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Factors Controlling the K^+ Conductance in *Chara*

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Summary. Previous current/voltage (*I/V*) investigations of the *Chara* K^+ state have been extended by increasing the voltage range (up to +200 mV) through blocking the action potential with La^{3+}. A region of negative slope was found in the *I/V* characteristics at positive PD's, similar to that already observed at PD's more negative than the resting level. These decreases in membrane currents at PD's more negative than −150 mV and at PD's close to 0 or positive are thought to arise from the K^+ channel closure. Both the negative slope regions could be reversibly abolished by 0.1 mM K^+, 20 mM Na^+, more than 10 mM Ca^{2+} or 5 mM tetraethylammonium (TEA). The K^+ channels are therefore blocked by TEA, closed by low $[K^+]_o$ or high $[Ca^{2+}]_o$ and are highly selective to K^+ over Na^+. With the K^+ channels closed, the remaining *I/V* profile was approximately linear over the interval of 400 mV (suggesting a leakage current), but large rectifying currents were observed at PD's more positive than +50 mV. These currents showed a substantial decrease in high $[Ca^{2+}]_o$, sometimes displayed a slight shift to more positive PD's with increasing $[K^+]_o$ and were unaffected by TEA or changes in $[Na^+]_o$. The slope of the linear part of the *I/V* profile was steeper in low $[K^+]_o$ than in TEA or high $[Na^+]_o$ (indicating participation of K^+, but not Na^+, in the leak current). Diethylstilbestrol (DES) was employed to inhibit the proton pump, but it was found that the leakage current and later the K^+ channels were also strongly affected.

Key Words *Chara corallina* · K^+ channels · *I/V* characteristics · leakage current · conductance

Introduction

The fact that the K^+ channels in giant algal cells close as the membrane PD is hyperpolarized (by a presence of an electrogenic pump or by voltage clamping) is well documented (e.g., Smith & Walker, 1981; Sokolik & Yurin, 1981; Bisson & Walker, 1982; Findlay, 1982; Findlay & Coleman, 1983; Bisson, 1984; Beilby, 1985). Clamping the membrane PD to a positive level, however, was expected to produce an increase (or saturation) in the K^+ conductance. A substantial drop has been observed instead (Fig. 6, Beilby, 1985), prompting the present investigation.

The excitation conductance is diminished in the K^+ state, probably due to a decrease in electrochemical gradient for Cl^- (Fig. 8 and 9, Beilby, 1985), but the transient conductance changes still render the *I/V* curve very difficult to interpret. (Such an *I/V* record is immediately recognizable by monitoring the *I* versus time data, where the current does not return to zero as the clamp potential is restored to resting level.) The use of low concentrations of La^{3+} irreversibly blocked excitation in the pump state (Beilby, 1984). The technique proved equally efficacious in this case and the complete PD dependence of the K^+ channels was revealed. The extended *I/V* curves also provide more information on the K^+ channel selectivity and the leakage current.

Materials and Methods

The methods were described previously (Beilby & Beilby, 1983; Beilby, 1985, 1986). The plasmalemma of young cells of *Chara corallina* was space clamped under computer control. The clamp potential and the clamp current were data logged at 2-msec intervals. To obtain the *I/V* characteristics, the voltage clamp command of bipolar staircase was generated by the computer. The pulses of the staircase were 40-msec wide and 400-msec apart. The conductance was calculated by numerical differentiation.

Cells were presoaked in 6 mM Na^+ APW (artificial pond water) for several days before the experiment. (The normal APW contains, in mM: 0.1 KCl, 1.0 NaCl, 0.5 $CaCl_2$, 1.0 HEPES buffer and sufficient NaOH to reach the pH of 7.5. Any changes to this basic recipe are designated by a prefix, e.g. 6 mM Na^+ APW has 6.0 mM NaCl instead of 1.0 mM NaCl). The K^+ state was induced by depolarization of the membrane PD in 5 mM K^+ APW. Figure 1 shows a typical K^+ state *I/V* curve with a negative conductance region between −100 and −200 mV. The *I/V* curve was truncated at −100 mV to prevent excitation. The action potential was then

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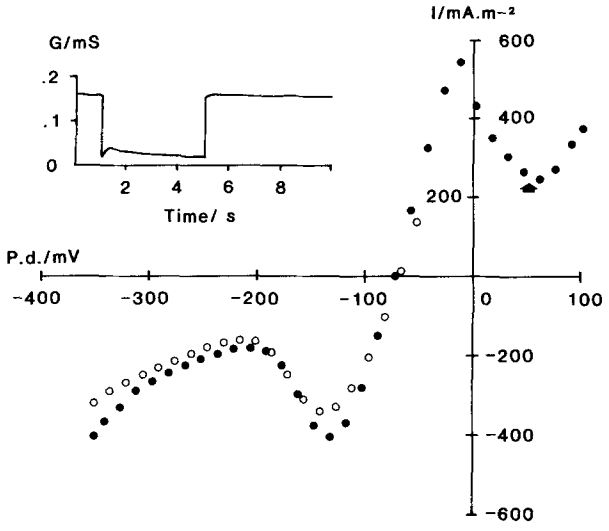

Fig. 1. Comparison of *I/V* characteristics before ○ and after ● application of 0.1 mM $LaCl_3$. ($LaCl_3$ was removed once the membrane became inexcitable.) The inset shows the response of the membrane conductance when the transmembrane PD is voltage-clamped to resting level (−80 mV) for 1 sec, +50 mV (indicated by an arrow on the *I/V* curve) for 5 sec and then back to resting PD. The conductance was obtained by superimposing a small sinusoidal perturbation (amplitude 15 mV, frequency 5 Hz) on the clamp command. Thus the membrane does show a steady low conductance at +50 mV and the minimum on the *I/V* characteristics is not an artifact produced by the bipolar staircase method

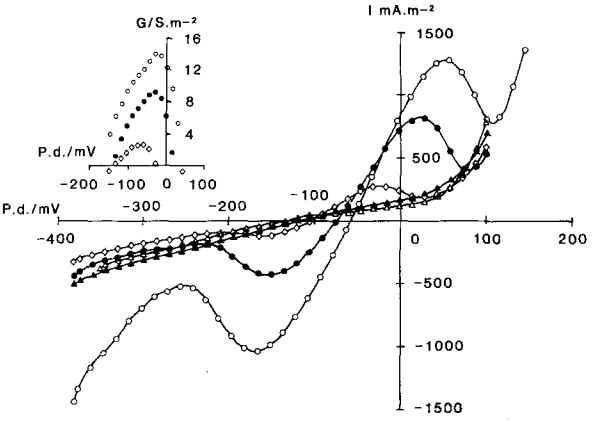

Fig. 2. *I/V* characteristics in 0.1 mM K^+ APW △, 2.0 mM K^+ APW ◇, 5.0 mM K^+ APW ●, 10.0 mM K^+ APW ○, 5.0 mM K^+, 5.0 mM TEA APW ▲. The Ca^{2+} concentration was kept at 0.5 mM. The resting PD (at 0 current) follows estimated E_K. The inset shows the K^+ conductances calculated by numerical differentiation from the *I/V* curves (same symbols are used). The lines were fitted to the data during the numerical differentiation procedure

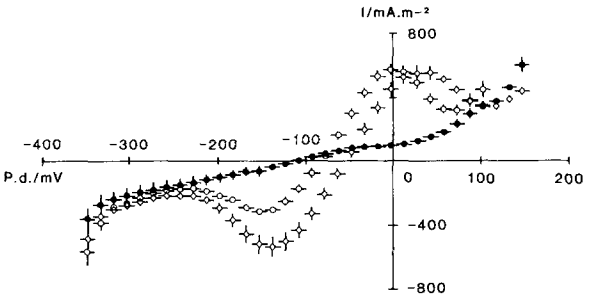

Fig. 3. Summary of data from seven cells in 5.0 mM K^+ APW ○, 0.1 mM K^+ APW ●, and 10 mM K^+ APW ◇. The horizontal bars represent grouping of data into intervals of 15 mV; the vertical bars are the standard error

irreversibly blocked by an exposure to 0.1 mM $LaCl_3$ for ~1 hr (Beilby, 1984). In the work on K^+ channels the La^{3+} was applied with some misgivings, as previous studies indicate some influence of La^{3+} on the K^+ fluxes. Keifer and Spanswick (1978) found that the K^+ permeability P_K decreased to a quarter of the control in 0.5 mM $LaCl_3$. However, the experiments were done at 0.5 mM K^+ and thus the cells were not in K^+ state. J.R. Smith (*in preparation*) observed that cells in K^+ state diminished their P_K from 25 nm/sec to 1 nm/sec in 1.0 mM $LaCl_3$. In the present work the $LaCl_3$ was withdrawn, once the excitation was abolished and perhaps that is why the *I/V* curve is little affected (*see* Fig. 1).

In an attempt to further elucidate the status of the proton pump in the K^+ state, DES (diethylstilbestrol) was applied and its effect on the *I/V* curves was observed for more than 30 min. DES was kept in stock solution of 40 mM in ethanol and was added to 5 mM K^+ APW in concentration of 10 μM.

TEA (tetraethylammonium) was used in 5 mM concentration in 5 mM K^+ APW. The cation and Cl^- concentration was held constant by changing the NaCl concentration.

To investigate the selectivity of the K^+ channels, the 5 mM K^+ APW was replaced by the 20 and 30 mM NaCl APW. In this case the cation and Cl^- concentrations were not kept constant.

Results

PD Dependence in Rising $[K^+]_o$

In K^+ state the extended *I/V* characteristics revealed another negative conductance region between 0 and +50 mV (Fig. 1). Figure 2 shows *I/V* profiles of a cell exposed to 0.1, 2.0, 5.0, 10 mM K^+ APW and 5.0 mM K^+, 5.0 mM TEA. The K^+ conductance (shown in the inset) increased with K^+ concentration and the resting PD followed estimated E_K.

At 0.1 mM K^+ the negative conductance regions disappeared and the *I/V* characteristics became linear over a large range of almost half a volt, but with a strong rectification at PD's more positive than +50 mV. This feature could be distinguished even

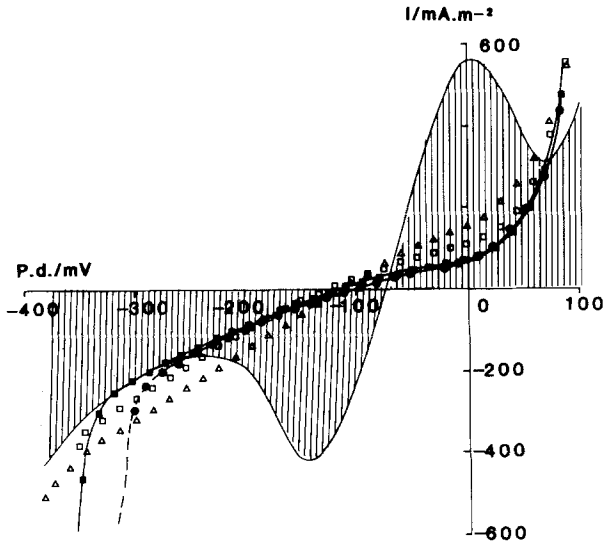

Fig. 4. Effect of the $[Na^+]_o$ on the leak current. The cell was initially in 5 mM K^+ APW (shaded region indicates the *I/V* profile), but 20 mM Na^+ APW resulted in characteristics given by ■. Further increase to 30 mM Na^+ is shown as ●. The profile in TEA □ (obtained minutes before the Na^+ exposure) and in 0.1 mM K^+ △ (recorded 30 min before the Na^+ exposure) are included

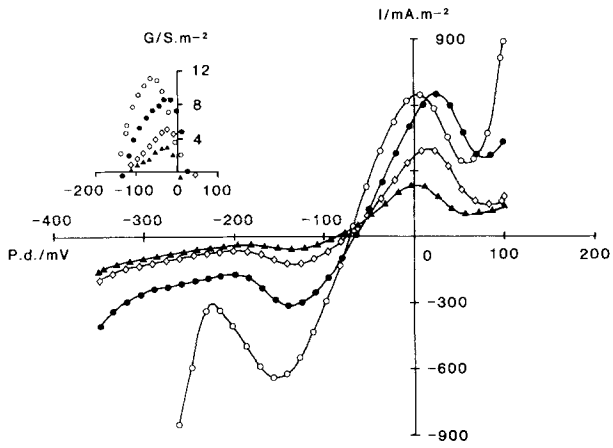

Fig. 5. *I/V* characteristics in 10.0 mM Ca^{2+} APW ▲, 5.0 mM Ca^{2+} APW ◇, 0.5 mM Ca^{2+} APW ●, "0" Ca^{2+} APW ○. The K^+ concentration was kept at 5.0 mM. Same cell as in Fig. 2. Note that the resting PD remained almost unchanged

in high $[K^+]_o$ (*see* Fig. 2). In some cells the increase in $[K^+]_o$ shifted the onset of the rectification to more positive PD's. Some cells showed an increase in the slope of the *I/V* profile at PD's more negative than −200 mV, as 10 mM K^+ APW was put on (Fig. 2). Figure 3 summarizes the data from seven cells exposed to 5 mM K^+ APW, 10 mM K^+ APW and 0.1 mM K^+ APW.

Effect of TEA

5.0 mM TEA added to 5.0 mM K^+ APW yielded an *I/V* profile similar to that found in low K^+, but with a slightly smaller slope. The block was readily reversible. The rectifying region of the *I/V* curve was not affected by the TEA (*see* Figs. 2 and 4).

Effect of $[Na^+]_o$

With the cell initially in the K^+ state, exposure to 20 mM Na^+ APW caused a quick hyperpolarization to −110 mV and an *I/V* profile close to that in TEA and 0.1 K^+. Further increase in Na^+ to 30 mM elicited a little change, apart from an earlier punchthrough. The rectifying region was again not affected (*see* Fig. 4).

PD Dependence in Rising $[Ca^{2+}]_o$

As the K^+ state is known to be sensitive to $[Ca^{2+}]_o$ (Keifer & Lucas, 1982; Bisson, 1984) the *I/V* characteristics at 10, 5.0, 0.5 and "0" mM Ca^{2+} APW were recorded (*see* Fig. 5). It was difficult to remove all the calcium, as there is a strong adsorption to the cell wall. This problem was partially overcome by a short exposure (5 min) to 30 mM NaCl. The increase in $[Ca^{2+}]_o$ achieved the opposite result to rise in $[K^+]_o$: waning in the K^+ conductance. The resting PD, however, stayed relatively constant. The sudden increase in positive current at positive PD's was no longer observed as $[Ca^{2+}]_o$ increased. Similarly, at negative PD's (beyond −200 mV) the *I/V* relationship became close to linear above 5 mM Ca^{2+}.

Effect of DES (Diethylstilbestrol)

Figure 6 shows the effect of 10 μM DES on the *I/V* profile: (*A*) before the exposure to DES; (*B*) after 9 min of DES, showing mainly inhibition of the rectifying region of the leak current; (*C*) and (*D*) indicate that after 20 and 25 min, respectively, the K^+ conductance also started to diminish and the punchthrough phenomenon waned. At this point of the inhibition, the resting PD dropped by ~20 mV. In (*D*) the cell was also exposed to 10 and 0.1 mM K^+, showing that the $[K^+]_o$ dependence of the *I/V* profile persisted despite the DES inhibition. The inhibition was reversible.

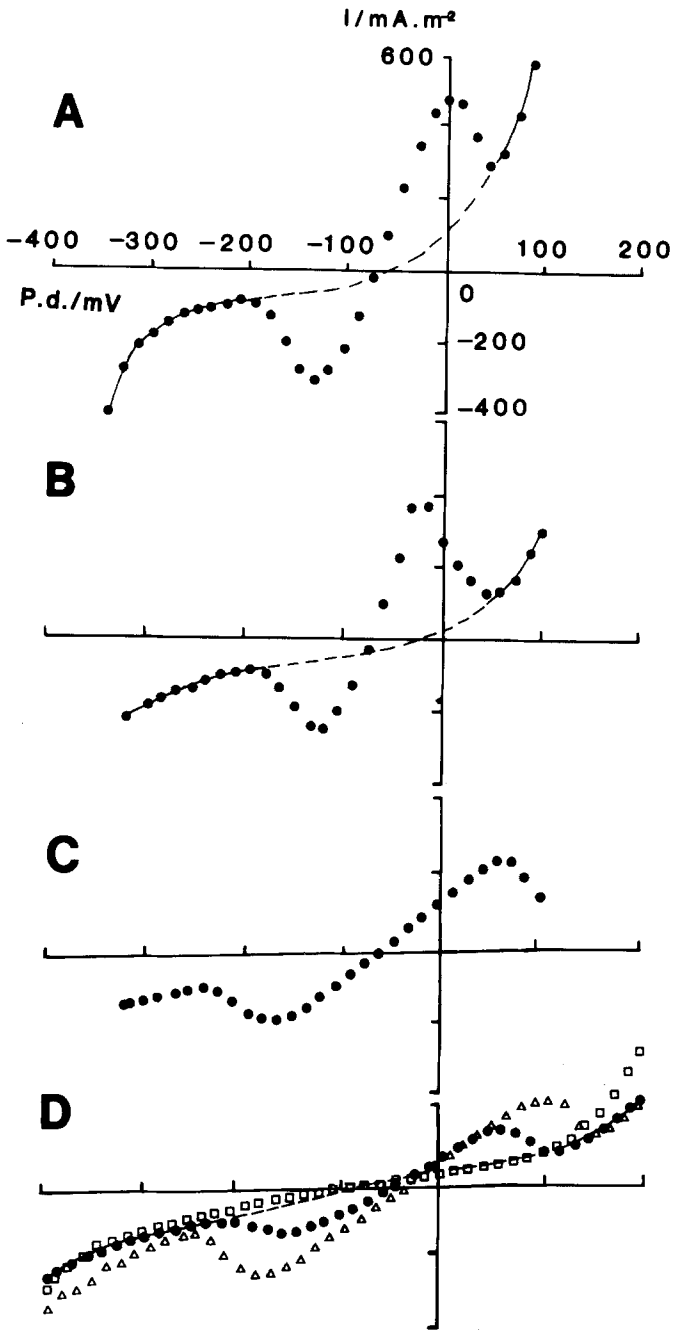

Fig. 6. Inhibition by DES. (*A*) Control in 5 mM K^+ APW. Resting PD was −74 mV, the conductance was ~7 Sm^{-2}. (The conductance was measured as the slope of the *I/V* profile at the resting PD.) (*B*) 9 min in the same medium with 10 μM DES. The resting PD and conductance were unchanged, but currents at positive PD's were diminished. (*C*) 20 min in DES. Resting PD depolarized to −64 mV and the conductance decreased to ~2 Sm^{-2}. (*D*) 25 min in DES. Resting PD was −58 mV, conductance close to 1 Sm^{-2}. Large currents at the PD extremes were inhibited (*compare with A*). $[K^+]_o$ was changed to 10 mM △ and 0.1 mM □ to see whether the $[K^+]_o$ dependence was retained. The broken lines were fitted by eye, using exponential slope french curve to approximate a "Goldmanian" leak. These are included merely to draw attention to the DES inhibition of the leak current. A detailed fitting of the leak is planned (Blatt & Beilby, *in preparation*). The scales on the axes of (*B*), (*C*) and (*D*) are as in (*A*)

Discussion

PD Dependence of the K^+ Channels

The negative conductance is usually associated with the excitation phenomenon. However, this effect can occur with any process where the conductance is a steep function of PD (Handbook of Physiology, 1977). For a relationship obeying the Ohm's law the slope of an *I/V* curve is given by

$$dI/dV = G + V(dG/dV).$$

The slope can become negative if the second term on the right is greater than the first term and V or dG/dV is negative. The last two conditions are satisfied at the K^+ conductance turn-on and turn-off, respectively. The K^+ channels thus open over a limited window of the transmembrane PD. The dynamics of the K^+ channels do not show total symmetry on each side of the resting PD. Upon clamping to hyperpolarized PD's the closure is much faster than subsequent opening as the membrane is returned to resting level (Beilby, 1986). Excursions to positive PD's result in fast closure and equally speedy reopening at resting PD (inset of Fig. 1). Thus it is hoped that the hyperpolarized arm of the *I/V* staircase does not interfere with the depolarized arm. However, investigations of the time dependence of the clamp currents at a range of positive PD's will be necessary to work out the detailed PD dependence of the channels. Such PD dependence of the channel conductance has not been observed before *in vivo* in plant or animal tissues. It is reminiscent of the VDAC channels from mitochondria (Colombini, 1979).

K^+ Conductance as Function of Concentration of Various Ions

Figure 2 indicates that a rise in $[K^+]_o$ increases the negative slope regions of the *I/V* profile, that is, the K^+ currents become larger. Numerical differentiation of the 0.1 mM K^+ and the TEA *I/V* curves yields approximately constant conductance of 1.5 S m^{-2} which begins to rise at +50 mV. The conductances of the other *I/V* characteristics are similar at PD's more hyperpolarized than −250 mV, become negative between −150 and −250 mV and rise to a peak between −150 and +20 mV (shown in the inset). These conductance peaks are attributed mainly to plasmalemma permeability to K^+ ions. It is interesting that the conductance maxima are located at PD's more depolarized than the resting level. (The fact that the total membrane current was differentiated rather than the K^+ current only

should not substantially change the magnitude nor the position of these maxima, as the leak current is linear in that PD interval.) Is the rise in conductance with rising $[K^+]_o$ due to more K ions being available (i.e., rise in each unitary K^+ conductance), or is the population of the open K^+ channels increasing? An equation based on Goldman's assumption (Hope & Waker, 1975) gives P_K's of 70 nm/sec at 10 mM K^+, 43 nm/sec at 5 mM K^+ and 4 nm/sec at 2 mM K^+, supporting the latter option. In any case the changes in $[K^+]_o$ would only be expected to affect the K^+ inflow, as the $[K^+]_i$ remains constant. Thus a decline in the open K^+ channel population as $[K^+]_o$ diminishes, with no channels opened at 0.1 K^+, seems a reasonable picture. This scheme would also explain, why the resting PD no longer behaves as K^+ electrode, when $[K^+]_o$ falls below 1.0 mM (e.g. Hope & Walker, 1975). The exact threshold $[K^+]_o$ for the channel opening was not investigated and may vary from one cell culture to another.

The fact that the elevation of $[Ca^{2+}]_o$ decreases the K^+ conductance without substantially changing the resting PD (*see* Fig. 5) also supports the view that the changes in K^+ conductance are mainly caused by the variation in the open K^+ channel population.

The effect of high Na^+ suggests that the K^+ channels are highly selective to K^+ over Na^+ (Fig. 4). It will be necessary, however, to expose the membrane to a combination of high Na^+ and high K^+ (to keep the channels open), to ascertain that Na^+ cannot pass through the channels.

The Leakage Current

In my previous work, the limited range of the *I/V* characteristics revealed an essentially linear profile under conditions where the K^+ channels were closed. Thus I suggested that a passive, nonspecific leak is involved (Beilby, 1985, 1986). While I have retained "leakage current" for lack of a more informed label, in the light of present results it seems no longer nonspecific and perhaps might not be entirely passive. The strong rectification near 0 and at positive PD's has been observed before, but was usually ascribed to K^+ channels. This characteristic remains unchanged, however, when K^+ channels are closed by low K^+ or blocked by TEA. The Goldman model or the "double fixed charge membrane" model (Coster, 1965) both predict rectification of the leakage current and anticipate contribution of both K^+ and Na^+, according to their permeabilities. Figure 4 shows, however, that addition of 30 mM Na^+ hardly changed the resting PD nor the slope of the *I/V* profile of the leak. (In Fig. 4 Na^+ seems to block some of the leak conductance similarly to TEA, but the data are too sparse to draw such conclusions.) Thus it might be possible, that the rectification currents arise from a different mechanism than the currents responsible for the linear part of the leak. This line of thought seems to be supported by the relatively fast inhibition of the rectifying currents by DES (Fig. 6). (At long exposures DES is rather indiscriminate and the slope of the linear *I/V* profile, K^+ conductance and punchthrough all decline.) As DES is reputed to be an effective inhibitor of membrane-bound ATPases (*see*, for instance, Keifer & Spanswick, 1979), the rectification may be associated with the proton pump. This is, however, a somewhat circular argument and more research is necessary. (For instance, it may be interesting to subject a cell in the "leak" state to a fast pH_o change.) If, on the other hand, the rectification is not associated with the pump, then DES makes a poor inhibitor for the pump and the method of obtaining the pump *I/V* characteristics (Beilby, 1984) must be re-thought.

Another possible origin of the leakage current may be another type of K^+ channel, as suggested by Sokolik and Yurin (1981). Both the linear and the rectifying part of the leak show some dependence on $[K^+]_o$. In Fig. 2 the leak currents at PD's more negative than −200 mV behave in such a way, but this effect is lost in the statistical analysis in Fig. 3. At 10 mM K^+ the research is frustrated by the evolution of the *I/V* profile with time (Beilby, 1986).

The future investigation of the leak current rectifying region will probably need to establish the time dependencies of the phenomenon, as the *I/V* technique may be misleading with the fast changing currents. Radioactive tracers together with voltage clamping (Walker et al., 1979) may facilitate the identification of the ions carrying the rectifying currents.

Comparison to Higher Plants and Animal Cells

The important proposal of this article, that the K^+ channels are opened by increasing $[K^+]_o$, can explain some aspects of the behavior of many higher plant tissues. For instance, Epstein (1976) described "dual pattern of ion transport," where K^+ influxes show a marked increase at $[K^+]_o$ less than 0.1 mM and more than 1.0 mM. Potassium absorption at high $[K^+]_o$ is inhibited by Na^+ and Ca^{2+}. Cheeseman and Hanson (1979) observed that cell potentials in corn roots follow E_K closely above $[K^+]_o$ of 0.5 mM. Such data can be explained by high $[K^+]_o$-activated K^+ channels.

As with the *Chara* action potential, the role of K^+ channels in *Chara* physiology is not clear, although in other plant tissues, such as stomata, the greatly increased permeability to K^+ is vital during

opening and closing (Raschke, 1979; MacRobbie, 1981).

In animal kingdom the K^+ channels show a great diversity (*see* Hille, 1984 for review). The classical delayed rectifier described by Hodgkin-Huxley equations is activated by depolarization and shows a constant conductance up to 100 mV (Cole & Moore, 1960). The calcium-dependent K^+ channels are activated by a rise of $[Ca^{2+}]_i$ and are insensitive to $[Ca^{2+}]_o$ (Meech, 1978; Marty, 1981). These channels show inactivation at positive PD's (Heyer & Lux, 1976; Meech, 1978), but this is imparted by the PD dependence of the calcium currents, which wane at 100 mV. Most K^+ channels are blocked by widely varied concentrations of TEA applied on the outside and/or inside.

The advent of patch clamping (Neher & Sakman, 1976) enables us to study the kinetics of a single ion channel in a living biomembrane. The availability of relatively large animal cells and pharmacological channel blockers provides a substantial body of data to draw upon. The electrophysiology of plant cells, however, is more obscure (MacRobbie, 1985), with the exception of that of giant algal cells. The patch clamp has now been applied to plant tissues (Moran et al., 1984; Schroeder et al., 1984), presenting us with data rather difficult to correlate to what is known of plant electrophysiology. The relatively high conductance and the clearly defined activation conditions, however, should make the *Chara* K^+ channel a good candidate for patch-clamp studies.

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