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Potassium Channels and Different States of *Chara* Plasmalemma

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Summary. The current-voltage (*I/V*) technique was employed to investigate the different electrophysiological states of the *Chara* plasmalemma and their interaction under a range of conditions. In K^+ state the membrane became very permeable (conductances >20 S m^{-2}) as $[K^+]_o$ increased to 10 mM. As the cells were then easily damaged by the voltage-clamp procedures, it was difficult to determine the saturation K^+ conductance. TEA (tetraethylammonium chloride) reversibly blocked the K^+ channels, but had no effect on the *I/V* curve of the pump state, indicating that the K^+ channels were not participating in this state. Acid pH_o (4.5) diminished the K^+ conductance, but did not alter the response of the K^+ channels to change in $[K^+]_o$. Alkaline pH_o (11.0) made *Chara* resting PD "bistable": the PD either stayed near the estimated E_K and the *I/V* curve showed a negative conductance region typical of the K^+ state, or it hyperpolarized and the near-linear *I/V* profile of the proton-permeable state was observed.

Key Words *Chara corallina* · K^+ channels · *I/V* characteristics · plasmalemma · voltage clamp

Introduction

The last decade has revealed that the *Chara* plasmalemma can exist in several electrophysiological states. Further, some of these states can be located on adjacent patches of the same cell (e.g. Bisson & Walker, 1981; Smith & Walker, 1985). Transitions between the states are governed by the outside conditions.

At pH_o less than about 10.0, $[K^+]_o$ less than 2.0 mM and with Ca^{2+} present, the membrane conductance is dominated by the electrogenic proton pump. The resting PD can be very large at neutral pH_o (~−250 mV), but drops considerably at acid pH_o. This depolarization was thought to be caused by the pump inhibition (e.g. Richards & Hope, 1974), but can also be explained by a change in the pump kinetics (Beilby, 1984). The conductance remains at ~2.0 S m^{-2} throughout the pH_o range.

Above pH_o 10.0, the membrane becomes highly permeable to H^+ (or OH^-) and behaves as a pH electrode (e.g. Bisson & Walker, 1980). The resting PD follows the estimated E_H. The conductance in this state can rise up to 20.0 S m^{-2} (Beilby, *unpublished*). After its original discoverer, this state is referred to as the "Bisson state."

As $[K^+]_o$ rises above ~2.0 mM, the permeability of the plasmalemma to K^+ increases and the resting PD becomes small and follows the estimated E_K. The conductances are again 15.0 to 20.0 S m^{-2}, depending on $[K^+]_o$. The existence of the K^+ state is well documented (e.g. Oda, 1962; Spanswick, 1972; Smith & Walker, 1981; Sokolik & Yurin, 1981; Keifer & Lucas, 1982; Bisson, 1984), but only recently have the current/voltage (*I/V*) characteristics of the space-clamped plasmalemma been obtained (Sokolik & Yurin, 1981; Beilby, 1985*a*). This approach showed that the K^+ channels in *Chara* close at hyperpolarized potentials and the *I/V* profile can be resolved into two straight lines: one due to the K^+ conductance, the other due to an unspecific leak (the null state).

The electrophysiology of the giant algae is thus emerging as an increasingly complex picture. The measurements of the resting PD and conductance alone are no longer sufficient to identify the various factors contributing to the electrical characteristics. A refined *I/V* analysis (Beilby & Beilby, 1983; Beilby, 1985*a*) was found to provide a promising tool for the discrimination among the states of the plasmalemma. The *I/V* curves of the pump state, Bisson state (Beilby, 1984) and K^+ state (Beilby, 1985*a*) are strikingly different. The *I/V* technique can thus be exploited to observe the three principal states under a range of conditions and to establish the interactions between them. This communication is therefore exploratory in nature, rather than setting out to test a specific hypothesis.

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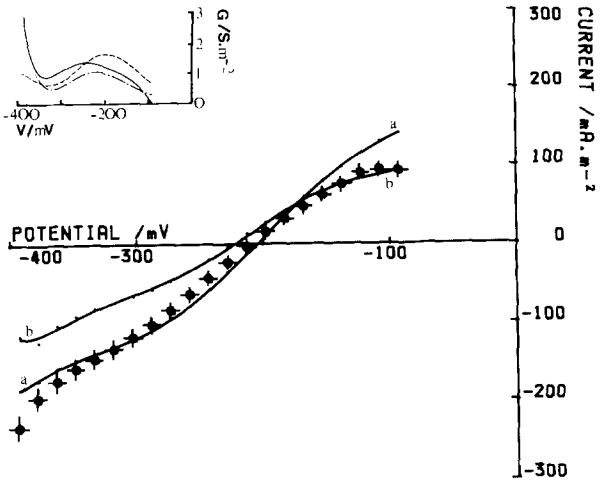

Fig. 1. Comparison of the pump *I/V* characteristics in different $[Na^+]_o$. The closed circles show the data from cells pretreated in 10.0 mM Na^+ APW. The data have been grouped into intervals of 15 mV, represented by the horizontal bars; the vertical bars are the standard error for eight cells. The curve (a) shows a polynomial fitted to the data from four cells conditioned in normal APW (1.0 mM Na^+)—for experimental points and standard errors *see* Beilby (1984). The curve (b) represents a polynomial fit to the data gathered from five cells pretreated in 3.0 to 5.0 mM Na^+ APW—*see* Beilby (1985*a*) for experimental points and standard errors. To make the three data sets easier to distinguish, polynomials fitted to the previously published data, rather than the experimental points themselves, are shown. (The data are included as dots, but are usually obscured by the lines, because the fits are good.) The conductances in the inset are: ——— 10 mM Na^+ APW; –·–·–·– 3.0 to 5.0 mM Na^+ APW; ------- 1.0 mM Na^+ APW

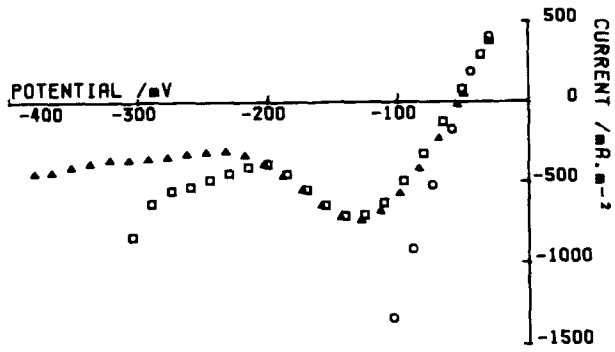

Fig. 2. Time dependence of the *I/V* characteristics in 10.0 mM K^+ APW. ▲ at 6 min, □ at 8 min, ○ at 60 min

Materials and Methods

Young *Chara* cells without calcifications were stored in high NaCl artificial pond water (APW). This APW consisted of the normal APW (KCl, 0.1 mM, $CaCl_2$, 0.5 mM, zwitterionic buffer, 1.0 mM, NaOH to adjust to required pH_o, NaCl, 1.0 mM—the final Na^+ concentration after the pH was adjusted was between 1.0 and 2.0 mM) with 4.0 to 9.0 mM NaCl added according to experimental requirements. HEPES buffer was used at pH_o 7.5, MES buffer at pH_o 4.5 and CAPS buffer at pH_o 11.0. The APW was made just before the experiment and the flow through the experimental chamber was fast. KCl was added to APW to change the K^+ concentration.

In the initial experiments cells were presoaked in 10 mM Na^+ APW for several days prior to impaling, so that the total cation concentration and $[Cl^-]_o$ could be kept constant throughout the experiments. Upon exposure to this $[Na^+]_o$ the transient depolarization and slow recovery after action potentials described previously (Keifer & Lucas, 1982; Beilby, 1985*a*) were more marked. However, once the cells adjusted to 10.0 mM Na^+ APW, they displayed the typical sigmoid *I/V* characteristics and the large resting PD's of the pump state. In Fig. 1 the *I/V* curve statistics gathered from eight cells pretreated several days in 10.0 mM Na^+ APW are compared to cells from normal APW (Fig. 4; Beilby, 1984) and cells presoaked in 2.0 and 5.0 mM Na^+ APW (Fig. 1; Beilby, 1985*a*). As $[Na^+]_o$ increased, there seemed to be a slight shift of the conductance maximum to more hyperpolarized PD's. However, both the position and the magnitude of the conductance maximum may vary due to seasonal changes in the *Chara* cultures.

In experiments involving changes of pH_o, the K^+ state was usually induced by 5.0 mM K^+ APW and the cells were conditioned in 5.0 mM Na^+ APW. The pH of the pretreatment APW was kept at 7.5 at all times. During the experiment, the exposure to different pH_o was kept to a minimum to avoid changes in cytoplasmic pH. An exception to this regime was pH_o 11.0, where it took up to 60 min for the Bisson state to develop. Between various pH_o values the cells were returned to pH_o 7.5 until the resting PD recovered to its initial value.

TEA (tetraethylammonium chloride) was obtained from Sigma. The TEA APW was made just before the experiment and consisted of 5.0 mM TEA, 5.0 mM K^+ and the normal APW with 1.0 mM Na^+ replaced by 0.1 mM to keep the total cation concentration constant. For the TEA experiments the cells were pretreated in 10.0 mM Na^+ APW.

At the time of the experiment the cells were illuminated by a fiber optics source Intralux H150 Volpi, light intensity of 50 to 100 $\mu Em^{-2}\ sec^{-1}$.

The methods have been described previously in detail (Beilby & Beilby, 1983; Beilby, 1985*a*). The voltage clamp was controlled by a MINC 11 computer. The *I/V* curves were obtained by using the bipolar staircase clamp commands. Conductance was calculated by differentiation of polynomials fitted to *I/V* curves, by numerical differentiation (as the polynomial fit could not handle the negative conductance region of the K^+ state) or by a sine perturbation method.

The cells were space-clamped and the PD measuring electrode was kept in the cytoplasm. Both these measures were vital in the present experiments. In the pump state the *I/V* characteristics of the plasmalemma and tonoplast in series are very similar to those of the plasmalemma alone (Beilby, 1984), because the plasmalemma resistance is considerably higher than the tonoplast one (Coster & Smith, 1977). However, in the K^+ state and the Bisson state the conductance of the plasmalemma can be very large and the electrical properties of the tonoplast are likely to contribute significantly to the *I/V* curve, making the results difficult to interpret. The space clamp is necessary, as the distortion due to the cable properties of the cell tends to linearize the *I/V* curves (Smith, 1984). This is particularly relevant to the K^+ state, where the conductance is a strong function of the PD.

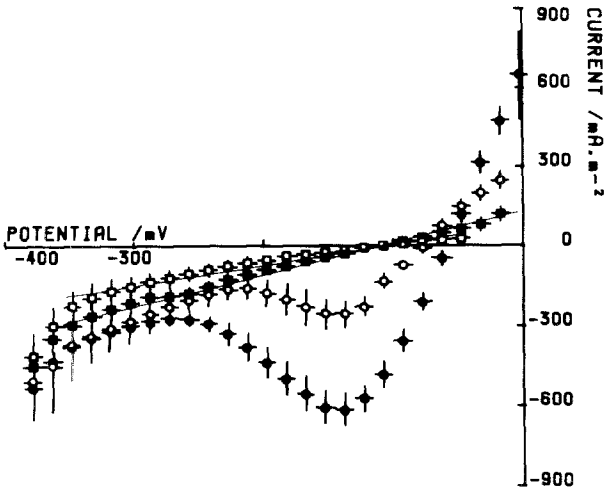

Fig. 3. Effect of $[K^+]_o$ and 5.0 mM TEA on the K^+ state *I/V* characteristics. The data were processed as in Fig. 1. ● 10.0 mM K^+ APW, data collected from nine cells immediately after the resting PD stabilized in response to medium change. ○ 5.0 mM K^+ APW, data from four cells. ■ 0.1 mM K^+ APW, data from five cells. The cells were in K^+ state prior to being exposed to low K^+ APW and the *I/V* curves were recorded after 3 to 5 min in this medium. □ 5.0 mM TEA and 5.0 mM K^+ APW, data from three cells. The straight lines were fitted by eye, giving conductances of 0.9 S m^{-2} for the TEA profile and 1.1 S m^{-2} for the 0.1 K^+ APW profile

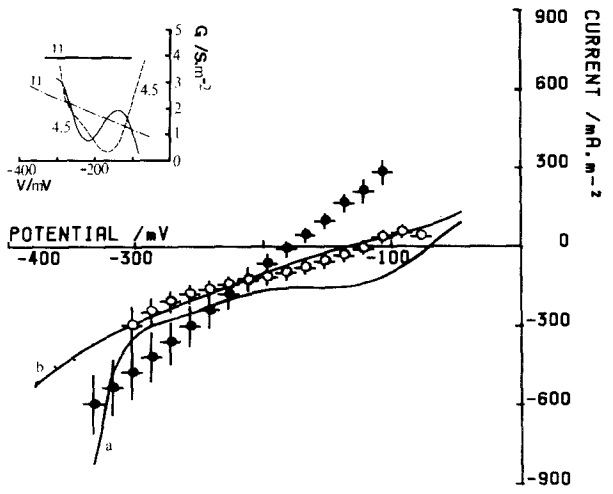

Fig. 4. *I/V* characteristics at the extremes of pH_o in normal APW ($[K^+]_o$ = 0.1 mM): ○ pH_o 4.5, data from six cells; ● pH_o 11.0, data from seven cells. Data were processed as in Fig. 1. The *I/V* profiles at the same pH_o values (curve (*a*) pH_o 4.5, curve (*b*) pH_o 11.0), but in 5 mM K^+ APW have been included for comparison. These polynomials were fitted to the data shown in Fig. 6. The conductances are shown in the inset. The continuous lines are the low K^+ conductances, the broken lines show the conductances in 5.0 mM K^+ APW. At acid pH_o the *I/V* curves were truncated at PD's near −300 mV, as the punchthrough occurs at more depolarized levels (Coster, 1969)

Results

Effect of Increasing $[K^+]_o$ on K^+ State

At pH_o 7.5 the resting PD in 10.0 mM K^+ APW remained steady at −60.3 ± 3.4 mV (seven cells) for many hours, but the *I/V* characteristics evolved with time (*see* Fig. 2). The typical profile (which could be resolved into a low conductance at hyperpolarized PD and higher conductance due to the K^+ channels at PD's more positive than −200 mV) changed gradually into a single conductance with a large slope of 20 S m^{-2} and the *I/V* clamping procedure often became lethal to the cell. The time taken to arrive at this point varied widely from cell to cell. With some cells it was impossible to run the *I/V* procedure, others changed more gradually (such as in Fig. 2). In most cells, however, an *I/V* profile could be recorded immediately after the resting PD had stabilized in response to the medium change. Figure 3 shows a summary of such data from nine cells exposed to 10.0 mM K^+. Data from four cells at 5.0 mM K^+ and five cells at 0.1 mM K^+ (where the K^+ channels are closed) are included for comparison. As the *I/V* procedure was often deleterious to the cells at 10.0 mM K^+, higher concentrations were not investigated.

Effect of TEA

Three cells were exposed to 5.0 mM TEA and the average *I/V* curve is shown in Fig. 3. As TEA was introduced into the outside medium, the resting PD hyperpolarized immediately to −120 mV. *I/V* characteristics were then obtained showing a total absence of the negative conductance region, typical of the K^+ state. If the cells were left in TEA, the transmembrane PD continued to hyperpolarize gradually. This behavior was similar to that seen in cells exposed to 0.1 mM K^+ APW while in the K^+ state. The *I/V* profiles in TEA APW and 0.1 mM K^+ APW were very close, yielding conductances 0.9 and 1.1 S m^{-2}, respectively.

Upon removal of TEA, it was often necessary to depolarize the cell by voltage clamping, to generate the K^+ state again. The TEA effect was completely reversible. The addition of the same amount of TEA to high Na^+ APW, while the cell was in the pump state, had no effect.

Effect of pH_o

To evaluate the effect of pH_o on the K^+ state, it was necessary first to explore the *I/V* characteristics at the extremes of pH_o in high Na^+ APW. Figure 4

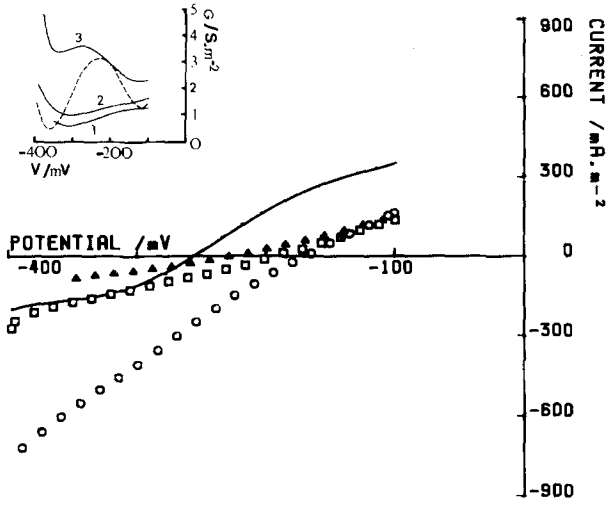

Fig. 5. Transition into Bisson state. The *I/V* profile of the pump state is represented by a polynomial fitted to the data. (The data points are included as dots, but these are mostly obscured by the line because of the good fit.) The cell was exposed to normal APW, pH_o 11.0 for 10 min ▲, 30 min □ and 45 min ○. The conductances in the inset: broken line is the pump state profile, the Bisson state profiles are numbered consecutively according to the time in pH_o 11

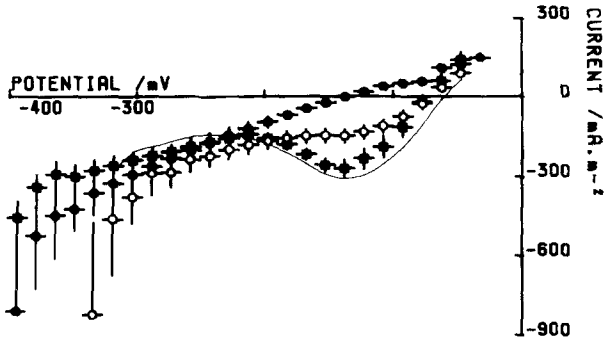

Fig. 6. Effect of pH_o on the 5.0 mM K^+ state *I/V* characteristics. ○ 4.5 pH_o, data from seven cells; ■ 7.5 pH_o, data from seven cells; ● 11.0 pH_o, data from five cells which hyperpolarized upon exposure to this pH_o spontaneously or after an *I/V* procedure. Data were processed as in Fig. 1. The continuous line is a polynomial fitted to data (three cells) at pH_o 11.0, which either stayed in or were forced by voltage clamping into K^+ state

shows a summary of the data from six cells at pH_o 4.5 (empty circles) and seven cells at pH_o 11.0 (full circles). The conductances are presented in the inset. The acid pH_o *I/V* profile became established within a few minutes after the solution was introduced into the chamber. At pH_o 11.0, however, in most cells the *I/V* characteristics continued to evolve for up to an hour after exposure. Only then was the Bisson state (with high conductance and a resting PD close to the estimated E_H) attained (*see* Fig. 5).

Figure 6 shows the effect of the pH_o extremes on the 5.0 mM K^+ state. The data have been gathered from seven cells at pH_o 4.5 and 7.5, and five cells at pH_o 11.0. Upon exposure to acid pH_o the resting PD depolarized by 6.1 ± 5.4 mV (eight cells). The scatter was large, but the depolarization was observed in every cell. The negative conductance region became less marked, disappearing altogether in some cells.

The data gathered at pH_o 11.0 could be divided into two groups. Upon exposure to this pH_o, some cells kept their PD's almost unchanged, while others hyperpolarized to −146.2 ± 9.5 mV (four cells). The *I/V* characteristics of the former group were very similar to those at pH_o 7.5. The procedure of recording these *I/V* curves, however, shifted the cells into the hyperpolarized state. 40-msec hyperpolarizing pulses of the bipolar staircase of the *I/V* procedure were sufficiently long to close the K^+ channels. The subsequent *I/V* curves and those from the latter group showed the near-linear profile typical of the Bisson state. To return the cells into the K^+ state, a prolonged clamping (at least 0.5 min) to depolarized levels (near 0) was necessary. It was thus possible to alternate between K^+ state and Bisson state by clamping the transmembrane PD to 0 potential or to −150 mV. When the clamp was switched off, the resting PD tended to E_K or E_H, respectively. The *I/V* procedure always transferred the cell from the K^+ state into the Bisson state.

To investigate the time and PD dependencies of the K^+ channels over a range of pH_o, the cells were clamped to their resting PD's for 1.0 sec, then the clamp level was changed to −150 mV for 4.0 sec and then the cells were returned to the resting PD. The conductance was measured by the sine perturbation method. A typical set of results for the conductance is shown in Fig. 7. Throughout the pH_o range the K^+ channels closed in about a second, as the transmembrane PD shifted to −150 mV. At acid pH_o the conductance returned to the original level within 2.0 sec of depolarizing the PD to the resting level. At pH_o 7.5, the recovery of the conductance was slower. At pH_o 11.0, the conductance stayed low as the cell underwent transition from the K^+ state to the Bisson state.

The response of K^+ channels to $[K^+]_o$ at pH_o extremes was also explored. Figure 8 shows the *I/V* characteristics at pH_o 4.5 and K^+ concentrations of 10.0, 5.0 and 0.1 mM. *I/V* curves at 0.1 and 5.0 mM K^+, pH_o 7.5, are included for comparison. The response to $[K^+]_o$ was similar to that at pH_o 7.5, but the slopes of the *I/V* curves in the K^+-conducting region were smaller than those at more basic pH_o (*see* Fig. 9).

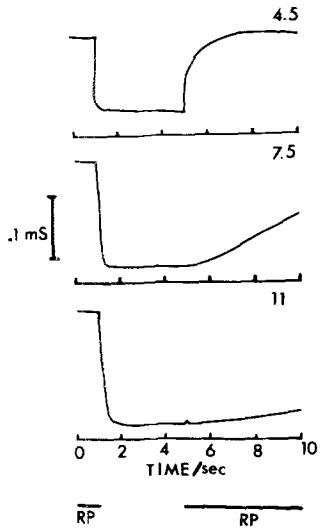

Fig. 7. Time and PD dependence of the membrane conductance (the ordinate) in the K^+ state at pH_o 4.5, 7.5 and 11.0. The cells were clamped to the resting PD (−73 mV at pH_o 4.5; −77 mV at pH_o 7.5 and −62 mV at pH_o 11.0) for 1 sec, then the clamp level was changed to −150 mV for 4 sec and finally returned to the resting level for 5 sec. A sine wave of 10 mV and 5.0 Hz was superimposed on this voltage-clamp command. The conductance was calculated as described in Beilby & Beilby, 1983. Cell area was 0.16 cm^2. Regardless of the pH_o, the conductance diminished within a second of the PD being hyperpolarized. The K^+ channel closure thus seems relatively insensitive to changes in pH_o. The conductance rise upon subsequent depolarization (due to the reopening of the K^+ channels) is faster as the pH_o becomes more acid

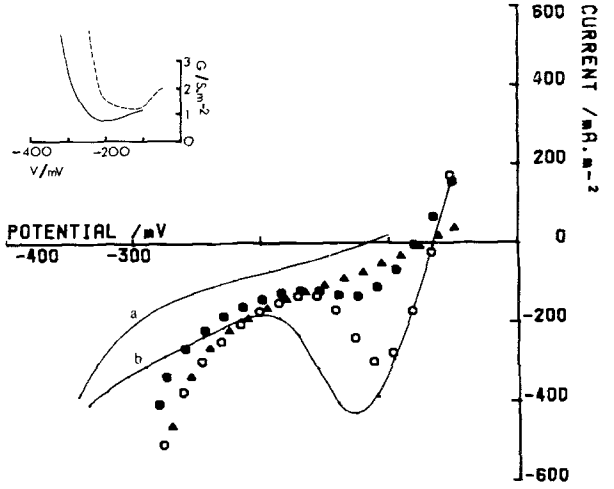

Fig. 8. Effect of $[K^+]_o$ at pH_o 4.5. ○ 10 mM K^+ APW, ● 5.0 mM K^+ APW and ▲ 0.1 mM K^+ APW. The cell showed a strong tendency to punchthrough at ~−280 mV. The continuous lines are polynomials fitted to data at pH_o 7.5 from the same cell: curve (*b*) at 5.0 mM K^+ APW, curve (*a*) at 0.1 mM K^+ APW. These are included for comparison. The conductances in 0.1 mM K^+ APW are shown in the inset, ---- pH_o 4.5 and —— pH_o 7.5

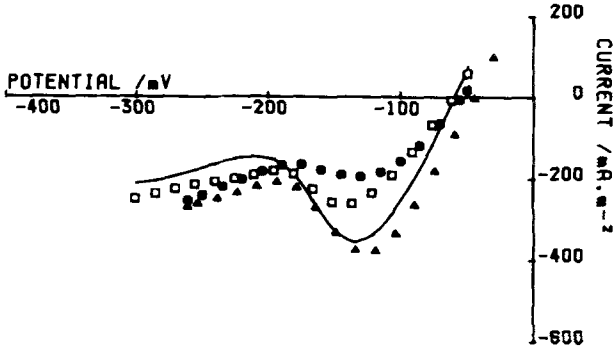

Fig. 9. Effect of changes in pH_o on the K^+ state *I/V* characteristics ($[K^+]_o$ = 5.0 mM): ● pH_o 4.5, □ pH_o 5.5, — pH_o 7.5 (the polynomial fitted to the data is shown for clarity, the data are included as dots). ▲ 10.0 mM K^+, pH_o 4.5 *I/V* curve was included to compare the K^+ conductance (given by the slope of the *I/V* curve in the K^+ channel region) to that at pH_o 7.5 and 5 mM K^+

At pH_o 11.0, increasing $[K^+]_o$ from 5.0 to 10.0 mM did not change the resting PD until the cell was clamped to 0 potential for ~30.0 sec (Fig. 10). The resting PD then depolarized to −55 mV and the *I/V* characteristics showed the negative conductance region typical of the K^+ state. Although at $[K^+]_o$ of 5.0 mM the K^+ conductance was comparable to that of pH_o 7.5, at $[K^+]_o$ of 10.0 mM the K^+ conductance did not increase further (*compare* with Fig. 3).

Discussion

The plasmalemma of the giant algae contains a diversity of transport systems. Different channels or pumps are activated according to the conditions in the outside medium. There are three principal states: the proton pump state at pH_o less than ~10.5 (e.g. Spanswick, 1972), the H^+-permeable state at pH_o above 10.5 (Bisson & Walker, 1980) and the K^+ state in $[K^+]_o$ greater than ~2.0 mM (e.g. Smith & Walker, 1981; Sokolik & Yurin, 1981). The *I/V* characteristics of these states are strikingly different. The *I/V* technique is therefore a useful tool in exploring the properties of these states and their relationships. In this communication the previous work on K^+ state (Beilby, 1985*a*,*b*) is extended and the relationship of the K^+ channels to the other two states is explored.

Factors Controlling the K^+ Conductance

Patch-clamping technique (e.g. Neher & Sakmann, 1976; Neher, 1982) confirms the general picture of the K^+ conductance as a statistical distribution of

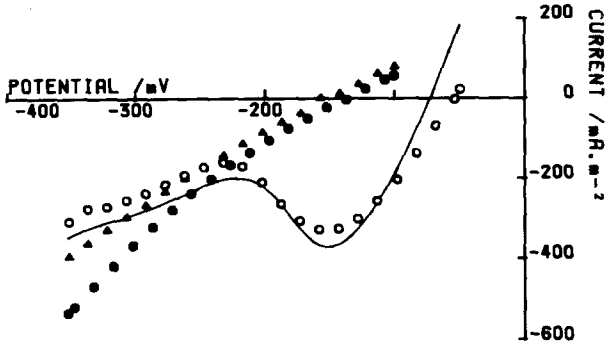

Fig. 10. In 5.0 mM K^+ state (pH_o 7.5) the resting PD was −74.1 mV and the *I/V* characteristics are shown by the continuous line. As the pH_o was increased to 11.0, the resting PD hyperpolarized to −160.0 mV and the *I/V* profile is shown as ▲ after 5 min. The $[K^+]_o$ was increased to 10.0 mM and the cell was clamped for 30 sec to 0 potential and the resting PD subsequently depolarized to −55.0 mV and the *I/V* profile is given by ○. The *I/V* procedure hyperpolarized the resting PD to −140.0 mV with *I/V* profile given as ●

the population of open K^+ channels, each channel contributing a unitary conductance. In giant algae, the K^+ channels are activated by an increase in $[K^+]_o$ (e.g. Oda, 1962; Smith & Walker, 1981; Sokolik & Yurin, 1981; Findlay & Coleman, 1983; Bisson, 1984). In *Chara* the K^+ conductance continues to increase as $[K^+]_o$ rises from 2.0 to 5.0 mM (Beilby, 1985*a*). With the above view in mind, is it possible to find a $[K^+]_o$ where all the K^+ channels are opened? The attempt to estimate this saturation point by the *I/V* technique has been frustrated by a time-dependent conductance increase, which made further *I/V* measurements impossible.

While in 5.0 mM K^+ APW the *I/V* profile remains steady for many hours (Beilby, 1985*a*), in 10.0 mM K^+ APW the threshold potential for K^+ channel opening (Findlay & Coleman, 1983; Beilby, 1985*a*) gradually disappears. The currents at clamp levels only ~50 mV removed from the resting PD become very large (*see* Fig. 2). Before this effect sets in, short exposures (few min) to this $[K^+]_o$ revealed an *I/V* profile with a negative conductance region seen previously at lower $[K^+]_o$ (Beilby, 1985*a*) with maximum conductance of 12 S m^{-2} (Fig. 3). The 5.0 mM K^+ APW data included for comparison yielded a conductance of 5 S m^{-2}. The latter is rather low, compared to an earlier study (Beilby, 1985*a*). The group of cells described in Fig. 3 was pretreated in 10.0 mM NaCl APW, and this was, at first, thought to be the cause. However, in later experiments the pretreatment $[Na^+]_o$ was lowered to 5.0 mM with the 5.0 mM K^+ conductance unchanged. The changing sensitivities to $[K^+]_o$ of different *Chara* populations might be due to fluctuations in the potassium concentration of the growth medium. For instance, a considerable increase in K^+ transport can be observed in *Neurospora*, which has been grown under K^+ starvation regime (M.R. Blatt, *in preparation*). It would be interesting, therefore, to conduct experiments, where the potassium concentration of the growth medium can be controlled.

What happens to K^+ conductance after prolonged exposure to 10.0 mM K^+ APW? Since the resting PD remains steady, no changes in $[K^+]_o$ are implicated. It seems, however, that the PD dependence of the K^+ channels changes and they no longer close at hyperpolarized potentials. The large conductances >20 S m^{-2} might mean that most of the channels are opened, but more measurements will be necessary. The use of the sine perturbation method, which does not take the membrane too far from the resting PD, might be less damaging than the *I/V* technique. The increase in conductance from 1.0 S m^{-2} at 0.1 mM K^+ APW to ~20 S m^{-2} at 10.0 mM K^+ APW (after long exposures) is of the same order as an increase in $^{42}K^+$ influx measured by J.R. Smith (*in preparation*). Lucas, Spanswick and Dainty (1978) found that Ca^{2+}-free APW or 10.0 mM K^+ APW inhibited bicarbonate uptake. The uptake was restored, however, if the concentration of Ca^{2+} in the 10.0 mM K^+ APW was increased from 0.2 to 2.0 mM. They suggested that with low Ca^{2+} (0.5 mM in this study) in the APW, 10.0 mM K^+ displaced a critical amount of Ca^{2+} from the membrane sites. Keifer and Lucas (1982) proposed that Ca^{2+} is necessary for the K^+ channel closure. Similar conclusions have been reached by Bisson (1984). Finally, recent *I/V* studies (Beilby, 1985*b*) showed that in Ca^{2+}-free APW (the Ca^{2+} from the cell wall was removed by a 5.0-min exposure to 20 to 30 mM NaCl) the conductance gradually increased in a fashion similar to that seen in 10.0 mM K^+ APW. The *I/V* studies also explain why the clamp currents at −200 mV in Ca^{2+}-free APW are much less $[K^+]_o$ sensitive, than the ones at −100 mV (Bisson, 1984): the K^+ channels are closed at hyperpolarized PD's. As this PD dependence seems to disappear with Ca^{2+} removed, it also indicates that some Ca^{2+} was still present in the medium.

The Ca^{2+} dependence of the plant K^+ channels differs from that found in nerve tissue. There the increase in inner Ca^{2+} activates the channels, while the outside Ca^{2+} has no effect (e.g. Meech, 1978; Marty, 1981).

As can be seen in Fig. 6, low pH_o (4.5) decreases the K^+ conductance. The response of the *I/V* characteristics to $[K^+]_o$ remains similar to that at neutral pH_o: the negative conductance region be-

comes more pronounced with increasing $[K^+]_o$, but disappears in 0.1 mM K^+ APW. It thus seems that a smaller population of K^+ channels is opened at pH_o 4.5. The K^+ conductance decreases gradually, as pH_o is lowered (*see* Fig. 9). This result is in disagreement with the measurements of $^{42}K^+$ influx into *Chara* cells in 10.0 mM K^+ APW, where no change was found between 7.0 and 5.0 pH_o (J.R. Smith, *in preparation*).

Blocking the K^+ Channels

The classical K^+ channel blocker, TEA, which abolishes K^+ currents in nerve (Armstrong, 1971), *Hydrodictyon* (Findlay & Coleman, 1983), *Chara inflata* (Coleman & Findlay, 1985) and *Nitella flexilis* (Sokolik & Yurin, 1981) is also effective in *Chara corallina* (*see* Fig. 3). The inhibition is completely reversible. It is interesting to note that the TEA *I/V* profile yields a lower conductance of 0.9 S m^{-2} than the 0.1 mM K^+ APW profile (1.1 S m^{-2}). If the overall scatter in the data is considered, such a small change in the slopes of the *I/V* lines is barely significant, but the error bars are small near the crossover of the two data sets (~ -110 mV) and the effect was observed in all the experiments. Perhaps TEA also blocks part of the leak conductance.

The lack of any effect of TEA on the pump state indicates that the K^+ channels are closed in that state, at least at transmembrane PD's more negative than -100 mV. As Shimmen and Tazawa (1983) found no effect of TEA on *Chara corallina* action potential, the K^+ channels are probably inactivated even at more depolarized PD's.

Further experiments are planned to investigate the effects of other blockers, such as Cs^+ and nonyltriethylammonium (C9).

Status of the Proton Pump

At neutral pH_o it is difficult to determine whether the proton pump is inactivated or merely short-circuited by the K^+ conductance. Keifer and Lucas (1982) argued the latter case, as the resting PD's they observed in the K^+ state were more hyperpolarized than the predicted E_K and further depolarization could be elicited by application of inhibitors. I argued for the pump inhibition, as upon K^+ channel closure by 0.1 mM K^+ APW (or TEA) low resting PD and linear *I/V* characteristics suggest that the membrane electrophysiology is dominated by a nonspecific leak (Beilby, 1985*a*).

The acid pH_o (4.5) measurements seem to suggest that the answer may be somewhere between these two extremes. At this pH_o, the K^+ conductance is no longer much greater than the pump state conductance and the resting PD's of the two states are close together (*see* Fig. 4). If the pump action remained unchanged in the K^+ state, one would expect that resting PD to hyperpolarize as the pH_o is made more acidic in the K^+ state, but a small depolarization is observed (*see* Fig. 6). The reason for this depolarization is at present unknown. When the K^+ channels are closed by 0.1 mM K^+ APW, the two "null" states at pH_o 7.5 and 4.5 cross the potential axis 40 mV apart (*see* Fig. 8). Such behavior would be expected if either the proton pump or a proton flux (as suggested by Kitasato, 1968) was involved in the "null" state. The conductances (inset, Fig. 8) show an increase at depolarized PD's similar to those in the pump state (Beilby, 1984). Further, the leak conductance obtained by exposure to DES (diethylstilbestrol), was pH_o independent (Beilby, 1984) and thus it does appear, that the proton pump contributes to the membrane resting PD, but is greatly suppressed in the K^+ state. [The normal response of the pump to pH_o shift from 7.5 to 4.5 is $\sim$140 mV depolarization of the membrane PD (Beilby, 1984).] The change in the pump operation must be caused by factors other than the low transmembrane PD imposed by the K^+ state, as at pH_o 4.5 the resting PD's of the pump state and K^+ state are very close (*see* Figs. 4 and 6). If the PD is clamped to resting levels, then to -150 mV and then back to resting level (*see* Fig. 7), the conductance at pH_o 4.5 changes fast on restoring the resting clamp level. This behavior is more typical of the pump state, than the slow reopening of the K^+ channels at higher pH_o. However, it is possible that the low pH_o changes the time dependence of the K^+ channel gating.

High pH_o and High $[K^+]_o$: "Bistable" State

The proton-permeable Bisson state has been extensively studied (e.g., Bisson & Walker, 1980; Bisson & Walker, 1982) but space-clamped plasmalemma *I/V* curves have not been investigated before. As the plasmalemma conductance in this state is high, measurements which include the tonoplast may introduce substantial error.

In 0.1 mM K^+ APW (pH_o 11.0), the *I/V* profile is almost linear and the resting PD close to -180 mV [which matches the estimated E_H for protons (Bisson & Walker, 1980)]. The conductance is $\sim$4.0 S m^{-2} throughout the PD range (*see* Fig. 4). Despite the fast flow of APW through the chamber, in my hands the change to the Bisson state took up to 60 min (rather than seconds, as described by Bisson & Walker, 1981) and the cells always passed through a

low-conductance state (*see* Fig. 5). In this transition region the proton pump appears to contribute no conductance, but the resting PD is still well in excess of any Nernst PD. Such behavior could be explained by the pump becoming a constant current source, the current drop across the membrane resistance providing the excess PD. Once stabilized, the *I/V* characteristics of the Bisson state are close to a straight line, but a first derivative of a polynomial fit reveals that the conductance maximum, typical of the pump state (Beilby, 1984), is still present. The proton pump thus seems to make a small contribution to the *I/V* characteristics of the Bisson state. The near-linear *I/V* profile with the resting PD at the predicted E_H suggests that the proton conductance is passive (e.g. Bisson & Walker, 1980).

Upon exposure to pH_o 11.0, the K^+ state (5 mM K^+ APW) gives way to the Bisson state in most cells. The transition is fast and the resultant *I/V* characteristics give a lower conductance and a more depolarized resting PD than the pure Bisson state (*see* Figs. 4 and 6). Upon clamping the resting PD to 0 level the *I/V* characteristics and PD return to those of the K^+ state. It is easy to understand that transition into the Bisson state closes the K^+ channels, but it is more difficult to appreciate why the proton conductance turns off, when the membrane is forced into the K^+ state. The *I/V* characteristics of the proton-permeable state certainly show no indication of diminishing currents at depolarized PD's. Increasing $[K^+]_o$ to 10.0 mM does not alter the Bisson state greatly and does not increase the K^+ conductance (Fig. 10). Some degree of interference between the K^+ and the H^+ (or OH^-) channels is thus implicated.

Finally one might care to ponder why the circulating currents disappear in high K^+ APW (Lucas et al., 1978). As the proton-permeable region still functions with somewhat reduced conductance, the alkaline zones should still appear. So, perhaps, the pump is indeed not sufficiently operative to create the acid bands.

Conclusions

The plasmalemma of *Chara* becomes greatly conductive after prolonged exposure to 10.0 mM K^+ APW, preventing the gathering of further *I/V* data and determination of the saturation K^+ conductance.

TEA blocks the K^+ conductance completely and reversibly, but has no effect on the pump state. Thus no involvement of K^+ channels in the pump state is indicated.

Acid pH_o (4.5) diminishes the K^+ conductance, but the response of the K^+ channels to increase in $[K^+]_o$ is unchanged. The conductance studies (using the perturbation method) indicate that the K^+ channels close within a second upon the membrane hyperpolarization to −150 mV, regardless of pH_o. The opening time of the channels upon subsequent depolarization, however, is shorter at acid than at neutral pH_o.

The alkaline pH_o (11.0) proton-permeable state was found to attain a near-linear *I/V* profile with 0 current at the estimated E_H, following a transition state of low conductance.

The combination of 5.0 mM K^+ APW and pH_o 11.0 resulted in a bistable state with a resting PD near E_K and *I/V* characteristics similar to those of 5.0 mM K^+ state at 7.5 pH_o, or with a resting PD more hyperpolarized and *I/V* profile similar to the Bisson state.

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