifier, as well as demonstrating the existence of the electrogenic H^+ extrusion pump, in the plasmalemma of isolated wheat protoplasts. Some of the properties of a cation outward rectifier have already been described by Schachtman et al., 1991, and a rapidly activating outward Cl^- rectifier has been described in detail by Skerrett & Tyerman (1993).



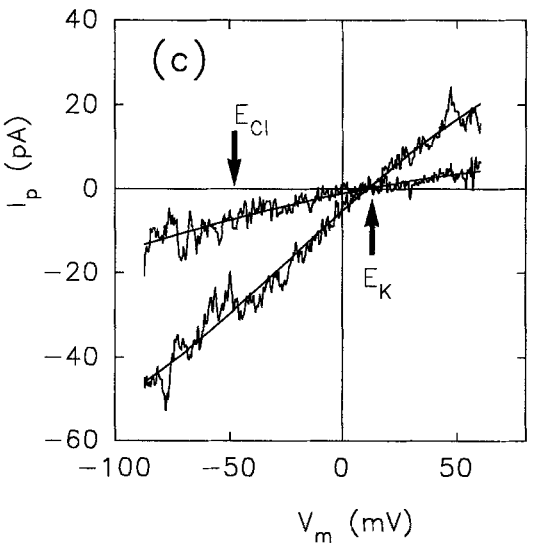


Fig. 9. (*a*) Current flow through an outside-out patch from Kharchia, held at −168 mV, showing two types of channel activity. (*b*) Current flow through a detached outside-out patch from Kharchia (lower traces) during the application of eight pairs of linear ramps in PD (upper traces) showing various levels of conductance. The arrows show a transition, from the highest level of conductance to a lower level, lasting about 6 msec. (*c*) Current-voltage curves for the largest two conductance states. Linear regression lines yield conductances of 115 and 463 pS. The arrow shows E_K and E_{Cl}. Solutions: bath = (in mM) KCl 100, $CaCl_2$ 10, MES 5, sorbitol 462, KOH 2.5, pH 6; pipette = (in mM) K glutamate 10, remainder as for standard solution.

We used three varieties of wheat: Kharchia, a salt-tolerant variety, Modoc, a salt-sensitive variety, and Machete, a commercial variety. While the experiments were not deliberately designed to test differences between the varieties, but were more focused on the general physiological behavior of the plasmalemma, it became apparent that there were no striking differences between the varieties, supporting the findings of Schachtman et al. (1991). In fact, the data shown in the Table show quite close similarities in the types of conductance present in the plasmalemma and their frequencies of occurrence. It is because of these similarities that we have presented data from all three varieties throughout the paper.

Membrane Conductance as a Function of V_m, and the Activity of the Proton Extrusion Pump

The data of Fig. 1 show that in protoplasts in the whole cell mode, V_m can attain very negative values, down to −180 mV, compared with the Nernst PD for K^+ and Cl^-; indeed, considerably more negative than any of the ionic diffusion potentials in the system. The large negative values of V_m and the effects of DCCD strongly suggest the activity of an electrogenic pump, presumably the proton extrusion pump. However, the magnitude of the pump current, about 2 mA m^{-2} or 0.2 μA cm^{-2} is about 0.1% of that in root hairs (Lew, 1991).

While there seems to be some correlation between membrane conductance and PD, the data need to be interpreted with considerable care, because the measured conductance has two components, the conductance of the protoplast membrane and the conductance of the seal between the patching pipette and the membrane. Usually, the seal resistance was taken to about 5–10 GΩ before the whole cell mode was produced. However, in some whole cell preparations, the actual resistance between pipette and outside solution was very much greater than this. For example, in Fig. 1 the conductance for the most negative value of V_m, −180 mV, was about

0.0045 S m^{-2}, giving as the actual resistance of the whole protoplast (area 3.06×10^{-9} m^{-2}) 68 GΩ, or 15 pS. In this situation, the opening of only one channel of comparable conductance in the whole of the protoplast membrane will cause a considerable depolarization of the membrane PD. We have recorded instances where either single channel activity or the activity of a few channels can be seen quite clearly in a whole protoplast; *see* Fig. 7*d*.

Where the conductance of the plasma membrane is very low, the proton extrusion pump need have only low activity to maintain V_m at its very negative level. On the other hand, in quite a number of protoplasts from the data set of Fig. 1, even though the membrane conductance was higher, in the range 0.1 to 0.2 S m^{-2}, or 4 to 2 GΩ for the protoplast, the membrane was still hyperpolarized, and with PDs as negative as −100 mV. These conductances are considerably lower than those measured in cortical cells in the intact root, about 3 S m^{-2} (W.H. Zhang, *personal communication*).

Nevertheless, regardless of how the conductance in protoplasts was distributed between membrane and seal, it is clear that the proton pump was working, and in many protoplasts at an increased activity compared with its activity in those protoplasts with very negative PDs and low conductances of the membrane.

The Slowly Activating K^+ Inward Rectifier

Slowly activating K^+ inward rectifiers have been described in the plasmalemma of protoplasts from barley aleurone cells (Bush et al., 1988), cells of *Arabidopsis* (Colombo & Cerana, 1992), mesophyll cells of *Avena* (Kourie & Goldsmith, 1992), guard cells of *Vicia* (Schroeder & Fang, 1991; Blatt, 1992) and guard cells of *Zea* (Fairley-Grenot & Assmann, 1993). We have now shown that a similar rectifier occurs in the plasmalemma of wheat protoplasts. However, it only appeared in about 23% of protoplasts. There could be several explanations for this: (i) the rectifier does not occur in all cortical cells, (ii) it occurs at specific locations in the root cortex, or (iii) the variations could be due to the methods used in the preparation of the protoplasts. The last possibility is rather unlikely because we have found that not all protoplasts from the one batch possess the same complement of rectifiers. We are thus inclined to alternative (ii), although until such time as the source of protoplasts can be localized, it is not possible to determine whether there is radial or longitudinal asymmetry of distribution of the rectifier.

The currents flowing as a result of the activation of the slowly activating K^+ inward rectifier are fitted best by two rising exponential components. Kourie and Goldsmith (1992) found a similar result for *Avena* mesophyll protoplasts. Data from Schroeder (1988) for the protoplasts of guard cells of *Vicia* can be similarly fitted, but Fairley-Grenot and Assmann (1993), for their protoplasts of both *Zea* and *Vicia*, found that the best fit was one exponential component. It is not clear why there are such differences in *Vicia*.

The activation of the inward rectifier with two time constants can indicate either two closed states of a channel, or two populations of channel each with one closed state. In the three varieties of wheat, there was some voltage dependence of the longer of the two time constants. This contrasts with the data of Fairley-Grenot and Assmann (1993) for *Vicia* and *Zea*, where both time constants appear to be voltage independent. Kourie and Goldsmith (1992) do not present any data for *Avena*.

The deactivation of the inward rectifier is best fitted by a single exponential, and this result is also found by Fairley-Grenot and Assmann (1993) for *Vicia* and *Zea*. In *Avena*, however, Kourie and Goldsmith (1992) show that the deactivation is best fitted by two exponential components. The existence of a single time constant for the decay of the inward rectifier suggests that the channel has just one open state, and thus it is not surprising that Kouri and Goldsmith (1992) found more than one because their single channel data seemed to show two channels of different conductance as well as substates of at least one of the channels. The same remark can be made for wheat as a result of the recent findings of White and Tester (1992) who found a number of different types of K^+ channel in plasmalemma fragments from rye root cells incorporated into lipid bilayers, although there seemed to be one channel, of 49 pS, clearly contributing to a K^+ inward rectifier. This channel, however, was voltage independent, and open about 80% of the time, and is thus an unlikely candidate for a slowly activating K^+ inward rectifier of the type that we have observed in wheat.

Spiky Inward Current

The channel constituent of the spiky inward rectifier contrasts sharply with those of the two outward rectifiers. The channel components of the slowly activating inward K^+ rectifier have yet to be identified. The slowly activating K^+ outward rectifier has channels of about 30 pS (Schachtman et al., 1991) and the Cl^- outward rectifier has channels of about 3 pS (Skerrett & Tyerman, 1993).

The spiky inward rectifier, on the other hand, apparently consists either of a variety of channels with conductance up to about 450 pS, or of a channel with substates. The available data are insufficient to allow a distinction to be made between these two possibilities at present.

Distinctive features of the spiky inward rectifier are

the spiky nature of the current arising apparently from very short openings, the seemingly random nature of its activation and the magnitude of its conductance. Given that these currents tend to differ from the standard type of channel currents, it might be argued that the activation is caused either by electrical breakdown of the patch membrane, or by breakdown of the seal between the patching pipette and the patch. However, in some instances, the channels remained in the open state for a considerable time, and when this occurred, we have recorded instances, in detached patches, where the channel appeared to have made a transition directly from its "open" state to the "closed" state, or to intermediate substates. When the channel was open, substates became apparent, as shown in Fig. 9*c*. Furthermore, the reversal PD of the channel was very close to E_K, rather than close to the expected junction potential of 9.8 mV between the pipette and bathing solutions, the value expected if seal or membrane breakdown was involved. In Fig. 9*c*, V_m has already been corrected for the junction of PD and thus the expected reversal PD if seal breakdown were occurring would be 0 mV. In fact, the actual reversal PD is considerably removed from this value. But in any case, there have been other observations of channels with large conductance in plant membranes. Moran, Ehrenstein and Iwasa (1984) have described a 160 pS cation channel in wheat leaf protoplasts, which opened at small negative membrane PDs, while Terry et al. (1991) have described a multistate anion channel with a maximum conductance of about 200 pS in the plasmalemma of *Amaranthus* protoplasts.

Recently, Sachs and Qin (1993) have shown that it is possible to obtain what appears to be gated channel activity when patching pipettes are pushed against a surface of Sylgard®. These channels were conventional in appearance and ion specific. However, the spiky activity recorded in the wheat plasma membrane is very different in form and magnitude from that shown by Sachs and Qin, and we believe that it is more likely to be a property of the actual membrane, rather than a property of the gap between the membrane and the tip surface of the micropipette, in the system described by Sachs and Qin.

Roles of the Various Types of Conductances in the Plasmalemma in Wheat

The Proton Pump

One of the major disadvantages of electrophysiology on plant protoplasts is the requirement to remove the cell wall, usually by enzymic methods, although in many animal cell preparations, enzyme treatments are also needed. The combination of both loss of turgor and the enzyme treatment might well have quite dramatic effects on the operation of the resultant protoplast. Our findings, however, that the PD in wheat protoplasts can be as large as −180 mV is a strong indication that the proton pump is still working.

Figure 1 shows that for PDs more negative than about −100 mV, the membrane conductance drops sharply. Given that the slowly activating inward rectifier is activated at about this PD, we would expect that the conductance would increase with increasingly negative PDs, rather than decrease as observed. It seems likely, therefore, that the very negative PDs, down to −180 mV, are only seen in protoplasts where this inward rectifier is absent or has a low conductance, and where the observed conductance would be due to the pump. Where the rectifier is present with a low conductance, it could manifest itself as noise in the PD, as seen in Fig. 2*a*. In some protoplasts where the PD is very negative, the effects of activation of the spiky inward rectifier are quite apparent, however (*see* Fig. 8*b*).

The Inward Rectifiers

The steady-state current-voltage curves are quite similar to those obtained by Schroeder and Fang (1991) for *Vicia* guard cells. In that paper it was argued that this conductance was the likely route for low affinity uptake of K^+ into plant cells. Here we show that a conductance with similar properties is actually present in root cortical cells. In contrast to the outward K^+ rectifier, there appears to be a fairly obvious role for both types of inward K^+ rectifiers in the uptake of K^+ from the external environment. Certainly, the slowly activating K^+ inward rectifier can remain in its activated state for long periods of time (Fairley-Grenot & Assmann, 1993) when the PD is more negative than about −100 mV, a state which exists when the proton pump is operating. The slowly activating inward rectifier could also act as a brake on the tendency of the proton pump to take the membrane PD to excessively high negative levels. It may also provide cation/proton exchange, which could be important during pH regulation. A similar role appears to be played by a slowly activating Cl^- inward rectifier, activated at large negative membrane PDs in plants such as *Chara inflata* (Tyerman, Findlay & Paterson, 1986*a,b*) and *Amaranthus* (Terry, Tyerman & Findlay, 1991).

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References

Blatt, M.R. 1992. K^+ channels of stomatal guard cells. *J. Gen. Physiol.* **99**:615–644

Bush, D.S., Hedrich, R., Schroeder, J.I., Jones, R.L. 1988. Channel-mediated K^+ flux in barley aleurone protoplasts. *Planta* **176:**368–377

Colombo, R., Cerana, R. 1992. Inward rectifying K^+ channels in the plasma membrane of *Arabidopsis thaliana*. *Plant Physiol.* **77:**1130–1135

Cram, W.J. 1973. Chloride fluxes in cells of the isolated root cortex of *Zea mays*. *Aust. J. Biol. Sci.* **26:**757–759

Epstein, E., Hagen, C.E. 1952. A kinetic study of the absorption of alkali cations into barley roots. *Plant Physiol.* **27:**457–474

Fairley-Grenot, K.A., Assmann, S.M. 1991. Evidence for G-protein regulation of inward K^+ channel current in guard cells of fava bean. *Plant Cell* **3:**1037–1044

Fairley-Grenot, K.A., Assmann, S.M. 1993. Comparison of K^+ channel activation and deactivation in guard cells from a dicotyledon (*Vicia faba*) and a graminaceous monocotyledon (*Zea mays*). *Planta* **189:**410–419

Glass, A.D.M., Shaff, J.E., Kochian, L.V. 1992. Studies of the uptake of nitrate in barley. IV. Electrophysiology. *Plant Physiol.* **99:**456–463

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Iijima, T., Hagiwara, S. 1987. Voltage-dependent K channels in protoplasts of trap-lobe cells of *Dionaea muscipula*. *J. Membrane Biol.* **100:**73–81

Ketchum, K.A., Shrier, A., Poole, R.J. 1989. Characterisation of potassium-dependent currents in protoplasts of corn suspension cells. *Plant Physiol.* **89:**1184–1192

Kochian, L.V., Lucas, W.J. 1982. Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear components. *Plant Physiol.* **70:**1723–1731

Kochian, L.V., Lucas, W.J. 1988. Potassium transport in roots. *Adv. Bot. Res.* **15:**93–178

Kochian, L.V., Xin-Zhi, J., Lucas, W.J. 1985. Potassium transport in corn roots. IV. Characterization of the linear component. *Plant Physiol.* **79:**771–776

Kourie, J.I., Goldsmith, M.H.M. 1992. K^+ channels are responsible for an inwardly rectifying current in the plasma membrane of mesophyll protoplasts of *Avena sativa*. *Plant Physiol.* **98:**1087–1097

Lew, R.R. 1991. Electrogenic transport properties of growing *Arabidopsis* root hairs. The plasmalemma membrane proton pump and potassium channels. *Plant Physiol.* **97:**1527–1534

McClure, P.R., Kochian, L.V., Spanswick, R.M., Shaff, J.E. 1990. Evidence for cotransport of nitrate and protons in maize roots. I. Effects of nitrate on the membrane potential. *Plant Physiol.* **93:**281–289

Moran, N., Ehrenstein, G., Satter, R.L. 1984. Ion channels in plasmalemma of wheat protoplasts. *Science* **226:**835–838

Pitman, M.G. 1982. Transport across plant roots. *Q. Rev. Biophys.* **15:**481–554

Reid, R.J., Dejaegere, R., Pitman, M.G. 1985. Regulation of electrogenic pumping in barley by pH and ATP. *J. Exp. Bot.* **36:**535–549

Sachs, F., Qin, F. 1993. Gated ion-selective channels observed with patch pipettes—novel properties of a gigaseal. *Biophys. J.* **65:** 1101–1107

Schachtman, D.P., Tyerman, S.D., Terry, B.R. 1991. The K^+/Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. *Plant Physiol.* **97:**598–605

Schroeder, J.I. 1988. K^+ transport properties of K^+ channels in the plasma membrane of *Vicia faba* guard cells. *J. Gen. Physiol.* **92:**667–683

Schroeder, J.I. 1989. Quantitative analysis of outward rectifying K^+ channel currents in guard cell protoplasts from *Vicia faba*. *J. Membrane Biol.* **107:**229–235

Schroeder, J.I., Fang, H.H. 1991. Inward-rectifying K^+ channels in guard cells provide a mechanism for low-affinity K^+ uptake. *Proc. Natl. Acad. Sci. USA* **88:**11583–11587

Skerrett, M., Tyerman, S.D. 1993. A channel that allows inwardly directed fluxes of anions in protoplasts derived from wheat roots. *Planta* **192:**295–305

Spanswick, R.M. 1972. Electrical coupling between cells of higher plants: a direct demonstration of intercellular communication. *Planta* **102:**215–227

Stoeckel, H., Takeda, K. 1993. Plasmalemmal, voltage-dependent ionic currents from excitable pulvinar motor cells of *Mimosa pudica*. *J. Membrane Biol.* **131:**179–192

Terry, B.R., Findlay, G.P., Tyerman, S.D. 1992. Direct effects of Ca^{2+}-channel blockers on plasma membrane cation channels of *Amaranthus tricolor* protoplasts. *J. Exp. Bot.* **43:**1457–1473

Terry, B.R., Tyerman, S.D., Findlay, G.P. 1991. Ion channels in the plasma membrane of *Amaranthus* protoplasts: one cation and one anion channel dominate the conductance. *J. Membrane Biol.* **121:**223–236

Tyerman, S.D., Findlay, G.P. 1989. Current-voltage curves of single Cl^- channels which coexist with two types of K^+ channel in the tonoplast of *Chara corallina*. *J. Exp. Bot.* **40:**105–117

Tyerman, S.D., Findlay, G.P., Paterson, G.J. 1986*a*. Inward membrane currents in *Chara inflata:* I. A voltage- and time-dependent Cl^- component. *J. Membrane Biol.* **89:**139–152

Tyerman, S.D., Findlay, G.P., Paterson, G.J. 1986*b*. Inward currents in *Chara inflata:* II. Effects of pH, Cl^--blockers and NH_4^+, and significance for the hyperpolarized state. *J. Membrane Biol.* **89:**153–161

Tyerman, S.D., Terry, B.R., Findlay, G.P. 1991. Multiple conductances in the large K^+ channel from *Chara corallina* shown by a transient analysis method. *Biophys. J.* **61:**736–749

Van Duijn, B. 1993. Hodgkin-Huxley analysis of whole-cell outward rectifying K^+-currents in protoplasts from tobacco cell suspension cultures. *J. Membrane Biol.* **132:**77–85

Walker, N.A. 1980. The transport systems of charophyte and chlorophyte giant algae and their integration into modes of behaviour. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas and J. Dainty, editors. Elsevier/North-Holland Biomedical, Amsterdam

White, P.J., Tester, M.A. 1992. Potassium channels from the plasma membrane of rye roots characterized following incorporation into planar lipid bilayers. *Planta* **186:**188–202