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Blockade of Potassium Channels in the Plasmalemma of *Chara corallina* by Tetraethylammonium, Ba^{2+}, Na^+ and Cs^+

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Summary. The outer membranes of plant cells contain channels which are highly selective for K^+. In the giant-celled green alga *Chara corallina*, K^+ currents in the plasmalemma were measured during the action potential and when the cell was depolarized to the K^+ equilibrium potential in high external K^+ concentrations. Currents in both conditions were reduced by externally added tetraethylammonium (TEA^+), Ba^{2+}, Na^+ and Cs^+. In contrast to inhibition by TEA^+, the latter three ions inhibited inward K^+ current in a voltage-dependent manner, and reduced inward current more than outward. Ba^{2+} and Na^+ also appeared to inhibit outward current in a strongly voltage-dependent manner. The blockade by Cs^+ is studied in more detail in the following paper. TEA^+ inhibited both inward and outward currents in a largely voltage-independent manner, with an apparent K_D of about 0.7 to 1.1 mM, increasing with increasing external K^+. All inhibitors reduced current towards a similar linear leak, suggesting an insensitivity of the background leak in *Chara* to these various K^+ channel inhibitors. The selectivity of the channel to various monovalent cations varied depending on the method of measurement, suggesting that ion movement through the K^+-selective channel may not be independent.

Key Words K^+ · ion channel · current-voltage curves · action potential · voltage clamp

Introduction

In the plasmalemma of giant cells of the green alga *Chara corallina*, there are K^+-selective channels which open during the action potential, thereby greatly increasing K^+ efflux (Kitasato, 1973). K^+ channels also dominate membrane conductance when the cell is depolarized in solutions with a high K^+/Ca^{2+} ratio, when the PD stays near E_K, the K^+ equilibrium potential ("K-state": Keifer & Lucas, 1982; Beilby, 1985, 1986*a,b*). It is thought that the same channels are responsible for the K^+ fluxes in both processes (Beilby, 1985), and that they may be similar to high unitary conductance Ca^{2+}-activated K^+ channels (high G $K^+(Ca^{2+})$) which are widespread in animal cells (*see* Tester, 1988*a*). In the present study, blockade by TEA^+, Ba^{2+}, Na^+ and Cs^+ of the K^+ channels dominating membrane conductance in the K-state of *Chara* was investigated; the properties are compared with those of various channels found in animal cells, and provide a preliminary investigation of the structure of the plant plasmalemma K^+ channel. It is also shown in this work that these blockers all lengthen the action potential of *Chara corallina*, by decreasing the outward K^+ current.

The blockade of K^+ channels in animal cells by Cs^+ is often strongly potential dependent (i.e., the blockade increases with membrane hyperpolarization), and has been fitted to a model of ion blockade developed by Woodhull (1973) and Hille and Schwarz (1978); this is done in detail for the *Chara* K^+ channel in the following paper (Tester, 1988*b*). Blockade of K^+ conductance by Cs^+ has been described in plant tissues (Sokolik & Yurin, 1981, 1986; Schauf & Wilson, 1987*a,b;* Findlay, Tyerman & Paterson, 1988), but usually not in detail. As far as is known, no effect of Cs^+ on plant action potentials has previously been shown.

Ba^{2+} is also a well-characterized blocker of K^+ current in various animal tissues (e.g., Armstrong, Swenson & Taylor, 1982; Vergara & Latorre, 1983; Gitter et al., 1987); the potential-dependent blockade of high G $K^+(Ca^{2+})$ channels has been recently described in detail by Miller, Latorre and Reisin (1987). Ba^{2+} has only been applied occasionally to plant cells, where it has been shown to slow repolarization of the action potential (Barry, 1968; Belton & van Netton, 1971; van Netton & Belton, 1978; Kikuyama, 1986). The slowing of repolarization is presumably due to inhibition of K^+ efflux, although this has not been explicitly suggested. It may also be due to the interaction of Ba^{2+} with Ca^{2+} channels, as has been often described in animal tissues (e.g. Tsien, 1983; Hess & Tsien, 1984), and has

recently been suggested to occur in plant cells (Thaler et al., 1987). Recent patch-clamp studies of K^+ channels in the plasmalemma of different plant tissues, including the charophyte, *Nitellopsis obtusa*, have provided direct evidence for the blockade by Ba^{2+} of K^+ channels in plants (Azimov, Geletyuk & Berestovsky, 1987; Schroeder, Raschke & Neher, 1987).

Na^+ has also been reported to be a blocker of K^+ channels in animal tissues (e.g. French & Wells, 1977; Yellen, 1984), and inhibits high G $K^+(Ca^{2+})$ channels in a voltage-dependent manner (Latorre, 1986). Smith and Kerr (1987) found that quite low concentrations of Na^+ (0.1 mM) greatly reduced K^+ permeability in cells of *Chara corallina*, and it is possible that this was due to an inhibition of the K^+ channels.

Tetraethylammonium (TEA^+) is a classic inhibitor of K^+ channels in numerous tissues (e.g. Armstrong, 1975; Stanfield, 1983), and clearly inhibits the K^+ conductance in charophytes (e.g. Sokolik & Yurin, 1981, 1986; Beilby, 1986*a,b;* Smith & Kerr, 1987). The blockade by TEA^+ has been shown to affect both inward and outward current, and the blockade is potential independent (Sokolik & Yurin, 1986). However, the affinity of the inhibition of K^+ current by TEA^+ has not been studied in detail, nor has the effect of external K^+ on the inhibition been investigated. TEA^+ has been shown to slow repolarization of the action potential in various species of *Nitella* (Belton & van Netton, 1971; Koppenhofer, 1972; van Netton & Belton, 1978; Shimmen & Tazawa, 1983), although no effect has been noticed on the action potential of *Chara* (Beilby & Coster, 1979; Shimmen & Tazawa, 1983).

TEA^+ inhibits the high G $K^+(Ca^{2+})$ channels in a variety of animal tissues (e.g. Hermann & Gorman, 1981; Vergara, Moczydlowski & Latorre, 1984; Yellen, 1984; Findlay, Dunne & Petersen, 1985), and appears to be much more effective when added externally than when added internally. The concentration required for 50% inhibition often appears to be around 0.1 to 1.0 mM. However, its mode of action varies between tissues, and the blockade may be voltage dependent or independent, and may reduce single-channel conductance only or may also reduce the probability of a channel being open. As Stanfield (1983, p. 45) says, "few general rules emerge as to TEA affinity" for the different classes of K^+ channel (*see also* Hille, 1984, p. 112); TEA^+ would not appear to be a very useful inhibitor for investigating the properties of channels for identification and comparison with those in other systems, although in combination with numerous other quaternary ammonium ions, can provide valuable insights on channel structure (Miller, 1982).

The selectivity of the *Chara* K^+ channel for different monovalent ions is unknown, although it has been measured for *Nitella* by Sokolik and Yurin (1986) and for single channels in the plasmalemma of *Nitellopsis* (Azimov et al., 1987). Classically, selectivity can be determined in two ways; one method is to measure the reversal potential (or resting potential, as long as the channels are open at the resting potentials involved) with the outside of the membrane exposed to different ionic species, and calculate permeability from the Goldman-Hodgkin-Katz relationship. Another method is to measure the conductivity of the K^+ channel to different ions. If ions move through the K^+ channel independently of each other, then the order of conductivity and of permeability should be the same, but it is often found that the two measures of selectivity provide different sequences (e.g. Eisenman, Latorre & Miller, 1986; Tsien et al., 1987). It was of interest to see if this was the case with the *Chara* K^+ channel, and to compare the selectivity sequences with those of various K^+ channels found in animal cells.

Abbreviations: APW, artificial pond water; E_K, equilibrium potential for K^+ ions; *I/V*, current-voltage; PD, potential difference; TEA^+, tetraethylammonium.

Materials and Methods

Current-voltage (*I/V*) relations of the plasmalemma of small leaf cells of *Chara corallina* were used to measure the K^+ and "leak" conductances (Beilby, 1985). Curves of whole cells were obtained with a bipolar staircase of voltage-clamp commands, with 60-msec pulses to various potentials separated by 180 msec at the baseline clamp potential (Beilby, 1985); experimental details are described in Tester (1988*a*). Cells were exposed to 0.1 mM $LaCl_3$ for about 10 min to reduce irreversibly the Ca^{2+} conductance, thus reducing excitability and enabling measurement over a wide voltage span. The $LaCl_3$ was thoroughly washed away, until curves restabilized (La^{3+} also inhibits, reversibly, the K^+ channels: *see* Tester, 1988*a*).

To get into the K-state, the cell was depolarized while in an external solution containing (in mM): 0.5 $CaCl_2$, 1 NaCl, *x* KCl and 2 HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid)/NaOH, pH 6.8 ("*x*K" solution). The concentration of K^+ (*x* mM) varied between 3 and 30 mM in various experiments, and this is denoted by "3K" to "30K" for each solution used. Solutions were flowed at 3 to 10 ml/min (i.e. 3 to 10 chamber volumes/min), but within an experiment, rates were not altered, to eliminate any possible flow effects on PD or *I/V* curves. Cells were illuminated by a fiber optics source, at an intensity of approximately 50-100 μmol $m^{-2}sec^{-1}$.

Action potentials were either allowed to run freely after a short electrical stimulation, or the potential was clamped as described by Beilby and Beilby (1983). Action potentials were fired regularly at either 10- or 15-min intervals, and no treatments were applied before the currents became relatively stable. Cells were kept in conditions as described above, in a solution of (in mM): 0.1 KCl, 0.5 $CaCl_2$, 1 NaCl and 2 HEPES/NaOH, pH 6.8 ("APW").

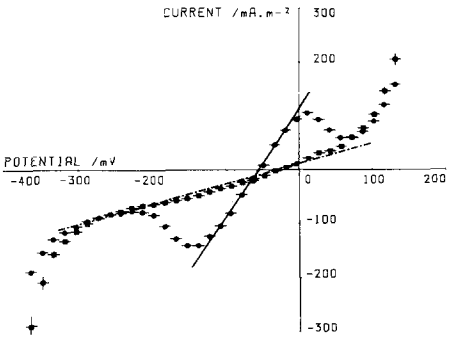

Fig. 1. Characteristics of the *I/V* relations of the plasmalemma of *Chara corallina*. Cell in "K-state," in a 5K solution only (●) or with 10 mM TEACl added (■). Solid line drawn by eye through the reversal potential, of slope G_{K+L}. The broken line is the estimated leak, of slope G_L. Each set of data is the average of four curves run during an experiment, with values averaged over 15 mV potential spans (horizontal bars), with standard errors of the mean (vertical bars)

When the addition of an inhibitor caused a substantial hyperpolarization, the staircase of voltage-clamp commands was run with a baseline potential at the resting potential, and then at the reversal potential found before hyperpolarization (i.e. near E_K, and K^+ equilibrium potential); the baseline voltage was held for about 10 sec before running the voltage staircase. The resulting *I/V* curves were always affected by the baseline clamp potential (Sokolik & Yurin, 1986; Blatt, Tester & Beilby, *in preparation*), and for the final analyses, the *I/V* curves measured with the baseline potential at E_K were used.

When the cell is in K-state, the steady-state *I/V* characteristics are remarkably stable for at least 24 hr (Beilby, 1985). The *I/V* relations show two characteristic regions of negative-slope conductance (e.g. Figs. 1, 4 and 5*a;* and Beilby, 1986*b*). The first is believed to represent the rapid voltage-dependent opening of K^+ channels as the potential moves from around −250 to −150 mV, and the second region may represent the closure of the same channels as the potential moves more positive of about 0 mV. (This second region may be due to the voltage-dependent blockade of the open channel by an internal ion, such as Na^+, rather than a voltage-dependent closure: *see* Discussion.) Between the two regions of negative-slope conductance, membrane conductance is dominated by K^+; beyond are regions with unspecified "leaks." The K^+ conductance across the plasmalemma at the resting potential (G_K) is estimated by subtracting the estimated leak conductance (e.g. the broken line in Fig. 1: G_L) from the slope of the curve through the resting (or reversal) PD (G_{K+L}). Data are presented here without lines drawn, so the raw data can be seen without directing the reader's eye. Leak conductance is assumed to be linear over the voltage range where the K^+ channels dominate plasmalemma conductance (i.e. between the two regions of negative-slope conductance); this is an approximation in some cases (e.g. Fig. 1), but is probably close to reality in others (e.g. Fig. 4 in Tester, 1988*a*).

Data from only one cell are usually presented, but experiments were usually repeated three or more times, and the results were at least qualitatively similar. Most inorganic chemicals were from BDH; organic chemicals were from Sigma. TlCl (from Aldrich) was dissolved on the day of the experiment and stored in the dark, although the solution was exposed to the light for the short time when in the cell chamber.

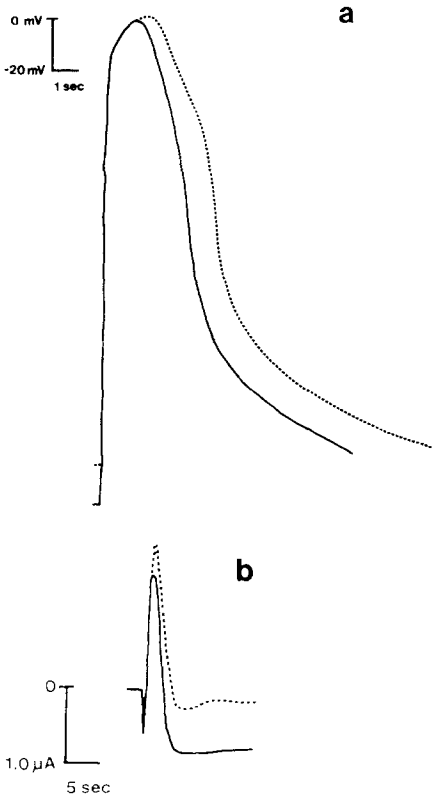

Fig. 2. Reduction of outward current during the action potential by TEA^+. (*a*) Free running action potential of the plasmalemma and tonoplast in series elicited by a short electrical stimulation, in APW only (solid line) and after addition of 5 mM TEACl (dotted line); effects were fully reversible. (*b*) Current required to clamp PD to −30 mV during an action potential, with cell in APW only (solid line), and after addition of 5 mM TEACl (dotted line). The large initial inward current flow (upstroke in the Figure) is carried by an inflow of Ca^{2+} and outflow of Cl^-, and the later, longer-lasting current is due to an outflow of K^+. Cell surface area 2.0×10^{-5} m²

Results

Action Potentials

TEA^+ reduced the K^+ efflux during the action potential. The action potential was clearly and reproducibly lengthened by application of these chemicals, and there was little effect on the potential at which the depolarization was reversed (Fig. 2*a*). When the potential was clamped during the action potential, the largest and most consistent effect of TEA^+ was on the late outward current, which was greatly reduced (Fig. 2*b*). These effects were readily reversed. Addition of CsCl or $BaCl_2$ had similar effects to that of TEA^+; they lengthened the action potential by reducing the late outward current (*results not presented*). However, the effects of Ba^{2+} took much longer to reverse, possibly due to

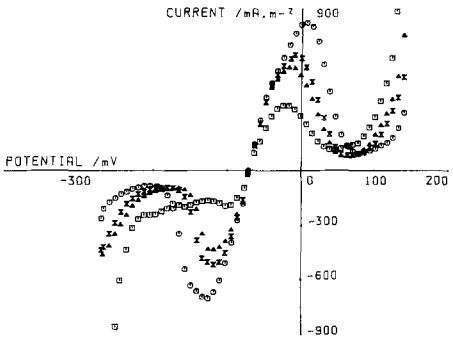

Fig. 3. Effect of Na^+ on K^+ conductance across the plasmalemma of *Chara corallina*. *I/V* relations with the cell in a 5K solution only (○) or with 3 (×), 10 (△) or 30 (□) mM NaCl added

the tight binding of the Ba^{2+} to the channel (cf. Miller et al., 1987), or possibly due to accumulation of Ba^{2+} in the cytoplasm (*see below*). Addition of 10 mM Na^+ also reduced the late outward current during action potentials (*results not presented*); although the effect was smaller than that found for the other ions, it was also repeatable and readily reversible.

Resting Potentials

Addition of Cs^+, TEA^+ or Ba^{2+} often hyperpolarized the cell (*data not shown*), as was found with TEA^+ by Keifer and Lucas (1982) and Smith and Kerr (1987), and for Cs^+ by Sokolik and Yurin (1986), although the latter reported hyperpolarizations of only 4 to 8 mV. For example, in our cells, upon addition of 10 mM TEACl, it was not uncommon to find a hyperpolarization of the order of 50 mV over 4 min; addition of lower concentrations of TEA^+ led to much smaller hyperpolarizations. It is likely that the amount of hyperpolarization is related to the activity of the proton pump in each cell—the cells used by Sokolik and Yurin had very little pump activity, whereas the cells used in these experiments had variable pump activity, but sometimes it appeared to be quite large.

Blockade in K-State

Relatively high concentrations of Na^+ reduced K^+ conductance in the K-state, in a clearly voltage-dependent manner (Fig. 3). Both inward and outward currents were reduced, and the effect on outward current was also clearly voltage-dependent, increasing with increasing outward current, suggesting that the effect on the outward current is from Na^+ that has accumulated in the cytoplasm, possibly entering via the Ca^{2+} channels (Lunevsky et al., 1983). The potency of the Na^+ appeared to be much lower than that reported by Smith and Kerr (1987), but the reason for this is unknown.

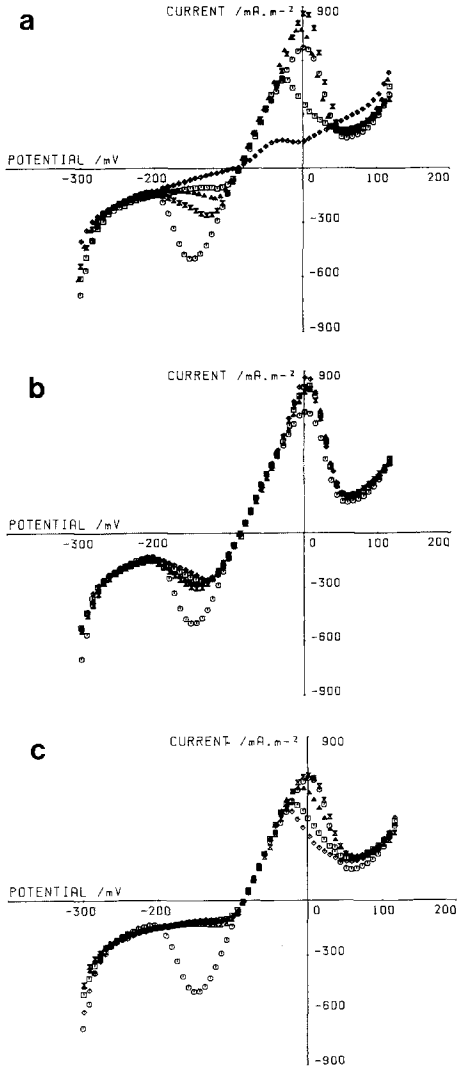

Fig. 4. Effect of Ba^{2+} on K^+ conductance across the plasmalemma of *Chara corallina*. (*a*) Effect of concentration. *I/V* relations measured with the cell in a 5K solution only (○) or with 0.03 (×), 0.1 (△), 0.3 (□) or 1.0 (◇) mM $BaCl_2$ added for 14 min (*b*) Effect of time on inward current. *I/V* relations measured with the cell in a 5K solution only (○) or with 0.03 mM $BaCl_2$ added for 1.5 (×), 4 (△), 8 (□) or 14 (◇) min. (*c*) Effect of time on outward current. *I/V* relations measured with the cell in a 5K solution only (○) or with 0.3 mM $BaCl_2$ added for 1 (×), 4 (△), 9 (□) or 14 (◇) min. The curve measured 25 min after addition of the $BaCl_2$ was indistinguishable from the final curve presented here

The K^+ conductance in the K-state was also reduced upon addition of $BaCl_2$ (Fig. 4). The characteristics of the blockade were similar to those for Na^+, but Ba^{2+} blockade increased more slowly with time, and Ba^{2+} was clearly a much more potent inhibitor than Na^+. Blockade by Ba^{2+} was voltage-

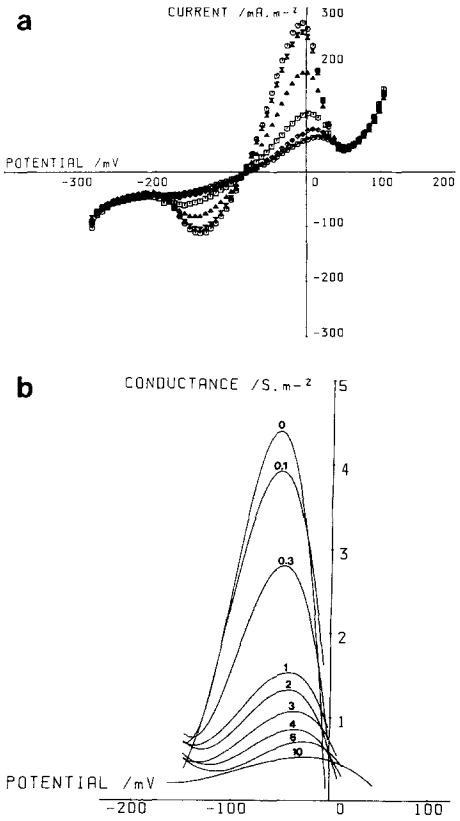

Fig. 5. Effect of TEA^+ on K^+ conductance across the plasmalemma of *Chara corallina*. (*a*) *I/V* relations measured with the cell in a 3K solution only (○) or with 0.1 (×), 0.3 (△), 1.0 (□), 3.0 (◇) or 10 (∅) mM TEACl added. All curves were run at a baseline potential of −87 mV. (*b*) Conductance-voltage (*G/V*) relations calculated from data in Fig. 5*a*, including some extra values excluded from Fig. 5*a* for clarity. Curves were edited to include only values between the regions of negative-slope conductance, then fitted to a 4th order polynomial and differentiated to obtain *G*. Cell in a 3K solution, with 0 to 10 mM TEACl added as indicated by the numbers next to each curve

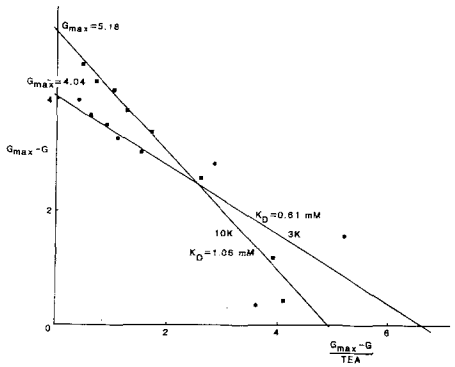

Fig. 6. Summary of the effect of external TEA^+ on K^+ conductance (G_K) across the plasmalemma of *Chara corallina*. Eadie-Hofstee plot, of the amount of K^+ current inhibited by TEA^+ ($G_{max}-G$) against ($G_{max}-G$) divided by the external concentration of TEACl. Data from the cell used in Fig. 5, with an external K^+ concentration of either 3 mM (○) or 10 mM (□). Apparent K_D's and maximum G_K's for this and two other cells summarized in Table 1

dependent, and inward currents were reduced more than outward currents. Blockade increased with concentration (Fig. 4*a*) and with time (Fig. 4*b* and *c*). When the Ba^{2+} was either applied in high concentrations (Fig. 4*a*), or for a longer period of time (Fig. 4*c*), a clear inhibition of inward current was seen, and this blockade was strongly voltage dependent. It is probable that the slow increase in the blockade of outward current is due to a slow increase of Ba^{2+} in the cytoplasm, possibly entering via the Ca^{2+} channels. Blockade was slowly reversible, but was often not completely reversible when high concentrations of Ba^{2+} were applied.

As with Na^+ and Ba^{2+}, addition of Cs^+ clearly reduced inward K^+ fluxes in a concentration- and voltage-dependent manner (*results not presented; see following paper*). The blockade of the inward K^+ current was rapid and completely reversible; the voltage-dependence of the blockade is analyzed in detail in the following paper (Tester, 1988*b*).

The blockade of K^+ conductance by TEA^+ appeared to be voltage independent, and increased with concentration above about 100 μM (Fig. 5). Both inward and outward currents were reduced and the effects were fully and rapidly reversible. The affinity of blockade of K^+ current by TEA^+ was easily determined, as inhibition of G_K was essentially PD independent; therefore G_{K+L} between the two regions of negative-slope conductance remained relatively linear in the presence of TEA^+. The K^+ conductance at the reversal potential could therefore be calculated (by differentiation of polynomial-fitted *I/V* curves: *see* Fig. 5*b*) for different TEA^+ concentrations. Data from one cell at two external K^+ concentrations are presented as Eadie-Hofstee plots in Fig. 6. From these were obtained approximate K_D's for TEA^+, and values from three cells are summarized in Table 1. There appears to be competition between TEA^+ and K^+, with the apparent K_D for TEA^+ increasing some 60%, from 0.67 to 1.09 mM with a greater than threefold increase in K^+, from 3 to 10 mM.

Channel Selectivity

The selectivity of the *Chara* K^+ channel for different monovalent ions was measured by changes in the resting potential upon changes in external monovalent cations. Rapid PD shifts occurred upon substitution of KCl for the Cl^- salt of several other ions (Fig. 7*a*), a shift to less negative potentials oc-

Table 1. The effect on K^+ conductance (G_K) of external TEA^+: Calculated from Eadie-Hofstee plots, such as presented in Fig. 6 (which was cell 963)

Parameter calculated	K_D (mM)		G_{max} (Sm^{-2})	
External [K^+] (mM)	3.0	10	3.0	10
Cell number				
910	0.54	0.94	2.03	3.06
961	0.87	1.27	5.20	11.9
963	0.61	1.06	4.04	5.18
Average	0.67	1.09	3.79	6.72

Table 2. The effect of biionic changes on the resting potential (ΔPD) across the plasmalemma of *Chara corallina*, averaged from two cells[a]

External ion	ΔPD (mV)	Average G_S/G_K
Tl^+	+15	0.93
Rb^+	−5	0.09
Cs^+	−18	0
TEA^+	−38	0
Na^+	−46	0.17
Li^+	−51	0.17

[a] The ratio of the current in a given ionic solution to that in K^+ only is also presented (G_S/G_K), averaged for the two sets of data. The orders of selectivity for both cells were the same, so raw data have not been presented.

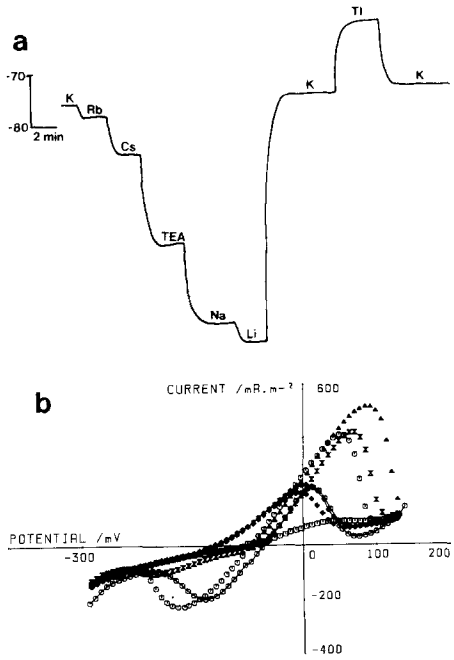

Fig. 7. Selectivity of the K^+ channel. (*a*) Measurement of permeability with biionic potential changes. Plasmalemma potential of a cell initially in a 5K solution, and then with the K^+ substituted for the ion indicated on the Figure. PD's (averaged with a second cell) are summarized in Table 2. (*b*) Measurement of current with changes of external monovalent cations. *I/V* relations with the cell in a 5K solution only (○), or with the K^+ substituted for Tl^+ (∅), Rb^+ (×), Cs^+ (△), TEA^+ (□), Na^+ (◇) or Li^+ (◊). The slopes of the inward currents immediately negative of the reversal potential are presented (averaged with a second cell) in Table 2

curring if the affinity of the channel for an ion was greater than that for K^+ (which only occurred upon addition of Tl^+), and to more negative potentials if the affinity was smaller. It should be noted that at all the resulting potentials, the K^+ channels were still dominating membrane conductance. The permeability sequence thus obtained (Table 2) appeared to be,

$$Tl^+ > K^+ > Rb^+ > Cs^+ > TEA^+ > Na^+ > Li^+.$$

Such sequences were obtained from cells which had low electrogenic pump activity, to remove the significant complication of extra hyperpolarization upon removal of K^+ due to the electrogenic H^+ pump. Pump activity was determined immediately before and after an experiment by addition of 10 mM CsCl to the 5K solution; if the cell hyperpolarized more than a few mV (often hyperpolarization was as much as 100 mV), this suggested a significant pump activity, but if there was little effect on the resting potential upon addition of the Cs^+, it was believed that the H^+ pump was not contributing significantly to total membrane current. Such a response (or lack thereof!) was found if the cell was left in K-state for several hours, or if the photon irradiance was kept low for several hours. A very different selectivity sequence to that above was found if the cell had a high pump activity, and the PD changed much more slowly, sometimes increasing for over 30 min. Hyperpolarization in such cases was clearly greater if the externally added ion was a more effective K^+ channel blocker.

Another method to measure channel selectivity is to measure the conductivity of the K^+ channel to different ions. In this study, this was done simply by measuring the inward current moving after substitution of external K^+ for another monovalent cation (Fig. 7*b*). This method is clearly limited, but until single-channel studies are performed, it is a reasonable approximation. This resulted in a quite different selectivity sequence to that found by measuring changes in resting potential (Table 2), and provided a conductivity sequence,

$$K^+ > Tl^+ > Na^+ = Li^+ > Rb^+ > Cs^+ = TEA^+.$$

Discussion

Action Potentials

The lengthening of the action potential by TEA^+, Cs^+, Ba^{2+} and Na^+ was most likely to be due to the blockade of the K^+ efflux which is at least partly responsible for the repolarization of the plasmalemma. The effect of Cs^+ on the outflow of K^+ during the action potential was perhaps unexpected, as most reports of Cs^+ blockade are of a blockade of only inward K^+ current (e.g. Gorman, Woolum & Cornwall, 1982). However, as demonstrated here (and shown in more detail in the following paper, Tester, 1988*b*), Cs^+ also inhibited outward K^+ current when found in high concentrations relative to external K^+ levels. During the action potentials, only 0.1 mM K^+ was present externally, so Cs^+ could effectively inhibit both inward and outward currents.

Blockade in K-State

The blockade by Na^+, Ba^{2+} and Cs^+ was clearly voltage-dependent, a characteristic which has also been found for Li^+ (Tester, 1988*a*). However, Li^+ was less potent than the blockers studied here. Other workers have also reported a blockade of the K^+ channels by external H^+ ions (Beilby, 1986*a*; Sokolik & Yurin, 1986; Plaks, Sokolik & Yurin, 1987), but the voltage dependence of this blockade is unclear.

Ba^{2+} and Na^+ also appeared to block outward K^+ current in a voltage-dependent manner, presumably after entering the cytoplasm and blocking from the inside of the channel. This suggests that the region of negative-slope conductance at positive potentials may be due not to a voltage-dependent closure of the channel, but to a voltage-dependent blockade of the outward current by an ion found normally in the cytoplasm, such as Na^+. This may be tested by perfusion of the cell with a Na^+-free cytoplasm, or by single-channel studies, although the latter are limited by the narrow voltage range over which the membrane patches can be exposed.

In contrast to blockade by Ba^{2+} and Na^+, the blockade of outward current by Cs^+ (Tester, 1988*b*) did not appear to increase as the difference between the clamped and reversal potentials increased. This difference may reflect a different mechanism of blockade by Cs^+ of the outward current (e.g. tight lodging of Cs^+ within the pore blocking outward movement of K^+, rather than entry of Cs^+ and then blocking from the inside), or it may reflect a true asymmetry of the pore structure, where Cs^+, Ba^{2+} and Na^+ all block in a strongly voltage-dependent manner from the outside of the plasmalemma, but only Ba^{2+} and Na^+ block in a voltage-dependent way from the inside. These differences will be best answered either by continuous perfusion of the cell cytoplasm, or from single-channel studies. Such studies would also be able to distinguish whether the voltage-dependent effects of the various blockers is due to effects on channel conductivity or due to effects on channel on-off times (*see* Tester, 1988*b*).

Part of the reason for this study was to screen several simple cations for their suitability for further detailed investigation of the channel structure. Cs^+ was chosen for more work (Tester, 1988*b*) because of its potency, its rapid and readily reversible action, its low permeability, and the strong voltage-dependence of its blockade. Na^+ and Li^+ were not particularly suitable for a more detailed study of the channel structure due to their low potency. TEA^+ has not yet been further used, as it does not inhibit in a voltage-dependent manner; this is discussed further below. More work was not done with Ba^{2+} due both to the slow time effects, and the incomplete reversibility. Also, when applying higher concentrations (i.e. over about 1 mM), cells often depolarized, and the "leak" conductance at negative potentials increased. Another disadvantage with the use of Ba^{2+}, especially when working with whole cells, is its high conductivity through Ca^{2+} channels (e.g. Tsien, 1983; Hess & Tsien, 1984), which could lead to secondary effects of unknown significance. This has already been implicated once in plant cells (Thaler et al., 1987).

The voltage independence of the blockade by TEA^+ agrees with other reports of a voltage-independent blockade of high G K^+ (Ca^{2+}) channels in animal tissues (Vergara et al., 1984), although a weakly voltage-dependent blockade of similar channels has also been reported (Hermann & Gorman, 1981; Yellen, 1984). It would be interesting to probe the K^+ channel with other quaternary ammonium ions (*cf.* Hermann & Gorman, 1981; Miller, 1982; Villarroel, Alvarez & Latorre, 1986), to see if smaller ions would block with a stronger voltage dependence, as they may be able to penetrate further into the channel pore. It should be noted that decamethonium blocked the *Chara* K^+ channel in a voltage-independent manner (Tester, 1988*a*).

The voltage-independence of TEA^+ blockade, and the competitive interaction between TEA^+ and K^+ suggests that there is a binding site for both TEA^+ and K^+ near the outside surface of the channel pore or antechamber. That external TEA^+ can inhibit both inward and outward current suggests that the binding is strong, as the outward flow of K^+

cannot "knock out" the TEA^+ from the channel pore (*see also* Sokolik & Yurin, 1986).

It is noteworthy that all the inhibitors reduced current towards a similar linear leak (cf. Figs. 3 to 5). This suggests that the background leak is insensitive to these various K^+ channel inhibitors, and is therefore likely to be dominated by ion movements other than through K^+ channels.

Channel Selectivity

If ions move through the K^+ channel independently of each other, then the order of conductivity and of permeability should be the same. However, the selectivity of the *Chara* K^+ channel was different with the two measures of selectivity (Table 2). This is taken as evidence for nonindependence of ion movement through the channel (e.g. Tsien et al., 1987). This is investigated further in the following paper (Tester, 1988*b*), where evidence is provided which strongly suggests that the channel is a multi-ion pore.

The permeability sequence measured here is different to that measured by Sokolik and Yurin (1986) for *Nitella*, but the reason for this is uncertain. Their sequence is, however, unusual, and does not correlate with any of the 11 common sequences for equilibrium ion exchange described by Eisenman (1962). The sequence for *Chara* is similar to many others measured for K^+ channels in animal tissues and for a series of synthetic ion-carrying molecules including nonactin and monactin (Eisenman & Krasne, 1975; Gorman et al., 1982; and references therein). From such a permeability sequence (known as Eisenman's Sequence IV: Eisenman & Krasne, 1975; Hille, 1984) can be drawn some tentative conclusions about the structure of the channel pore; Eisenman and Krasne (1975) suggest that the pore is lined with amide carbonyls and possibly some carboxylate groups. Structural elucidation of the channel will clearly not be done for many years, but it will be interesting to see if such predictions are correct.

Also of interest is the observation that Tl^+ conductance through the *Chara* K^+ channel was found to be less than that of K^+, but the permeability of Tl^+ measured by resting potential was greater. In contrast, Tl^+ conductance through most K^+ channels is greater than K^+ conductance, but recent work has shown that Tl^+ conductance through the very selective high $G\ K^+(Ca^{2+})$ channel is less than that of K^+, although Tl^+ permeability is greater (Eisenman et al., 1986). Azimov et al. (1987) have measured the conductivity of single K^+ channels in the plasma membrane of *Nitellopsis*, and obtained a similar sequence, but with Rb^+ more conductive than Na^+ and Li^+. More accurate measurements of ion permeability through the *Chara* K^+ channel are clearly needed, to confirm the result of Tl^+ conductivity, and also to check the surprisingly low conductivity of Rb^+.

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