J. Membrane Biol. 102, 235–246 (1988)

The Journal of
Membrane Biology

Potassium-Dependent, Bipolar Gating of K^+ Channels in Guard Cells

Michael R. Blatt
Botany School, University of Cambridge, Cambridge CB2 3EA, England

Summary. Guard cells of higher plants control transpirational water loss and gas exchange for photosynthesis by opening and closing pores in the epidermis of the leaf. To power these turgor-driven movements, guard cells accumulate (and lose) 200 to 400 mM (1 to 3 pmol/cell) K^+, fluxes thought to pass through K^+ channels in the guard cells plasma membrane. Steady-state current-voltage (*I-V*) relations of intact *Vicia* guard cells frequently show large, outward-going currents at potentials approaching 0 mV. Since this current could be carried by K^+ channels, its pharmacology and dependence on external K^+ (K_o^+) has been examined under voltage clamp over an extended potential range. Measurements were carried out on cells which showed little evidence of primary "electrogenic" transport, thus simplifying analyses. Clamping these cells away from the free-running membrane potential (V_m) revealed an outward-rectifying current with instantaneous and time-dependent components, and sensitive to the K^+ channel blocker tetraethylammonium chloride. The current declined also under metabolic blockade with NaCN and in the presence of diethylstilbesterol, responses which were attributed to secondary effects of these inhibitors. The putative K^+ current rose with voltage positive to V_m but it decayed over two voltage ranges, one negative to V_m and one near +100 mV, to give steady-state *I-V* relations with two regions of negative (slope) conductance. Voltage-dependent and kinetic characteristics of the current were affected by K_o^+ and followed the K^+ equilibrium potential. Against a (presumably) low background of primary ion transport, the K^+ current contributed appreciably to charge balance at V_m in 0.1 mM as well as in 1 to 10 mM K_o^+. Thus, gating of these K^+ channels compensates for the prevailing K^+ conditions to ensure net K^+ movement out of the cell.

Key Words stomatal guard cell · K^+ channel · outward rectifier · voltage-depending gating · tetraethylammonium · cyanide · *Vicia*

Introduction

That potassium channels occur in the membranes of plant cells is well established now, as it is in animal cells. The channels are found, not only in the giant algae (Coleman & Findlay, 1985; Lühring, 1986, Homblé, Ferrier & Dainty, 1987), in which a K^+ conductance was implicated early on in excitation recovery (Gaffey & Mullins, 1958; Kitasato, 1973) and a K^+-sensitive condition (Oda, 1962; Beilby, 1986), but they are known in yeast (Gustin et al., 1986) and in the cells of higher plants (Moran et al., 1984; Kolb, Köhler & Martinoia, 1987).

The presence of these channels raises fundamental questions about their physical properties and physiological functions in the intact plant cell. Whereas K^+ channels in animal cells may serve in sensory encoding, nervous signal conduction, cell volume regulation and to "stabilize" the membrane potential (Hille, 1984), the structures and habits of plants are likely to entail quite different roles and, possibly, divergent characteristics for ion channels.

One thought is that K^+ (as well as other ion) channels could participate in osmotic- or turgor-driven events. Potassium makes a major contribution to the osmotic pressure difference (turgor pressure) of cells with walls, from bacteria to higher plants (Harold, 1987). Dramatic changes in cellular K^+ content and turgor are known correlates, particularly to specialized "motor" tissues which drive a variety of "movements" in higher plants (*cf.* Edwards & Pickard, 1987).

For stomatal guard cells, varying the cellular K^+ content facilities guard cell volume changes which open and close the stomatal pore (Outlaw, 1983). Potassium must be transported across the plasma membrane, because guard cells are isolated from the surrounding tissues of the leaf (Wille & Lucas, 1984). Furthermore, the cation flux, at least during stomatal closure, can be large; estimates for K^+ loss from guard cells are as high as 100 pmol cm^{-2} sec^{-1} (Outlaw 1983; MacRobbie 1987). Such high rates intimate a device capable of sustaining a high ionic throughput, such as an ion channel.

Demonstrating K^+ channels in *Vicia* guard cell protoplasts (Schauf & Wilson, 1987; Schroeder, Raschke & Neher, 1987) has focussed attention on roles for channels in stomatal movement; but evidence that these channels contribute to guard cell K^+ flux is slight. Indeed, very little information on

guard cell electrical properties per se has been available until now. So, a basic issue is whether the K^+ channels described are expressed in the intact cell and, if so, under what conditions they might be observed.

Recently, it became possible to explore the physiology of stomatal guard cells using conventional electrical recording techniques (Blatt, 1987*a*,*b*). Once deleterious salt loading from the microelectrode was minimized, guard cells showed attributes common to other higher plant and algal cells including, near 0 mV, the rising current often thought to result from a Goldman-like diffusion regime or channel activity dominated by intracellular K^+ (*cf.* Gradmann, 1975).

I focus, now, on properties of this rising current [a preliminary report has appeared in abstract (Blatt, 1987*c*)]. Data for the present study were compiled at the end of the growing season (September-October, 1986) when guard cells showed little indication of primary pump activity (Blatt, 1987*b*), thus greatly simplifying analysis of whole-cell recordings. The current shows a conductance maximum near 0 mV, and is attributed to K^+ channels with unusual gating characteristics. An appreciable K^+ current occurs at the free-running membrane potential[1], and additional kinetic features make the channel a plausible candidate as a pathway for K^+ loss during stomatal closure.

Abbreviations

K_o^+, extracellular potassium (concentration); V_m, free-running membrane potential (difference); *I-V*, current-voltage (relationship); *G-V*, (slope) conductance-voltage (relationship).

Materials and Methods

Plant Culture and Experimental Protocol

Vicia faba L., cv. (Bunyan) Bunyard Exhibition, was grown hydroponically in Hoagland's Salts medium and epidermal strips were prepared as described before (Blatt, 1987*a*). Measurements were carried out in rapidly flowing solutions (Blatt, 1987*a*) containing 5 mM Ca^{2+}-HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid), pH 7.4 (5 mM HEPES buffer titrated to pH 7.4 with $Ca(OH)_2$, final $[Ca^{2+}] \simeq 1$ mM). For buffering pH below 7.4, HEPES was replaced with 2-(N-morpholino)propanesulfonic acid (MES) while keeping K^+ and Ca^{2+} constant. Potassium salts were added as indicated. Ambient temperatures were 20 to 22°C.

In some experiments, cyanide was introduced as the sodium salt to give a final concentration of 0.3 mM, in which case 0.4 mM salicylhydroxamic acid (SHAM) was included to block alternate oxidase pathways for electron transport. NaCN and SHAM were prepared as 1 M and 0.4 M (in ethanol) stock solutions, respectively. Diethylstilbesterol (DES) was added from a stock solution in ethanol to a final concentration of 40 μM (0.1% ethanol). Tetraethylamonium chloride was used at a concentration of 10 mM.

Surface areas and volumes were calculated assuming a cylindrical geometry (Blatt, 1987*a*), and the orthogonal dimensions (diameter, length) of impaled cells were measured with a calibrated eye-piece micrometer. Cell dimensions typically varied over 13 to 17 μm (diameter), and 35 to 45 μm (length). Estimated surface areas thus fell between 1.6×10^{-5} and 2.9×10^{-5} cm^2. For technical reasons, apertures were not recorded for individual stomatal pores adjacent to impaled guard cells, but stomata in these preparations were probably closed or only partially open; mean apertures measured on epidermal strips prior to experiments were 2 to 4 μm.

Electrical

Mechanical, electrical and software design have been described in detail (Blatt, 1987*a*,*b*). Recordings were obtained by the two-electrode method using double-barrelled microelectrodes. Electrodes were filled with 200 mM K^+-acetate, pH 7.2 to minimize microelectrode salt leakage and salt-loading artifacts (Blatt, 1987*a*).

Current-voltage relations were obtained by voltage clamp under microprocessor control. Steady-state *I-V* relations were determined by clamping cells to a bipolar staircase of command voltages (Blatt, 1987*b*). Steps alternated positive and negative from V_m (generally 20 bipolar pulse pairs, 150-msec step duration) and were separated by equivalent periods when the membrane was clamped to V_m. The current signal was filtered by a 4-pole Bessel filter at 3 kHz (−3 dB) before sampling, and currents and voltages were recorded during the final 20 msec of each pulse.

For time-dependent characteristics, current and voltage were sampled continuously, nominally at 1 kHz, while the clamped potential was driven through cycles of 1 to 4, programmable pulse steps. Recordings at 10 kHz (above the Nyquist limit) were restricted by available data storage space to 200-msec "windows" within each cycle; otherwise data taken at both frequencies gave similar results.

It was expected that direct measurement of the series resistance (R_s) to ground (Hodgkin, Huxley & Katz, 1952) would be complicated by electrode capacitance. However, estimates for R_s indicated that it was unlikely to pose a serious problem in measurements of clamp potential, despite the high resistivity of the bathing media (= 2.5 kΩ cm for 5 mM Ca^{2+}-HEPES with 0.1 mM KCl). In practice, R_s is likely determined by the cell wall (mean depth approx. 3 μm) which, if it depressed ionic conductivity by 10- to 30-fold (calculated assuming K^+ and Cl^- as the principle current-carrying species and based on estimates for K^+ and Cl^- self-diffusion coefficients in Ca^{2+}-containing bulk solution; Briggs, Hope & Robertson, 1961; Hope & Walker, 1975), might place R_s near 1 to 2 MΩ. Cell input resistances near V_m ranged between 1 and 4 GΩ and, in the worst case for the K^+ conductance maximum near 0 mV, (slope) input resistance never fell below 140 MΩ. Hence, the maximum voltage error likely was 1 to 2% of the clamp potential, or less than 2 mV on clamping a

[1] For reasons detailed previously (Blatt, 1987*a*), electrical recordings from the guard cells are assumed to reflect the characteristics of the plasma membrane alone. I use the terms guard 'cell' and 'membrane' potential and conductance in this context.

cell to 0 mV. The presence of a series resistance would, in any case, lead to an underestimate of changes in *I-V* and *G-V* relations, for example with K_o^+. So, no attempt was made to compensate for R_s.

Numerical Analysis

Guard cell *I-V* relations were fitted to polynomials, using a nonlinear, least-squares algorithm (Marquardt, 1963). In most cases, 8th-(occasionally 10th-) order polynomials coped with the rapid changes in slope at positive potentials. Exponential fittings, likewise, were by nonlinear, least squares.

Chemicals and Solutions

Sodium cyanide, SHAM. DES and tetraethylammonium chloride were from Sigma Chemical Co. (St. Louis, Mo.). The buffers HEPES and MES were also from Sigma. Otherwise, all chemicals were Analytical Grade from BDH Ltd. (Poole, Dorset, UK).

Where appropriate, results are reported ± standard errors of (n) observations.

Results

Activity of the Pump

Stomata examined in the autumn often failed to open, or responded only weakly on transfer to light, even in 1 to 10 mM external K^+ (K_o^+); however, they opened uniformly on treatment with fusicoccin (H. Brindley, G. Clint and M. Blatt, *unpublished*), a fungal toxin known to promote stomatal opening and reputed to stimulate the H^+-ATPase of plant plasma membranes (Marrè, 1985). Although an unequivocal explanation for the physiologic status of the tissue is lacking, one reason for weak responses to light appears to have rested with a low level of primary pump (H^+-ATPase) activity. The conclusion is supported by three additional observations.

First, mean membrane potentials (V_m) and conductances (G_m, = slope conductance, G, at V_m) of the guard cells were -130 ± 3 mV and 36 ± 7 μS cm^{-2} ($n = 11$) in the standard 5 mM Ca^{2+}-HEPES buffer, pH 7.4, with 0.1 mM KCl, or significantly lower ($P < 0.05$) than equivalent measurements from the spring and early summer of the same year (Blatt, 1987*a,b*). Membrane potential appeared largely insensitive to external pH in the range 7.4 to 5.5 (V_m response to acid-going shifts, 12 to 18 mV/pH unit, $n = 3$), whereas near-Nernstian responses to K_o^+ were recorded for concentrations of 1 mM and above (55 ± 2 mV/K_o^+ decade, $n = 8$).

Second, steady-state *I-V* profiles failed to reveal conductance characteristics of the pump. In 0.1 mM K_o^+, *I-V* curves were essentially linear at voltages negative to V_m (Fig. 1) before large inward (negative) currents were recorded at potentials near -300 mV (*not shown*); *G-V* curves, derived by differentiating polynomial fittings to the *I-V* data, showed little evidence of the conductance maximum near -200 mV associated with the pump (Blatt, 1987*b*).

Finally, large and rapid (half-times near 10 sec), positive-going shifts in V_m, attributed to ATP-dependent charge transport (Blatt, 1987*b*), were not observed when guard cells were challenged with 0.3 mM NaCN plus 0.4 mM SHAM against a background of 0.1 mM K_o^+. Instead, guard cell potentials decayed with apparent half-times of 1.5 to 3.2 min ($n = 4$) to values positive of -95 mV. This slower shift in V_m toward positive potentials was identified subsequently with a reduced K^+ conductance and I return to this point below.

Steady-State Characteristics

On first inspection outward- (positive-) rectifying currents and rising conductances at voltages positive to V_m appeared consistent with a Goldman-like diffusion regime expected for a cell with a high cation content and bathed in dilute medium (*cf.* Gradmann, 1975). However, at voltages positive to +50 mV, the steady-state currents declined, giving rise to a maximum near 0 mV in the *G-V* profile and to a region of negative slope conductance near +100 mV. Extending *I-V* scans beyond +100 to +120 mV proved hazardous, but in five cells (*see* Fig. 1, curve in 10 mM K_o^+; *also* Fig. 8) outward-going currents could be shown to rise steeply at still more positive potentials. Stepping K_o^+ to 3.0–10.0 mM [as K_2SO_4; also as KCl and K^+-HEPES, *data not shown*] introduced a region of negative slope conductance near -150 mV as well (Fig. 1). The conductance maximum near 0 mV increased in magnitude, rising roughly in proportion with the logarithm of the K_o^+ concentration between 1 and 10 mM. For potentials negative to approx. -150 mV, however, little change in conductance was observed over the entire K_o^+ range.

Dependence on Voltage, Time and K_o^+

The near-Nernstian response of V_m to K_o^+ pointed to a substantial fraction of membrane current passing through a K^+ channel. Most striking, the conductance maximum bounded by regions of negative conductance intimated unusual gating characteristics of a channel operating over a finite voltage range. Nonetheless, a voltage-dependence to the current rise-time might have distorted measure-

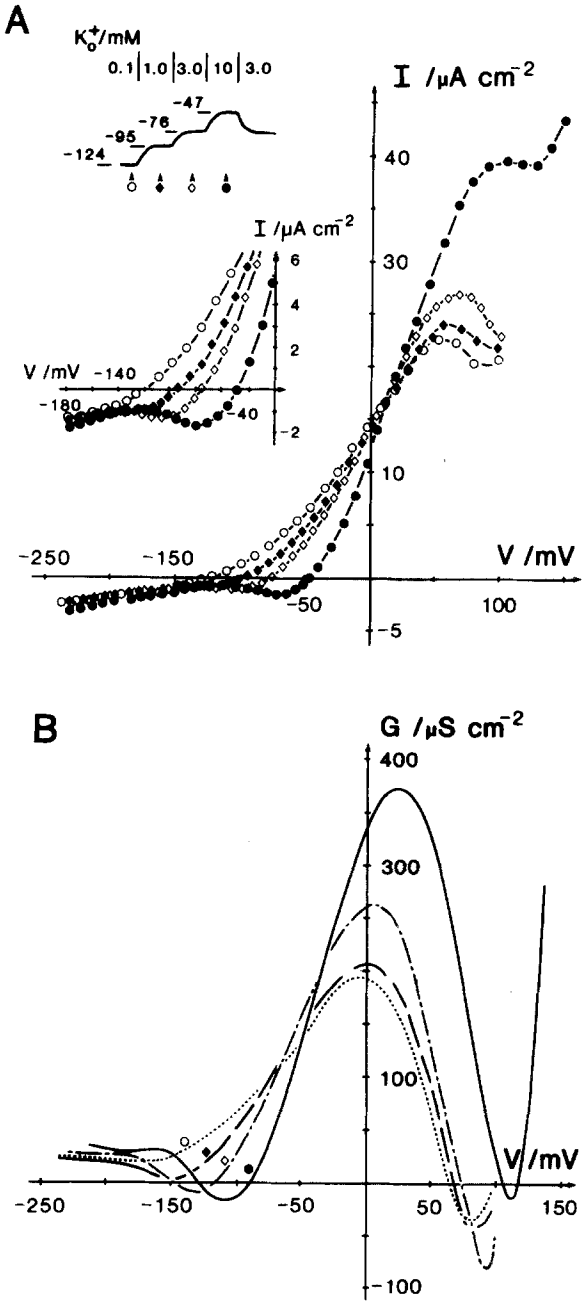

Fig. 1. Steady-state response to external potassium. (*A*) Current-voltage (*I-V*) curves. Current and voltage sampled at the end of 150-msec-long clamp steps from the free-running membrane potentials (V_m, V-axis intercepts). Inset, top left: voltage trace (recorded potentials in mV) with times of *I-V* scans (masked from trace) and K_o^+ exposures (carats) as indicated [(○) 0.1, (♦) 1.0, (◇) 3.0, (●) 10 mM K_o^+; time window shown, 8.9 min]. *Inset* (left): *I-V* curves replotted on expanded axes. Curves were fitted to 8th order polynomials. In 1 to 10 mM K_o^+, *I-V* curves typically crossed over one another three times, twice negative to V_m and once near 0 mV. (*B*) Conductance-voltage (*G-V*) curves for the data in (*A*) obtained by differentiating the fitted polynomials. Negative conductances (slopes) appeared over two voltage ranges, one negative to V_m and one near +100 mV. Increasing K_o^+ led to a rise in conductance near 0 mV (*see also* Fig. 6) and an overall shift of the *G-V* profile to the right along the voltage axis

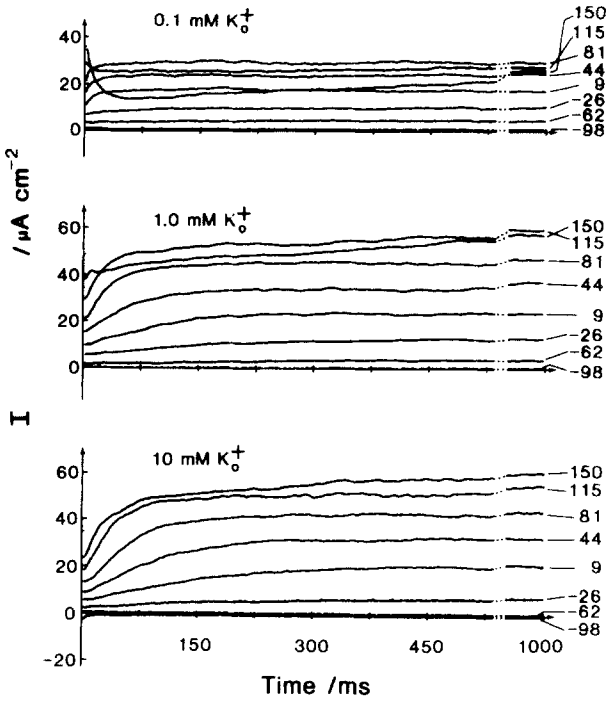

Fig. 2. Clamp current response to external potassium. Current and voltage sampled once per msec over 1-sec clamp steps from V_m (−125, −101 and −47 mV in 0.1, 1.0 and 10 mM K_o^+, respectively) to potentials (indicated on right) between −100 and +150 mV. In 0.1 mM, but not in 10 mM K_o^+ the current declined at +115 and +150 mV. Currents were seen to rise slowly over seconds at potentials near +150 mV

ments taken after a fixed-pulse duration to give a false impression of the true conductance profile.

Evidence for current decline and, hence, negative slope conductances over two voltage spans was afforded by recording currents (and voltages) continuously, both during single-clamp steps away from V_m and, using double or triple pulse protocols, during steps away from a common (conditioning) clamp potential (V_{cond}, *see recording scheme in* Fig. 6). Figure 2 shows clamp currents from one cell bathed in 0.1, 1.0 and 10 mM K_o^+. Comparable results were obtained from all other cells examined. Positive voltage steps were followed by a sigmoidally rising outward current with an instantaneous component. On clamping the membrane to voltages between +100 and +150 mV, however, the current fell, often precipitously within 20 to 50 msec, from its initial (instantaneous) high value. Likewise, tail currents recorded at potentials negative to V_m (*see* Fig. 3) decayed rapidly with apparent first-order kinetics (τ, 19 to 24 msec at −150 mV in 3 mM K_o^+, n = 4). [It is important to recognize that K^+ accumulating in the cell wall during the positive-going clamp steps could not account for current decline at positive potentials for two reasons. First, the cell wall is unlikely to pose a significant barrier to diffu-

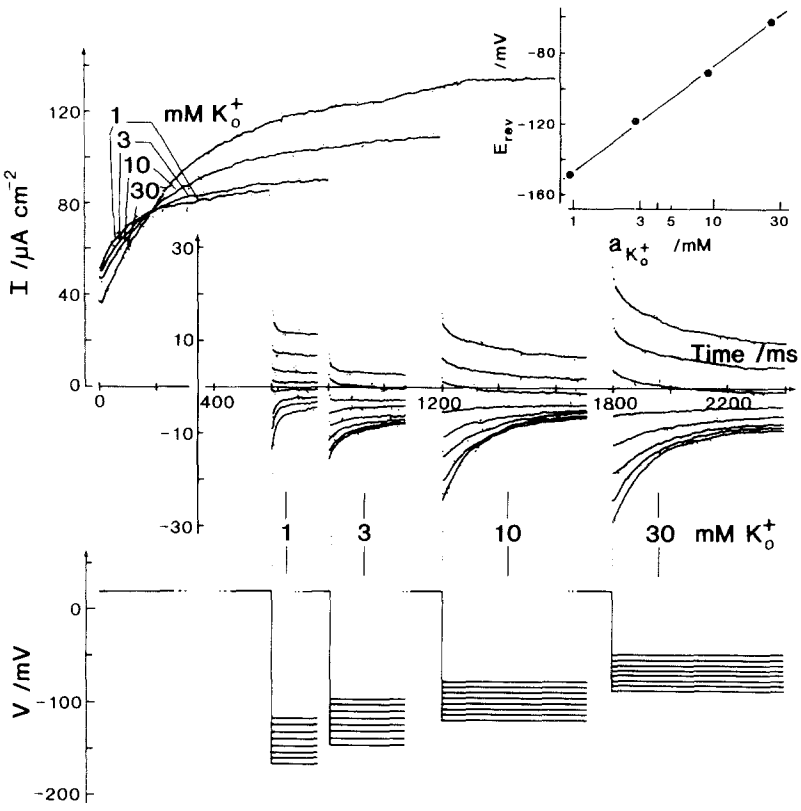

Fig. 3. Tail current reversal potential (E_{rev}) as a function of external potassium. Data from one cell exposed to 1, 3, 10 and 30 K_o^+. Current and voltage sampled at 1 kHz (1 and 3 mM K_o^+) or 0.5 kHz (10 and 30 mM K_o^+) during successive clamp cycles, stepping to a conditioning voltage of +20 mV and then to one of eight test voltages (below; vertical lines linking the voltage records are added as a visual aid). Holding potentials (= V_m): −131 (1 mM), −109 (3 mM), −78 (10 mM) and −54 mV (30 mM K_o^+). Records are overlaid along the time axis relative to the start of the conditioning voltage step (t = 0) and, for clarity, only one of each eight current traces for the conditioning step is shown. The expanded current axis applies to the tail currents. Current rise half-times at +20 mV were calculated from the time-dependent component following the voltage and instantaneous current step and gave 104 (1 mM), 147 (3 mM), 190 (10 mM) and 239 msec (30 mM K_o^+). *Inset:* Reversal potential (E_{rev}) plotted as a function of external K^+ activity (a_{K^+}). Values for E_{rev} were found by interpolating from the peak tail currents shown. The straight line through the points was drawn by eye and gives a slope of 58 mV/a_{K^+} decade. Predicted intracellular a_{K^+}, 373 mM

sion. Best estimates for K^+ salt diffusion coefficients in plant cell walls (*cf.* Gaffey & Mullins, 1958; Barry, 1970; Hope & Walker, 1975) are approx. 1% of values for diffusion in free solution. So, even the largest currents recorded would alter K_o^+ immediately outside the plasma membrane by no more than 10 nM in the steady state (i.e. 0.01% against a background of 0.1 mM K_o^+). Second, increasing K_o^+ was observed to *increase* the steady-state current at extreme positive potentials (*cf.* Fig. 1). So, any K^+ accumulating externally should have had quite the opposite effect, namely to *enhance* the outward current apparent at these potentials.]

Tail currents also provided direct evidence for K^+ as the principle charge carrier in the current. For these experiments, the impaled cell was first clamped to 0 or +20 mV, near the conductance maximum, until the current approached steady state. On stepping to a more negative potential, the current could then be expected to relax to a new, lower conductance state—and toward its equilibrium potential.

Figure 3 illustrates typical results from one cell exposed to 1, 3, 10 and 30 mM KCl. Visually inspecting the current tails recorded following the conditioning pulse at +20 mV shows that the *sign* of the tail (toward more positive or more negative) current reversed at a potential close to the expected E_K, and that this potential roughly followed E_K as K_o^+ varied. Reversal potentials (E_{rev}) were determined by plotting peak tail currents as a function of the clamp potential and then interpolating to zero current. After correcting for external K^+ activities [activity coefficients, δ_o: 0.95 (1 mM), 0.92 (3 mM), 0.89 (10 mM), 0.85 (30 mM K_o^+); Robinson & Stokes, 1959], E_{rev} showed an essentially Nernstian dependence on K_o^+ (Fig. 3, inset). Comparable results were obtained from 10 other cells. The mean value for E_{rev} in 10 mM K_o^+ was −77 ± 4 mV (n = 11, range −93 to −54 mV), thus corresponding to a mean intracellular K^+ activity of 238 ± 34 mM.

Finally, recordings such as those in Figs. 2 and 3 revealed voltage- and K_o^+-dependent features to the current kinetics. At any one K_o^+ concentration, currents rose sigmoidally with time, accelerated by increasingly positive clamp potentials (Fig. 2). Analogously, decreasing K_o^+ quickened the rising current at any one clamp potential.[2] The latter response is most easily seen by comparing the currents during the conditioning pulse at +20 mV in

[2] Clearly, some distortion from the true steady state might be expected in isochronal *I-V* profiles taken at 150 msec, particularly in 1 to 10 mM K_o^+. However, the errors did not seriously compromise these records (*compare* Figs. 6 and 7) for two reasons: (1) the proportional error declined as voltage steps from V_m increased and the current rise half-time decreased; and (2) an appreciable fraction of the true steady-state current was realized instantaneously on stepping away from V_m.

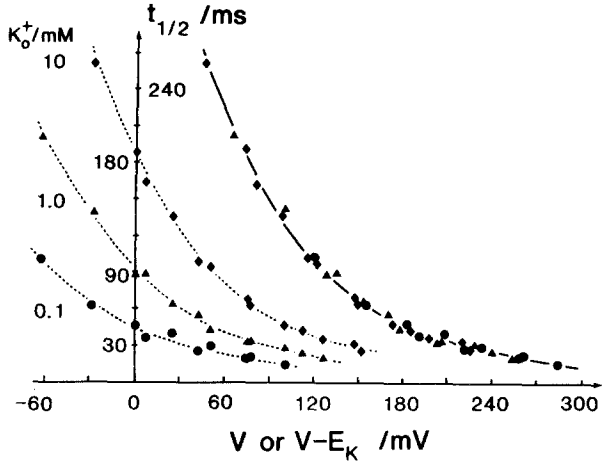

Fig. 4. Rising current dependence on potassium and voltage. Data from Fig. 2. Double-pulse measurements following steps from a common 1-sec conditioning clamp to 0 mV gave equivalent results and are included. Rise half-times ($t_{1/2}$, calculated from the time-dependent component following the voltage and instantaneous current step) are plotted against clamp voltage (V, dotted lines) for 0.1 (●), 1.0 (▲) and 10 (♦) mM K_o^+, and are replotted as a function of the voltage displacement V-E_K (solid line) assuming 200 mM $K_i^+$and a voltage response of 55 mV/[K^+] decade (58 mV/a_{K+} decade). The dotted curves are a joint fitting to single exponentials with a common factor in the exponent (*e*-fold rise in $t_{1/2}$ per −75.3 mV); the solid curve was fitted separately (*e*-fold rise in $t_{1/2}$ per −73.6 mV). Increasing K_o^+ 10-fold or shifting V by *c.* −55 mV effected approx. a twofold rise in $t_{1/2}$. Comparable results from five other cells gave rise half-times which varied by less than 22%

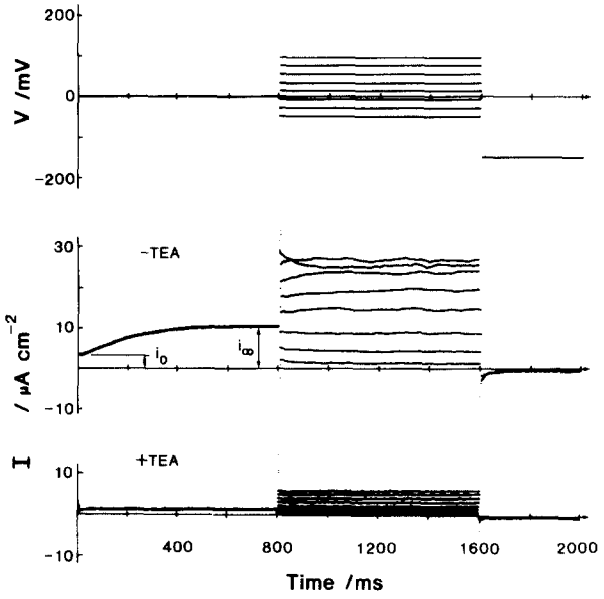

Fig. 5. Current response to K^+ channel block with TEA. Background K_o^+, 3 mM. Data from eight, successive clamp cycles. *Upper frame:* triple pulse protocol with recorded voltages (conditioning voltage, 0, mV; eight voltage steps, one per clamp cycle, between −50 and +100 mV; tailing voltage, −150 mV). Cell held at V_m (= −64 mV) between cycles. *Middle frame:* clamp current, without TEA present. Instantaneous (i_0) and final steady-state (i_∞) current on stepping from V_m as indicated. Note the current decay on stepping to +100 and to −150 mV. *Lower frame:* clamp current, with 10 mM TEA present, showing the overall reduction in conductance and loss of the rising current

Fig. 3. Half-times for the rising currents shown were 104, 147, 190 and 239 msec in 1, 3, 10 and 30 mM K_o^+, respectively. Rise-times were independent of the voltage "history" of the cell, since preceding clamp steps with conditioning pulses gave equivalent results (*see* Fig. 4).

These findings point to an apparent "electrochemical" equivalence between voltage and K_o^+: current rise-times followed the voltage displacement from E_K, not the voltage itself (Fig. 4). The conclusion extends, at least qualitatively, also to features of the conductance relations which were rightshifted along the voltage axis with K_o^+ (Fig. 1), and to the current which decayed on stepping to extreme positive voltages in 0.1 mM, but not in 10 mM K_o^+ (*compare* currents at +115 and +150 mV in Fig. 2). [A dependence on the voltage difference, V-E_K, does not extend to current inactivation at potentials negative to E_K. Careful scrutiny of the current tails at a near-constant displacement from E_{rev} (approx. E_{rev}-20 mV in Fig. 3: from the bottom, first trace in 1 mM K_o^+ and second traces in 3, 10 and 30 mM K_o^+) shows that the current declined more slowly with increasing K_o^+ (τ: 9.3 msec, 1 mM; 23.8 msec, 3 mM; 47.6 msec, 10 mM; 66.7 msec, 30 mM K_o^+). Details of the current kinetics and conductance, and their dependence on the external ionic environment are subjects of forthcoming publications (*manuscripts in preparation*).]

TEA

One remarkable feature of the guard cells examined in this study draws on the relationship between V_m and K_o^+. The near-Nernstian response of V_m to K_o^+ suggested a membrane characteristic dominated by the K^+ conductance near the free-running membrane potential; yet, the free-running potential invariably lay positive to E_{rev}, frequently in excess of 15 mV even in 10 mM K_o^+. For the data in Fig. 3, V_m was −131 (1 mM), −109 (3 mM), −78 (10 mM) and −54 mV (30 mM K_o^+), or displaced +17, +10, +13 and +11 mV from E_{rev}, respectively. This relationship points to an appreciable K^+ current (net K^+ efflux) occurring at the free-running potential and balanced by a background of inward-going current (at V_m the net membrane current, i_m = 0).

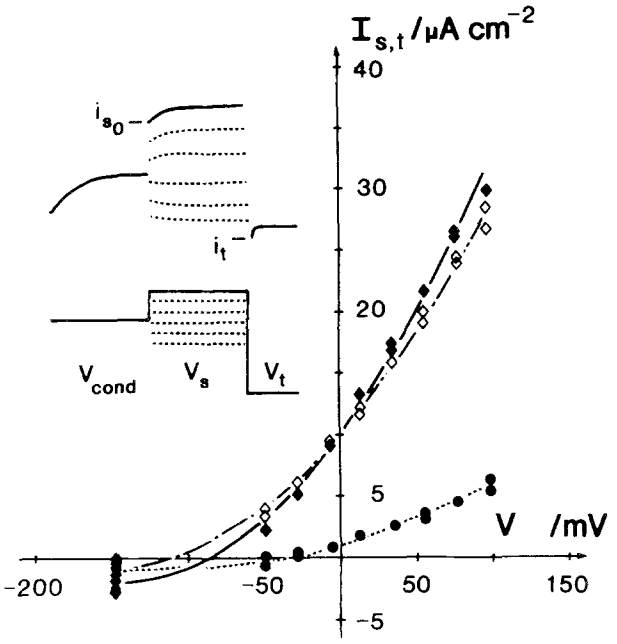

Fig. 6. Instantaneous *I-V* relations with and without TEA. Data from Fig. 5 (♦) and similar measurements carried out in 1 mM K_o^+ (◇) from the same cell. Points taken as shown in the inset (points between −50 and +100 mV at i_{s0}; points for −150 mV at i_t on stepping to V_t from V_s at −6 and +14 mV). Measurements in 10 mM TEA were superimposable and data points have been combined (●). Curves are empirical fittings to 2nd order polynomials by nonlinear least-squares (Marquardt, 1963). The instantaneous conductance at 0 mV (= V_{cond}) increased by 26% with passage from 1 to 3 mM K_o^+ (127 to 160 μS cm^{-2}), after accounting for the background conductance in TEA

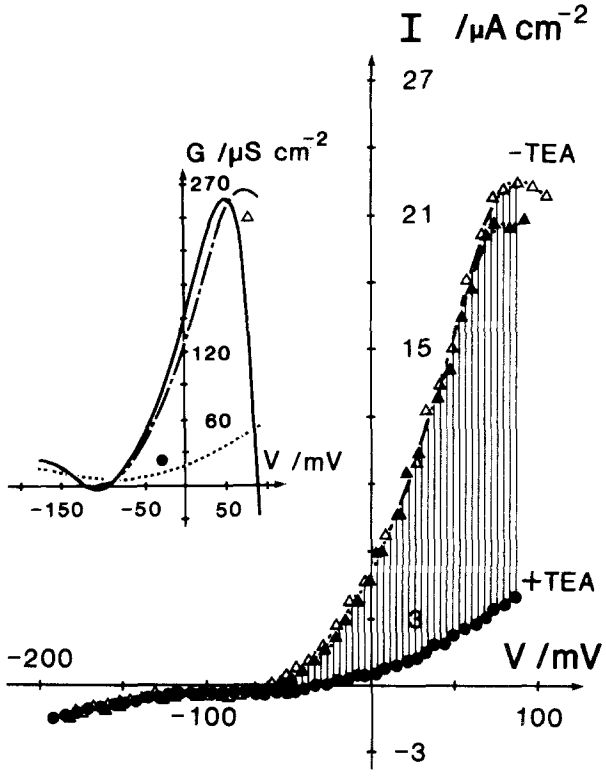

Fig. 7. Steady-state response to TEA. Current and voltage sampled in scans from V_m (150 msec, bipolar staircase). Background K_o^+, 3 mM. Measurement in 10 mM TEA (●, V_m = −26 mV) bracketted by scans run before (△, V_m = −64 mV) and after (▲, V_m = −62 mV) the treatment (3.1 min total elapsed time). Subtracting *I-V* curves ±TEA yields the TEA-sensitive current (shading). *Inset:* corresponding *G-V* curves. Also plotted is the chord conductance relation (-●-) for the K^+ subtraction (shaded) current (assumed E_K = −100 mV). Chord conductances have been scaled by the factor 2.7 (true peak conductance estimated, 102 μS cm^{-2}) for comparison with the slope conductance profile. The close similarity arises because the background conductance (+TEA) is small and largely voltage insensitive, and because the K^+ current rectifies strongly. For the same reasons, equivalent relationships can be drawn at the other K_o^+ concentrations

As a test of this prediction, membrane potentials and the *I-V* characteristics for three cells were determined in, and bracketing treatments with the classic K^+ channel blocker tetraethylammonium (TEA) chloride. Provided that the channel blocker affected only the K^+ conductance, adding TEA would be expected to evoke a positive-going shift in V_m if a significant K^+ current were present in the control. The approach also offered a preliminary means to extracting the K^+ current profile from the whole membrane *I-V* relations; subtracting currents recorded in TEA from those measured without the blocker would yield the *I-V* curve for the K^+ current, assuming that block was essentially complete across the accessible voltage spectrum and, again, that the remaining background current was unaffected.

The voltage traces and *I-V* records, indeed, indicated TEA block of an outward-directed K^+ current at V_m. Adding 10 mM TEA in the presence of 1 to 3 mM K_o^+ shifted V_m positive 28–45 mV to potentials between −26 and −40 mV, and recovery was complete on washing out the channel blocker (*not shown*). The *I-V* relations and current records taken during these maneuvers confirmed the link to the rising current and conductance, which were lost in TEA (Figs. 5–7).

Four additional conclusions were drawn from these studies and are illustrated with representative data from one cell in Figs. 5–7. (1) In TEA, a residue only of instantaneous current was recorded, thus supporting the notion that an appreciable fraction of channels were open at V_m. Subtracting these currents at each clamp step from the corresponding controls (−TEA) showed that 23 to 27% of the final steady-state K^+ current [= $100 \cdot (i_0^{-TEA} - i_0^{+TEA})/(i_\infty^{-TEA} - i_\infty^{+TEA})$; *see* Fig. 5] was realized instantly on stepping from V_m in 1 and 3 mM K_o^+. (2) The background current recorded in TEA showed only weak

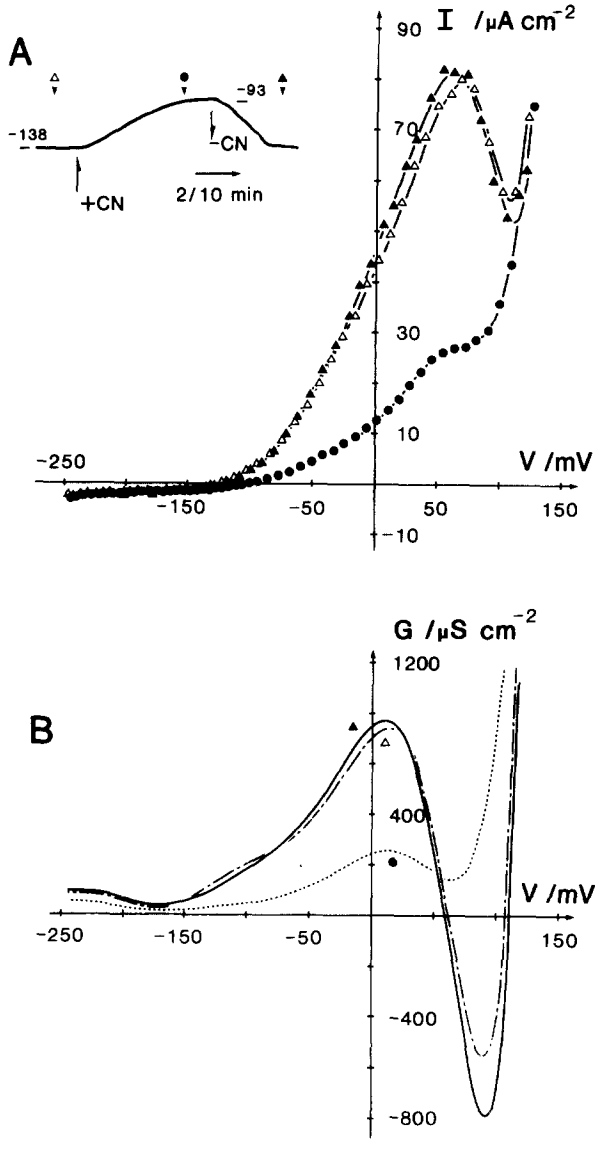

Fig. 8. Steady-state response to 0.3 mM NaCN (plus 0.4 mM SHAM). Background, 5 mM Ca^{2+}-HEPES and 0.1 mM KCl. Current-voltage (*A*) and conductance-voltage (*B*) curves before (△), during (●) and after (▲) cyanide exposure. *Inset:* corresponding voltage trace with recorded potentials (in mV) and times of cyanide addition (↑, +CN) and washout (↓, −CN) as indicated. Symbols indicate times of *I-V* scans (masked from trace) and cross-reference the *I-V* and *G-V* curves. Time scale is 2 min up to, and 10 min following cyanide washout (↓). A slow drift to positive potentials continued up to cyanide washout. Note the residual K^+ channel current in cyanide, as indicated by the *I-V* "shoulder" (near +50 mV) and conductance maximum (near 0 mV). Subtracting currents at V_m in the control (−CN) suggested a K^+ current near 0.5 to 0.6 μA cm^{-2}, assuming charge movement through the K^+ channel only was affected

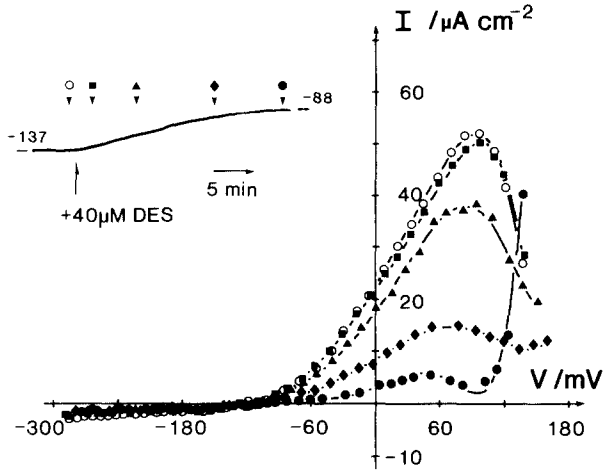

Fig. 9. Block of K^+ current by diethylstilbesterol (DES). Steady-state *I-V* scans taken before and after adding 40 μM DES to the background 5 mM Ca^{2+}-HEPES, pH 7.4, plus 0.1 mM KCl. Symbols cross-referenced to the voltage trace (*Inset*, recorded potentials in mV) indicate times the *I-V* scans (masked from trace) were run

sensitivity to voltage (Figs. 6, 7) and, at potentials negative from the expected E_K, overlapped with those recorded without TEA. These results affirmed use of the whole-cell *I-V* relations in identifying the K^+ conductance (*see* Fig. 7) and provided a check against nonspecific effects of TEA. (3) Values for E_K near −100 mV in 3 mM K_o^+ and −130 mV in 1 mM K_o^+ were indicated by intersections of instantaneous *I-V* curves (±TEA, Figs. 6); correcting for K^+ activity [activity coefficients, $\sigma_o = 0.92$, $\sigma_i = 0.61$, based on free solution standards (Robinson & Stokes, 1959)] gave reasonable concentrations of 250–270 mM for K_i^+, as well (*compare* with Fig. 3 and discussion). Finally, (4) subtractions (±TEA, *see* Fig. 7) yielded K^+ currents at V_m in the controls (−TEA; at V_m, in the steady state, $i_m = 0 = i_{K^+} + i_{bkgd}$) of 0.38 μA cm^{-2} at 3 mM K_o^+. For the three cells the range was 0.31 to 0.76 μA cm^{-2} with 1 and 3 mM K_o^+, equivalent to a K^+ flux (outward) of 2.9 to 7.3 pmol cm^{-2} sec^{-1}. TEA also depolarized (positive-going) V_m in 0.1 mM K_o^+, hence pointing to the outward-going K^+ current at potentials as negative as −178 mV, as well.

Cyanide

It is clear that guard cell potentials, in some instances, may be determined primarily by an outward-directed K^+ current balancing, as yet undefined, inward-directed charge movements. So, the slow decay in V_m of these cells with cyanide, which I remarked on before, raises the issue of K^+ channel sensitivity to the inhibitor.

Figure 8 shows data from one cell exposed to NaCN plus SHAM (+CN) in 5 mM Ca^{2+}-HEPES

and 0.1 mM. KCl[3]. Quantitatively similar results were obtained from three other cells, while no effect was observed on adding SHAM alone (*not shown*). Cyanide-induced depolarizations followed decays in outward current, the conductance maximum near 0 mV and the negative (slope) conductance region near +100 mV, assigned to the putative K^+ channel; both steady-state and instantaneous (*not shown*) K^+ currents were reduced in scalar fashion. Current and conductance characteristics—and V_m—recovered on washing out the inhibitors. Note, in Fig. 8, that the rapidly rising current at voltages positive to +100 mV, and currents negative to V_m (−CN) were largely unaffected. Also recorded was an analogous response (Fig. 9) to diethylstilbesterol (DES), an inhibitor often used to block the plant plasma membrane H^+-ATPase (*cf.* Cheeseman et al., 1980; but also Beilby, 1984, 1986; Bisson, 1986).

Discussion

Gating Characteristics

Negative slope conductances are commonly associated with the early depolarizing currents of excitable cells, but they can occur whenever membrane conductance becomes a steep function of voltage (Finkelstein & Mauro, 1977). The slope of a process obeying Ohm's law is defined by the relation

$$dI/dV = G + V(dG/dV) \quad (1)$$

and will drop below zero whenever the second term in the sum exceeds the first in magnitude, and V or dG/dV is negative. For the diffusive movement of an ion across a membrane, V must be referenced to the equilibrium potential, E_{ion}. So, negative slopes may be observed when G rises steeply with voltage negative to E_{ion}, or when G falls rapidly with voltage positive to E_{ion}. Both of these conditions are met for the K^+ conductance described, and the interpretation, thus, ties together a novel assortment of parallel kinetic and pharmacological properties.

One intriguing feature of the K^+ current is its dependence on K_o^+. Conductances following the voltage difference, V-E_K, and steady-state I-V curves which intersect above E_K ("crossover") are well documented for inward-rectifying K^+ channels (Hille & Schwarz, 1978; Hagiwara, 1983). These characteristics have been understood in terms of single-file, multi-site pores in which ions entering from one side of the membrane displace a blocking "molecule" coming from the other side (Armstrong, 1975; Hille & Schwarz, 1978). The concept accounts for enhanced outward currents when the transported ion is added outside, and might explain the K_o^+-dependent conductance near 0 mV and K^+ current decay at positive potentials in the guard cells.

Analogous behavior can be observed of the classic outward rectifier (K^+ channel) in squid axons perfused with TEA (Armstrong, 1971) and with the physiologically relevant cation, Na^+ (Bezanilla & Armstrong, 1972; French & Wells, 1977). Furthermore, anomolous (inward) rectification of K^+ channels in mammalian heart muscle appears to require normal levels of intracellular Mg^{2+} (Horie, Irisawa & Noma, 1987; Vandenberg, 1987). So, one question is whether a blocking molecule within the guard cells might give rise to the conductance maximum and negative slope regions observed at positive potentials.

By itself, however, the concept of a multi-ion pore and internal blocking molecule fails to accommodate at least one feature of the guard cell K^+ channel, namely the shift in voltage-dependence with K_o^+ which must underly the I-V crossovers near and negative to E_K (*see* Fig. 1). An analogous dependence on external pH is known for H^+ channels of snail neurones, and there is good reason to suspect that H_o^+ interacts directly with H^+ channel gating (Byerly, Meech & Moody, 1984). This idea has the virtue of proposing that cation availability *outside* affects the population of open or available channels which normally pass the cation drawn from *internal* pools only. Again, the concept has been applied to inward rectifiers (Ciani et al., 1978). In the present context, K_o^+-bound channels might be disposed to close at potentials negative to E_K, especially if the regulatory binding site was within (and sensitive to) the membrane electric field. Such a proposal raises questions about interactions with other external cations, notably Ca^{2+} (Armstrong & Matteson, 1986), in gating as well.

The dual gating characteristic, itself, has not generally been known outside of mitochrondrial VDAC channels (Colombini, 1979), Cl^- channels of cultured muscle cells (Blatz & Magleby, 1983) and artificial membranes doped with cation-selective, Excitability Inducing Material (Mueller & Rudin,

[3] Adding 1 mM NaCl against a background of 0.1 mM KCl was observed to shift V_m positive (+) 2 to 3 mV in two cells, but without an appreciable change (within normal data scatter) in the I-V characteristic (*see also* Blatt, 1987*a*). The channel probably is highly selective for K^+ over Na^+, but the subject was not pursued further at this time in view of uncertainties about the Na^+ content of the cells.

1968). So, it is interesting that several features of the channels in guard cells may find parallels in one other cell type. When bathed in millimolar K_o^+, the giant alga *Chara* also exhibits a K^+ current, sensitive to TEA (and to DES) and bounded by regions of negative slope conductance (Beilby, 1986). Channel gating in *Chara*, likewise, may respond to K_o^+, although a quantitative assessment of the time dependence for the current remains to be sorted out (*but see* Laver & Walker, 1987). From steady-state *I-V* measurements the current does not appear to rectify strongly, as it does in the guard cells. Nonetheless, the resemblance raises the question of whether dual-gating and K_o^+-dependent characteristics might be common, at least among K^+ channels in plants.

Activation by Ca^{2+}?

There is a similarity also between the K^+ channel described above for guard cells and the Ca^{2+}-activated K^+ currents of snail pace-maker neurones (*cf.* Meech & Standen, 1975; Lux & Hofmeier, 1982). Most notably, steady-state currents through both channels rise rapidly positive to V_m but decline beyond approx. +50 to +100 mV. Current decline in the snail neuron is related to Ca^{2+} influx which falls off as the clamp potential approaches the Ca^{2+} equilibrium potential. Since the equilibrium potential for Ca^{2+} in the present experiments probably was close to +100 mV, an analogous relationship may hold also for the guard cell K^+ current.

One feature does distinguish the guard cell K^+ current from that of the snail. In the latter case, increasing (positive to V_m) clamp voltage *slows* the current rise-time (contrast with Fig. 4). This point aside, calcium influence on stomatal behavior is pronounced, if complex (MacRobbie, 1987), and Ca^{2+} effects on the K^+ channel deserve close examination.

Response to Metabolic Depression

That guard cell K^+ current is sensitive to cyanide is not wholly surprising, but the point is significant in context of assessing primary charge transport in vivo and its role in controlling stomatal movement. Rapid electrical responses to metabolic poisons have been used frequently to characterize ATP-dependent ion pumping (*above* and *see also* Gradmann et al., 1978; Felle, 1981, 1982), despite the fact that secondary consequences of metabolic blockade are undoubtedly diverse (*cf.* Sanders & Slayman, 1982; Blatt, Rodriguez-Navarro & Slayman, 1987). So, knowing the nature of the transporters affected and their response is valuable from the electrical standpoint alone. It is reassuring that cyanide depresses guard cell pump and K^+ channel conductance with markedly differing time dependencies. Nonetheless, a decline in K^+ conductance may have accounted for rising *difference* currents near 0 mV observed occasionally in pump *dI-V* curves from guard cells treated with NaCN (Blatt, 1987*b*) and from *Chara* exposed to DES (Beilby, 1984).

Cyanide block of the K^+ current bears on models for stomatal action, as well. Metabolic poisons are known to inhibit stomatal closure induced by the phytohormone abscisic acid (Weyers et al., 1982). This observation is difficult to reconcile with a drop in pump output and the notion that modulating pump activity alone might control K^+ flux in guard cells, but it can be understood if metabolic blockade also reduces K^+ conductance.

Implications of Channel Behavior for Guard Cell Physiology

In itself, outward-rectification implies a role in K^+ loss from guard cells during stomatal closure. The notion is not new (*cf.* MacRobbie, 1987; Schroeder et al., 1987), but it takes on an added dimension in the present context. Gearing channel opening to the voltage difference V-E_K, rather than to V alone, may be seen to ensure that net K^+ movement through the channel will be directed outward, regardless of E_K or external K^+ conditions. Early studies with guard cells in epidermal peels (Fischer & Hsiao, 1968) are a reminder of the remarkably wide concentration range (0.1 to 100 mM K_o^+) over which stomatal movements may take place. Clearly guard cells are able to take up *and to release* K^+, even as E_K varies over nearly 180 mV within the normal physiological voltage range!

A singular observation is that of outward-directed K^+ currents at the free-running membrane potential[4], even in 0.1 mM K_o^+. The corresponding K^+ flux is a few percent only of that estimated to occur during stomatal closure (*see* Introduction). However, the channel clearly is capable of passing

[4] Since guard cells in these experiments were probably closed, it might be asked why they should continue to lose K^+, albeit at a low rate. A flux of 7 pmol cm^{-2} sec^{-1} (cell surface area, 2×10^{-5} cm^2, volume 5 pl) is equivalent to a loss of 1.4×10^{-6} mol K^+ $cell^{-1}$ sec^{-1} or approx. 1.6 mM K^+ $cell^{-1}$ min^{-1}. This figure is close to that for steady-state salt leakage from microelectrodes (*cf.* Blatt & Slayman, 1983). Microelectrode impalement of small cells may, in some instances, "clamp" intracellular K^+ concentration (Rodriguez-Navarro, Blatt & Slayman, 1986). So one explanation is that the guard cells may have been dumping an excess of K^+ entering via the electrode.

10 μA cm^{-2} ($\simeq$100 pmol cm^{-2} sec^{-1}) and more over the entire K_o^+ range, provided that the membrane potential is driven sufficiently positive (*compare* Schroeder et al., 1987). The question now is whether, during stomatal closure, V_m *does* shift to the right along the voltage axis (e.g. in response to an anion channel opening, as Schroeder et al. suggest) or whether, additionally, the K^+ conductance itself might be affected. Some degree of control on the K^+ channel characteristic is implicated by the fact that previous *I-V* studies of guard cells (Blatt, 1987*b*), on occasion, failed to pick up the rising current and conductance of the channel.

Relationship to K^+ Channels in Protoplasts

It is tempting to associate K^+ currents recorded in intact *Vicia* guard cells with an outward-rectifying component to single-channel and whole-cell K^+ currents observed in the guard cell protoplasts (Schauf & Wilson, 1987; Schroeder et al., 1987). But apart from the similarities in voltage dependence, a quantitative comparison under similar K_o^+ and clamp conditions highlights two outstanding differences: (1) current kinetics in the protoplasts, and those reconstructed from single-channel records gave rise half-times 2- to 20-fold longer than in the intact cells; and (2) at any one potential, steady-state K^+ current in the protoplasts, likewise, was typically 3- to 10-fold lower.

There is an obvious physical distinction between the intact plant cell and its protoplast that portends divergent membrane physiologies. Quite apart from its physico-mechanical function in containing the plant cell, the wall may interact directly in the gating of channels, notably those sensitive to shearing force or stretch, as Edwards and Pickard (1987) have suggested. Clearly, it is premature to equate the K^+ channels in the intact guard cells and protoplasts. Nonetheless, the distinctions may not be fundamental; factors contributing to channel and current kinetics and gating—including Ca^{2+} (Armstrong & Matteson, 1986), intracellular K^+ or pH (Moody & Hagiwara, 1982), ATP (Bezanilla et al., 1986), and possibly distention of the membrane itself (Guharay & Sachs, 1984)—remain to be identified.

Dr. Peter McNaughton (Physiology, Cambridge), Mr. Mark Tester and Prof. E.A.C. MacRobbie (Botany, Cambridge) contributed to discussions as I was preparing this manuscript. Financial support came from the Science and Engineering Research Council (UK).

References

Armstrong, C. 1971. The interaction of tetraethylammonium ion derivatives with potassium channels of giant axons. *J. Gen. Physiol.* **58:**413–437

Armstrong, C. 1975. Ionic pores, gates and gating currents. *Q. Rev. Biophys.* **7:**179–210

Armstrong, C., Matteson, D. 1986. The role of calcium ions in the closing of K^+ channels. *J. Gen. Physiol.* **87:**817–832

Barry, P.H. 1970. Volume flows and pressure changes during an action potential in cells of *Chara australis*. II. Theoretical considerations. *J. Membrane Biol.* **3:**335–371

Beilby, M.J. 1984. Current-voltage characteristics of the proton pump at *Chara* plasmalemma: I. pH dependence. *J. Membrane Biol.* **81:**113–125

Beilby, M.J. 1986. Factors controlling the K^+ conductance in *Chara*. *J. Membrane Biol.* **93:**187–193

Bezanilla, F., Armstrong, C.M. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* **60:**588–608

Bezanilla, F., Caputo, C., DiPolo, R., Rojas, H. 1986. Potassium conductance of the squid giant axon is modulated by ATP. *Proc. Natl. Acad. Sci. USA* **83:**2743–2745

Bisson, M.A. 1986. Inhibitors of proton pumping: Effect on passive proton transport. *Plant. Physiol.* **81:**55–59

Blatt, M.R. 1987*a*. Electrical characteristics of stomatal guard cells: The ionic basis of the membrane potential and the consequence of potassium chloride leakage from microelectrodes. *Planta* **170:**272–287

Blatt, M.R. 1987*b*. Electrical characteristics of stomatal guard cells: The contribution of ATP-dependent, "electrogenic" transport revealed by current-voltage and difference-current-voltage analysis. *J. Membrane Biol.* **98:**257–274

Blatt, M.R. 1987*c*. Fusicoccin, K^+ channels and stomatal closure. *Plant Physiol.* **83:**174A

Blatt, M.R., Rodriguez-Navarro, A., Slayman, C.L. 1987. Potassium-proton symport in *Neurospora:* Kinetic control by pH and membrane potential. *J. Membrane Biol.* **98:**169–189

Blatt, M.R., Slayman, C.L. 1983. KCl leakage from microelectrodes and its impact on the membrane parameters of a nonexcitable cell. *J. Membrane Biol.* **72:**223–234

Blatz, A., Magleby, K. 1983. Single voltage-dependent chloride-selective channels of large conductance in cultured rat muscle. *Biophys. J.* **43:**237–241

Briggs, G.E., Hope, A.B., Robertson, R.N. 1961. Electrolytes and Plant Cells. Blackwells, Oxford

Byerly, L., Meech, R., Moody, W. 1984. Rapidly activating hydrogen ion currents in perfused neurons of the snail, *Lymnaea stagnalis*. *J. Physiol. (London)* **351:**199–216

Ciani, S., Krasne, S., Miyazaki, S., Hagiwara, S. 1978. A model for anomalous rectification: Electrochemical-potential-dependent gating of membrane channels. *J. Membrane Biol.* **44:**103–134

Cheeseman, J., LaFayette, P., Gronewald, J., Hanson, J. 1980. Effect of ATPase inhibitors on cell potential and K^+ influx in corn roots. *Plant Physiol.* **65:**1139–1145

Coleman, H.A., Findlay, G.P. 1985. Ion channels in the membrane of *Chara inflata*. *J. Membrane Biol.* **83:**109–118

Colombini, M. 1979. A candidate for the permeability pathway of the outer mitochondrial membrane. *Nature (London)* **279:**643–645

Edwards, K., Pickard, B.G. 1987. Detection and transduction of physical stimuli in plants. *In:* The Cell Surface in Signal

Transduction. E. Wagner, H. Greppin, and B. Millet, editors. pp. 41–66. NATO-ASI Series H12. Springer, Berlin

Felle, H. 1981. A study of the current-voltage relationships of electrogenic active and passive membrane elements in *Riccia fluitans*. *Biochim. Biophys. Acta* **646:**151–160

Felle, H. 1982. Effects of fusicoccin upon membrane potential, resistance and current-voltage characteristics in root hairs of *Sinapis alba*. *Plant. Sci. Lett.* **25:**219–225

Finkelstein, A., Mauro, A. 1977. Physical principles and formalisms of electrical excitability. *In:* Handbook of Physiology. Vol. 1, pp. 383–441. E. Kandel, editor. APS, Bethesda, Maryland

Fischer, R., Hsiao, T. 1968. Stomatal opening in isolated epidermal strips of *Vicia faba*. II. Responses to KCl concentration and the role of potassium absorption. *Plant Physiol.* **43:**1953–1958

French, R.J., Wells, J. 1977. Sodium ions as blocking agents and charge carriers in the potassium channel of the squid giant axon. *J. Gen. Physiol.* **70:**707–724

Gaffey, C., Mullins, L. 1958. Ion fluxes during the action potential in *Chara*. *J. Physiol.* (*London*) **144:**505–524

Gradmann, D. 1975. Analog circuit of the *Acetabularia* membrane. *J. Membrane Biol.* **25:**183–208

Gradmann, D., Hansen, U.P., Long, W.S., Slayman, C.L., Warncke, J. 1978. Current-voltage relationships for the plasma membrane and its principal electrogenic pump in *Neurospora crassa:* I. Steady-state conditions. *J. Membrane Biol.* **3:**333–367

Guharay, F., Sachs, F. 1984. Stretch activated single ion-channel currents in tissue-cultured embryonic chick skeletal muscle. *J. Physiol.* (*London*) **352:**685–701

Gustin, M., Martinac, B., Saimi, Y., Culbertson, M., Kung, C. 1986. Ion channels in yeast. *Science* **233:**1195–1197

Hagiwara, S. 1983. Membrane Potential-Dependent Ion Channels in Cell Membrane. Raven, New York

Harold, F.M. 1987. The Vital Force: A Study of Bioenergetics. Freeman, New York

Hille, B. 1984. Ion Channels of Excitable Membranes. Sinauer, Sunderland, Massachusetts

Hille, B., Schwarz, W. 1978. Potassium channels as multi-ion single-file pores. *J. Gen. Physiol.* **72:**409–442

Hodgkin, A., Huxley, A.F., Katz, B. 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol.* (*London*) **116:**424–448

Homblé, F., Ferrier, J., Dainty, J. 1987. Voltage-dependent K^+ channel in protoplasmic droplets of *Chara corallina*. *Plant Physiol.* **83:**53–57

Hope, A.B., Walker, N.A. 1975. The Physiology of Giant Algal Cells. Cambridge University Press, Cambridge

Horie, M., Irisawa, H., Noma, A. 1987. Voltage-dependent magnesium block of adenosine-triphosphate-sensitive potassium channel in guinea-pig ventricular cells. *J. Physiol.* (*London*) **387:**251–272

Kitasato, H. 1973. K^+ permeability of *Nitella clavata* in the depolarised state. *J. Gen. Physiol.* **62:**535–549

Kolb, H.A., Köhler, K., Martinoia, E. 1987. Single potassium channels in membranes of isolated mesophyll barley vacuoles. *J. Membrane Biol.* **95:**163–169

Laver, D.R., Walker, N.A. 1987. Steady-state voltage-dependent gating and conduction kinetics of single K^+ channels in the membrane of cytoplasmic drops of *Chara australis*. *J. Membrane Biol.* **100:**31–42

Lühring, H. 1986. Recording of single K^+ channels in the membrane of cytoplasmic drop of *Chara australis*. *Protoplasma* **133:**19–28

Lux, H.D., Hofmeier, G. 1982. Properties of a calcium- and voltage-activated potassium current in *Helix pomatia* neurons. *Pfluegers Arch.* **394:**61–69

MacRobbie, E.A.C. 1987. Stomatal guard cells. *In:* Ion Transport in Plant Cells and Tissues. J. Hall and D.A. Baker, editors. Pitman, London (*in press*)

Marquardt, D. 1963. An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Ind. Appl. Math.* **11:**431–441

Marrè, E. 1985. Fusicoccin- and hormone-induced changes of H^+ extrusion: Physiological implications. *In:* Frontiers of Membrane Research in Agriculture. J. St. John, E. Berlin, and P. Jackson, editors. pp. 439–460. Rowman and Allanheld, Ottowa

Meech, R.W., Standen, N.B. 1975. Potassium activation in *Helix aspersa* neurons under voltage clamp: A component mediated by calcium influx. *J. Physiol.* (*London*) **249:**211–239

Moody, W.J., Hagiwara, S. 1982. Block of inward rectification by intracellular H^+ in immature oocytes of the starfish *Mediaster aequalis*. *J. Gen. Physiol.* **79:**115–130

Moran, N., Ehrenstein, G., Iwasa, K., Bare, C., Mischke, C. 1984. Ion channels in plasmalemma of wheat protoplasts. *Science* **226:**935–938

Mueller, R., Rudin, D. 1968. Resting and action potentials in experimental bimolecular lipid membranes. *J. Theor. Biol.* **18:**222–258

Oda, K. 1962. Polarised and depolarised states of the membrane in *Chara braunii*, with special reference to the transition between the two states. *Tohoku Univ. Sci. Report IV* **28:**1–16

Outlaw, W. 1983. Current concepts on the role of potassium in stomatal movements. *Physiol. Plant.* **59:**302–311

Robinson, R., Stokes, R. 1959. Electrolyte Solutions. Butterworths, London

Rodriguez-Navarro, A., Blatt, M.R., Slayman, C.L. 1986. A potassium-proton symport in *Neurospora crassa*. *J. Gen. Physiol.* **87:**649–674

Sanders, D., Slayman, C.L. 1982. Control of intracellular pH: Predominant role of oxidative metabolism, not proton transport, in the eukaryotic microorganism *Neurospora*. *J. Gen. Physiol.* **80:**377–402

Schauf, C., Wilson, K. 1987. Effects of abscisic acid on K^+ channels in *Vicia faba* guard cell protoplasts. *Biochem. Biophys. Res. Commun.* **145:**284–290

Schroeder, J., Raschke, K., Neher, E. 1987. Voltage dependence of K^+ channels in guard cell protoplasts. *Proc. Natl. Acad. Sci. USA* **84:**4190–4112

Vandenberg, C.A. 1987. Inward rectification of potassium channel in cardiac ventricular cells depends on internal magnesium ions. *Proc. Natl. Acad. Sci. USA* **84:**2560–2564

Weyers, J.D., Patterson, N.W., Fitzsimons, P.J., Dudley, J.M. 1982. Metabolic inhibitors block ABA-induced stomatal closure. *J. Exp. Bot.* **33:**1270–1278

Wille, A., Lucas, W. 1984. Ultrastructural and histochemical studies on guard cells. *Planta* **160:**129–142

Received 5 October 1987; revised 19 January 1988