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Ion Channels in the Membrane of *Chara inflata*

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Summary. Voltage-clamped steps in the electric potential difference (PD) across the membrane in cells of the green alga, *Chara inflata*, cause voltage- and time-dependent current flows, interpreted to arise from opening and closing of various types of ion channel in the membrane. With cells in the light, these channels are normally closed, and the resting PD is probably determined by the operation of an H^+ efflux pump. Positive steps in PD from the resting level often caused the opening of K^+ channels with sigmoid kinetics. The channels began to show opening when the PD $\simeq -120$ mV for an external concentration of K^+ of 1.0 mM. Return of the PD to the resting level caused closing of the channels with complex kinetics. Various treatments of the cell could cause these K^+ channels to open, and remain open continuously, with the PD then lying closer to the Nernst PD for K^+. The K^+ channels have been identified by the blocking effects of TEA^+. Another group of channels, probably Cl^- and Ca^{2+} associated with the action potential open when the PD is stepped to values less negative than $\simeq -50$ mV. Negative steps from the resting PD cause the slow opening, with a time course of seconds, of yet another type of channel, probably Cl^-.

Key Words K^+ channels · ion channels · *Chara inflata* · membranes · TEA^+ effects

Introduction

Findlay (1982) and Findlay and Coleman (1983) have shown that in the cells of the green alga *Hydrodictyon africanum* the electrical properties of the membrane are appreciably determined by a voltage-dependent K^+ conductance. This conductance is determined by the state of K^+ channels, whose existence is inferred from the threshold behavior of the conductance and its similarities to the K^+ conductances in other membranes where actual channel systems have been demonstrated (*see* Latorre, Coronado & Vergara, 1984). In *Hydrodictyon*, the membrane PD, when the K^+ channels are open, is usually close to the Nernst potential for K^+, and when closed the membrane is hyperpolarized because ψ_m is then determined by the operation of an electrogenic H^+ efflux pump. In another green alga, *Chara corallina*, there is similar evidence for K^+ channels (Smith & Walker, 1981; Keifer & Lucas, 1982), but the characteristics of the channels are not well understood because their opening appears to be linked to the occurrence of the action potential, and the resulting K^+ current is obscured by other ionic currents. In contrast, in *Hydrodictyon* there is no action potential, and K^+ currents can be seen clearly. The action potential in *Chara corallina* arises from the opening and closing of voltage-dependent Cl^-, Ca^{2+} and K^+ channels (Beilby & Coster, 1979). During the action potential there is a net outward current of K^+, and net inward currents of Cl^- and Ca^{2+}.

Coster (1969) also has investigated another type of inward current carried by Cl^- in *Chara corallina*, for large negative values of membrane PD, during a phenomenon of apparent electrical breakdown, or "punch-through" of the membrane. Beilby and Coster (1979) and Beilby and Beilby (1983) have shown that this inward current is time dependent and for a negative step in membrane PD takes some seconds to reach a steady value. Findlay and Coleman (1983) and Findlay and Tyerman (1984) have shown time-dependent inward currents in *Hydrodictyon africanum* and *Chara inflata*, respectively.

We have used the cells of the charophyte plant, *Chara inflata*, for further measurement of membrane currents, with voltage-clamp techniques, because in this species inward and outward membrane current through K^+ channels in particular, as well as other inward currents, can be observed more easily than in other charophyte species; the results are described in this paper.

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Materials and Methods

Materials

Plants of *Chara inflata*, originally collected from Fairview Conservation Park in South Australia were grown in cultures consisting of sandy loam, with a solution consisting of (in mM): KNO_3, 1.0; $MgSO_4 \cdot 7\ H_2O$, 1.0; Na_2HPO_4, 0.1; NaH_2PO_4, 0.5; $CaCl_2$, 0.1; FeEDTA, 0.002; together with trace elements. The temperature was about 25°C, and the culture was illuminated with light from fluorescent tubes for 16 hr per day. Generally whorl cells from plants which had recently appeared above the surface of the loam were used, as these cells were more spherical than whorl cells from higher up on more mature plants.

Samples of vacuolar sap for analysis of ionic content were obtained by first soaking cells in a solution of 10 mM $CaCl_2$ for 30 sec to remove extracellular K^+ and Na^+. The cells were then blotted dry and cut open with a razor blade on a greasy surface. A 5- to 10-μl sample was collected and added to 2 ml of distilled water for determination of K^+ and Na^+ concentrations by flame photometry.

Methods

The methods have been described previously by Findlay and Coleman (1983). Cells for experimental use were soaked overnight in the light, in a solution of artificial *Chara* pond water (CPW) consisting of (in mM): KCl, 0.1; $CaCl_2$, 0.1; $NaHCO_3$, 0.4; TAPS(3-[[Tris-(hydroxy methyl)methyl]amino] propane sulfonic acid), 4.0, adjusted to pH 8.5 with NaOH 2.4. This pretreatment solution also formed the basic control solution with changes in $[K^+]_o$ being made by increasing the concentration of KCl in the solution. However, some experiments were done with different values of external pH (pH_o). Details of such experiments are given specifically in the text. Cells were mounted on a Perspex® holder and irrigated with continuously flowing solution. Light from the microscope provided illumination.

Electrical methods have been described previously (Findlay & Coleman, 1983). Briefly, a 3 M KCl micropipette to measure ψ_m and a Pt-Ir (70 to 30%) electropolished wire, insulated to 50 μm of the tip, to pass current into the cell, were used. Both micropipette and current electrode were inserted into the cell to a depth of between 100 and 200 μm. Although no gushing of the cell sap into the microelectrode occurred, which would ensure that the tip of the microelectrode was in the vacuole, we think that after a short time, the tip would effectively measure the vacuole PD, although we are not certain of this; [*see* Findlay and Hope (1976) for further discussion on this point]. As the cells were approximately spherical, the diameter was estimated as the mean of two orthogonal diameters, and the surface area calculated accordingly. The TEA cation was provided by tetraethylammonium chloride (BDH).

Results

Transitions in Membrane Potential Difference (ψ_m) and Conductance (g_m)

Within 15 to 20 min after the insertion of current and PD electrodes, the intracellularly recorded PD had usually reached a steady value. For the reason given in the Methods, we believe that this PD is measured across the two membranes, tonoplast and plasmalemma, although we will refer to it as the membrane PD, ψ_m, throughout the paper. In 29 cells bathed in CPW with $[K]_o^+ = 0.1$ mM and pH = 8.5 the membrane PD was in the range -122 to -270 mV, with a mean value and standard error of -163 ± 6 mV. Flame photometric analysis of the vacuolar sap of a group of cells similar to those in which electrical measurements were made gave $[K^+] = 76 \pm 10(6)$ mM and $[Na^+] = 87 \pm 10(6)$ mM. The number in parentheses is the sample size. Thus for $[K^+]_o = 0.1$ mM, $\psi_K = -166 \pm 10(6)$ mV and for $[Na^+]_o = 2.8$ mM, $\psi_{Na} = -86 \pm 3(6)$ mV where ψ_K and ψ_{Na} are the Nernst potentials for K^+ and Na^+. While the mean value of ψ_m was close to ψ_K, in a number of cells ψ_m was more negative than ψ_K. This was particularly so for one subgroup of nine cells, which, after pH_o was changed to 7.0, had $\psi_m = -196 \pm 8$ mV. In these cells the membrane was hyperpolarized where we define the hyperpolarized membrane as one in which ψ_m is more negative than any possible ionic diffusion potential (*see* Findlay, 1982).

A change in $[K^+]_o$ from 0.1 mM to 1.0 mM had various effects. In a few cells, ψ_m became more negative, and in most it became less negative, but the change was less than the 58 mV that would be predicted from the Nernst equation for a K^+ diffusion potential. Where $[K^+]_o = 1.0$ mM, treatments, such as passing pulses of outward current of sufficient intensity across the membrane or turning off the light, which moved ψ_m above a threshold value, almost invariably caused a rise in the membrane conductance g_m ($=\partial I_m/\partial \psi_m$, where I_m is the membrane current) by a factor of up to four times (*see* Fig. 1*a*), with ψ_m settling to a level closer to ψ_K ($= -100$ mV). With the cell in darkness, ψ_m tended to settle to a value less negative than ψ_K. In some cells, the change in $[K^+]_o$ itself from 0.1 to 1.0 mM was sufficient to move ψ_m across the threshold level and produce the characteristic changes in g_m and ψ_m.

After the characteristic transition in ψ_m and g_m with $[K^+]_o \geq 1.0$ mM, the addition of TEA^+ at a concentration of 10 mM to the external solution caused g_m to fall and ψ_m to return to its more negative level (Fig. 1*b*).

Membrane Current for Positive Steps in ψ_m

In some cells, a positive voltage-clamp step in ψ_m caused a membrane current flow which increased from its initial level at the start of the step to a new level with a characteristic time course, provided ψ_m was stepped to a value which was less negative than a threshold value. Figure 2*a* shows membrane cur-

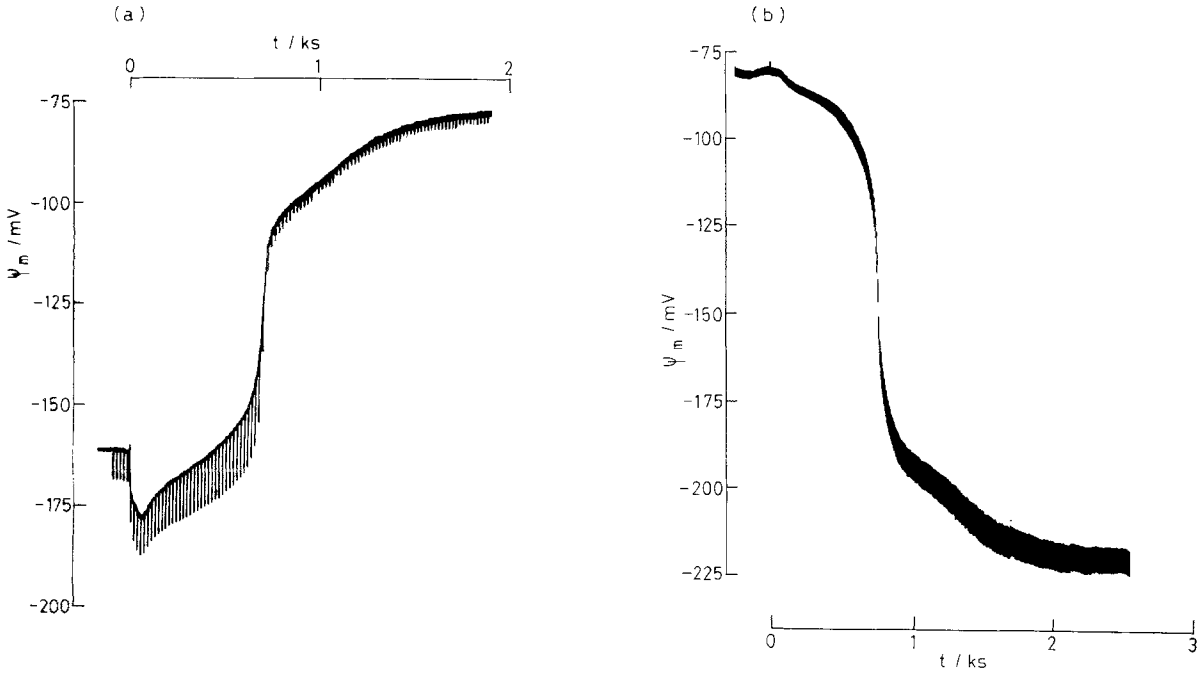

Fig. 1. (*a*) Response of membrane PD and conductance to light-off at $t = 0$. $[K^+]_o = 1.0$ mM, $pH_o = 7.0$. (*b*) Response of ψ_m to addition of 10 mM TEA^+ to external solution at $t = 0$. Initially $\psi_m \simeq \psi_K$. $[K^+]_o = 3.0$ mM, $pH_o = 8.5$. Test pulses of inward current of constant amplitude, producing short negative steps in ψ_m, were applied, and it can be seen that the membrane conductance decreased with increasing negativity of ψ_m

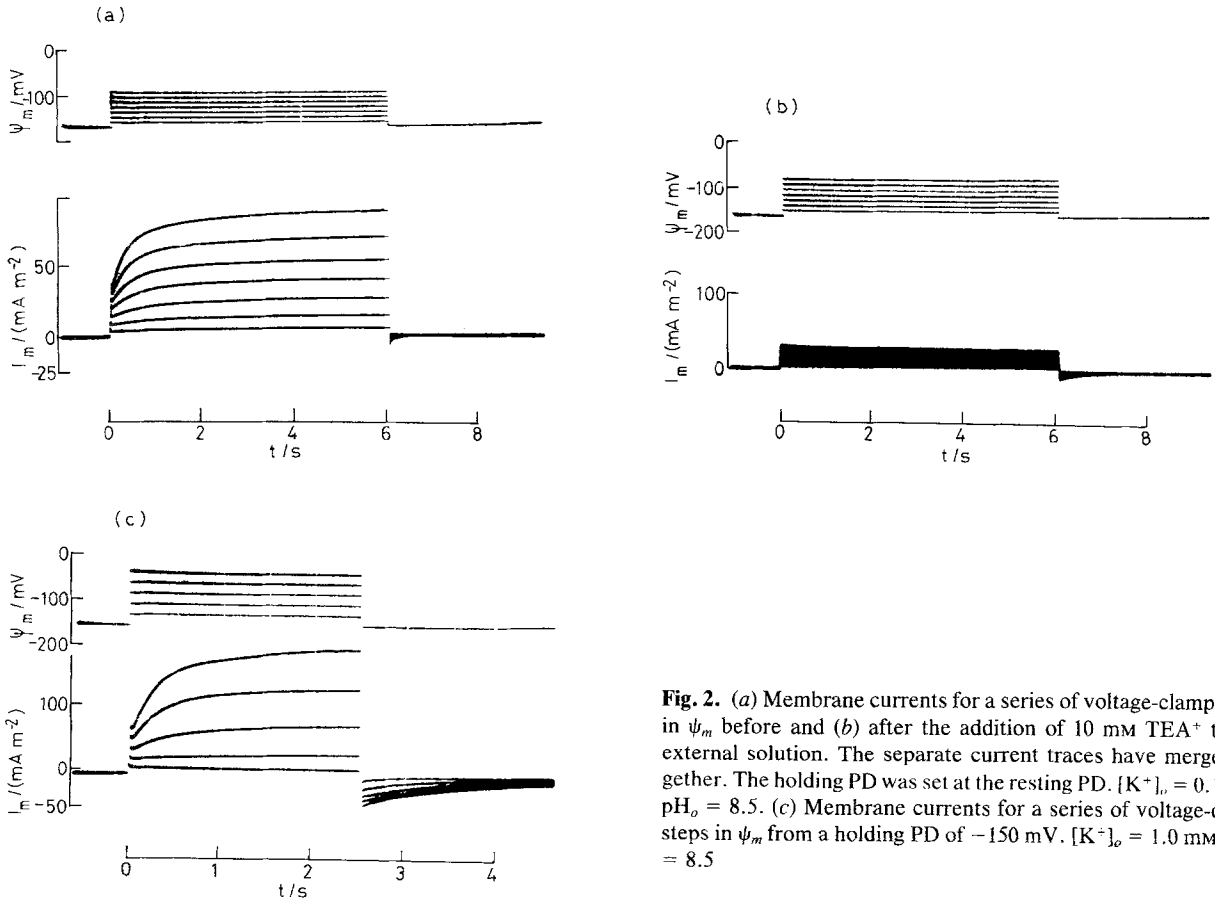

Fig. 2. (*a*) Membrane currents for a series of voltage-clamp steps in ψ_m before and (*b*) after the addition of 10 mM TEA^+ to the external solution. The separate current traces have merged together. The holding PD was set at the resting PD. $[K^+]_o = 0.1$ mM, $pH_o = 8.5$. (*c*) Membrane currents for a series of voltage-clamp steps in ψ_m from a holding PD of -150 mV. $[K^+]_o = 1.0$ mM, $pH_o = 8.5$

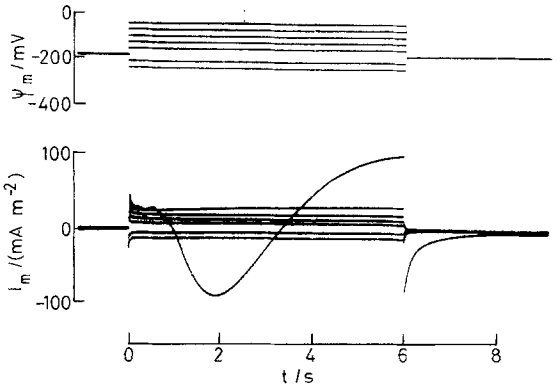

Fig. 3. Membrane currents for a series of voltage-clamp steps in ψ_m from a holding PD of −180 mV. The membrane was initially hyperpolarized, and the holding PD was set equal to the resting PD. $[K^+]_o$ = 0.1 mM, pH_o = 8.5

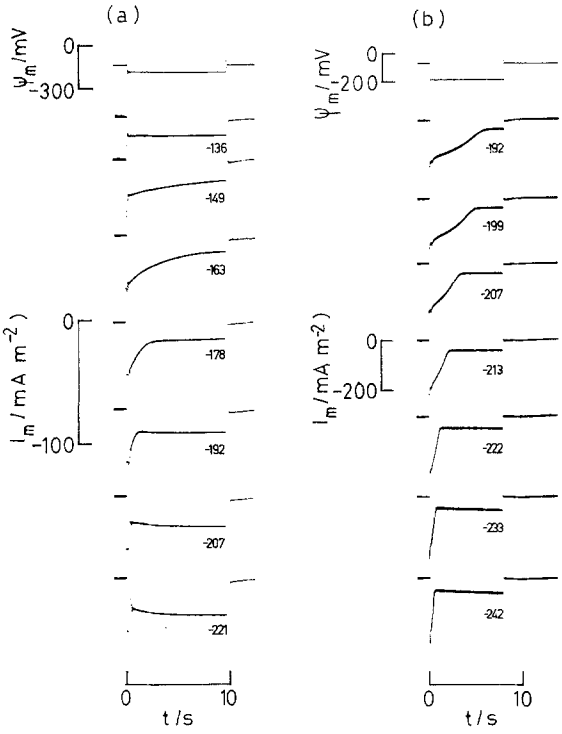

Fig. 4. Membrane currents for a series of negative voltage steps in ψ_m. The two curves are from different cells, and illustrate the range of shape of the current curves. (*a*) Holding PD (= resting PD) was −122 mV. $[K^+]_o$ = 0.1 mM, pH_o = 6.5. (*b*) Holding PD (= resting PD) was −65 mV. $[K^+]_o$ = 1.0 mM, pH_o = 8.5. Both cells were in the dark

rents for a series of voltage-clamp steps from the resting level of −165 mV, where $[K^+]_o$ = 0.1 mM and pH_o = 8.5. If 10 mM TEA^+ was added to the external solution, the membrane current flow during positive steps in ψ_m showed very little increase with time, during the voltage step (Fig. 2*b*). Figure 2*c* shows results from another cell where $[K^+]_o$ =

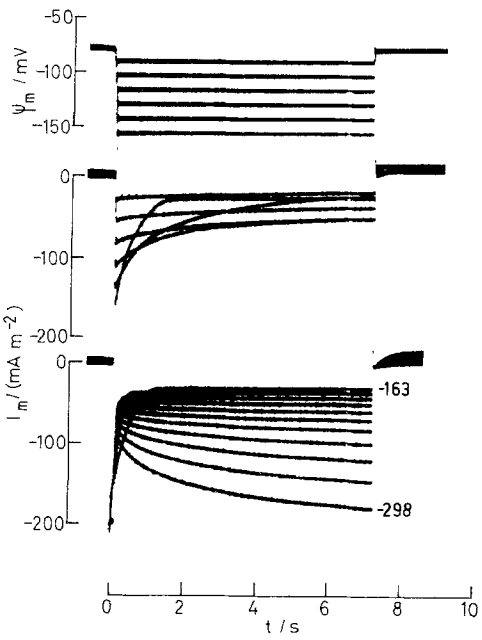

Fig. 5. Membrane currents for a series of voltage-clamp steps in ψ_m in one cell. The interval between each step in ψ_m was about 12.5 mV. The upper group of current curves was obtained for the PD steps illustrated. The lower set of current curves is for ψ_m in the range −163 to −298 mV. $[K^+]_o$ = 1.0 mM, pH_o = 6.5

1.0 mM. In this cell the resting PD, at −110 mV, was close to ψ_K. For the series of voltage-clamp steps, however, ψ_m was held at −150 mV. At the end of the pulse, when ψ_m was returned to holding level, current "tails" appeared with the current suddenly reversing direction, and its magnitude then decreasing to the original holding level. In other cells no increase in membrane current of the kind shown in Figs. 2*a* and 2*c* occurred. As the voltage-clamp steps were made increasingly more positive, the transient inward current associated with the action potential appeared when a second threshold level in ψ_m, usually about −50 mV, was exceeded. However, the membrane current, after the inward transient was over, was larger than the initial current (*see* Fig. 3) with a pronounced inward current "tail" following the cessation of the steps. In a few cells, the membrane was inexcitable.

Membrane Current for Negative Steps in ψ_m

In cells where $\psi_m \simeq \psi_K$, the membrane current for negative steps in ψ_m consisted of at least two discernible time- and voltage-dependent components. After the initial capacitative current flow, the inward current showed an initial step and then declined at a rate that was an increasing function of the clamped PD level. The time course of this decline was variable; sometimes complex, with a dis-

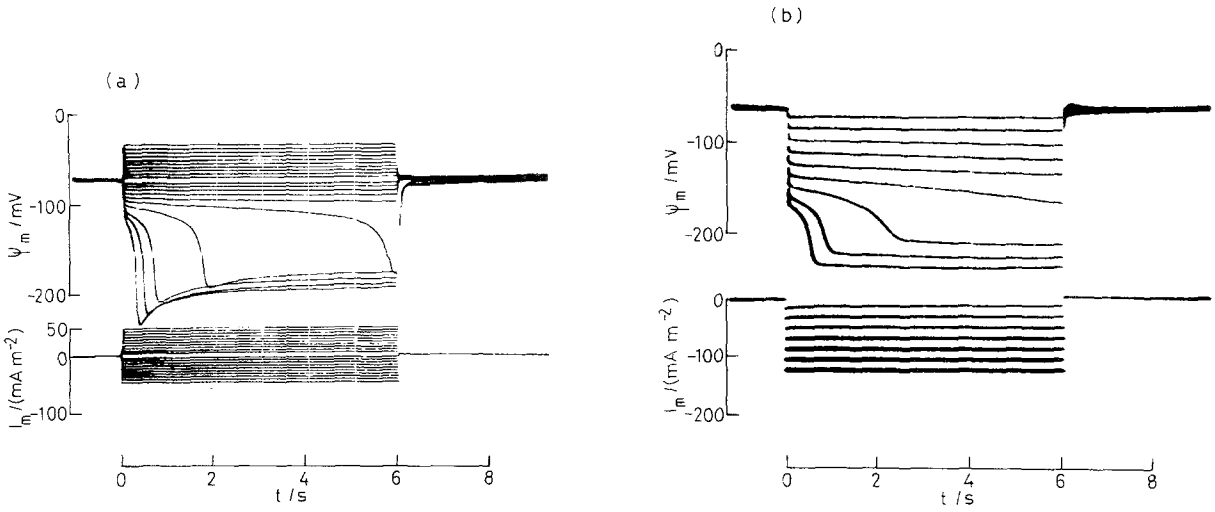

Fig. 6. (*a*,*b*) Two examples of the response of ψ_m (upper traces) to a series of constant current pulses of differing magnitudes. $[K^+]_o$ = 1.0 mM, pH_o = 8.5

tinct elbow, but never a simple exponential decline (*see* Fig. 4). The time for half change of this current, from its initial value to a steady value at the end of the pulse ranged from about 2 sec for $\psi_m = -160$ mV, to less than 130 msec for $\psi_m = -240$ mV. As the clamped PD was made increasingly more negative, a second component of inward current became apparent. This is particularly evident in Fig. 5.

In some cells, and particularly in those where the membrane was initially hyperpolarized, the decaying currents were absent.

Free Running Membrane; Response to ψ_m to Pulses of Constant Current

Several examples of the response of ψ_m, in cells where $\psi_m \simeq \psi_K$, to pulses of constant current are shown in Fig. 6. Positive steps in I_m produced fairly constant steps in ψ_m, although with some slow increase in magnitude.

Negative steps, however, produced a characteristic response in ψ_m, provided ψ_m was made more negative than a threshold level; about −93 mV in Fig. 6*a*. During the response $\partial\psi_m/\partial t$ increased, until ψ_m reached a peak value. Usually, ψ_m then declined in magnitude, and attained a steady value after a few seconds. In some cells, ψ_m remained steady at the initial peak level (*see* Fig. 6*b*). In a few cells, an oscillation in ψ_m appeared after the initial peak. In all of these responses, the time interval between the start of the pulse and the peak value of ψ_m decreased as the magnitude of I_m was increased.

The presence of 10 mM TEA^+ in the external medium eliminated the threshold behavior in ψ_m, with ψ_m responding to negative current steps in a simpler manner, as shown in Fig. 7.

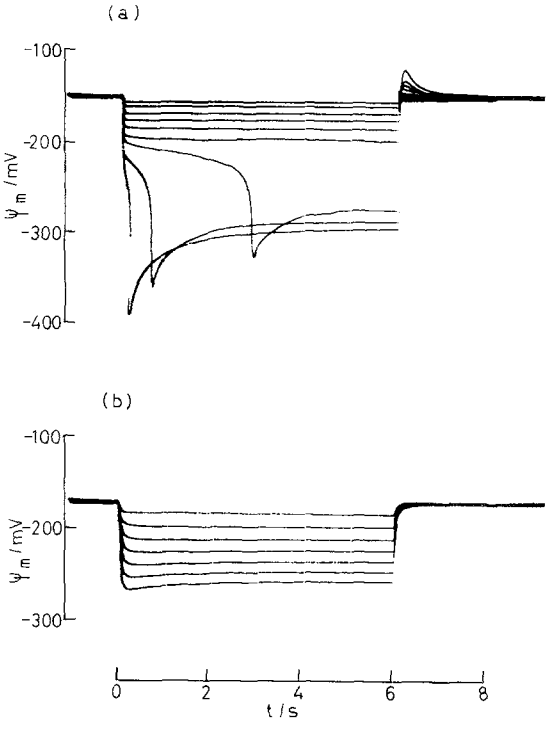

Fig. 7. The effect of 10 mM TEA^+ in the external solution on the response of ψ_m to a series of constant current steps (not shown) of differing magnitudes. (*a*) Before TEA^+, and (*b*) with TEA^+. $[K^+]_o$ = 0.1 mM, pH_o = 8.5. Initially $\psi_m \simeq \psi_K$. In (*a*) the current steps were multiples of 10.9 mA m^{-2}, and in (*b*), of 4.9 mA m^{-2}

Current-Voltage Curves

From the voltage-clamp data we define the following quantities. (a) I_1, the instantaneous current, which is the membrane current flowing immediately

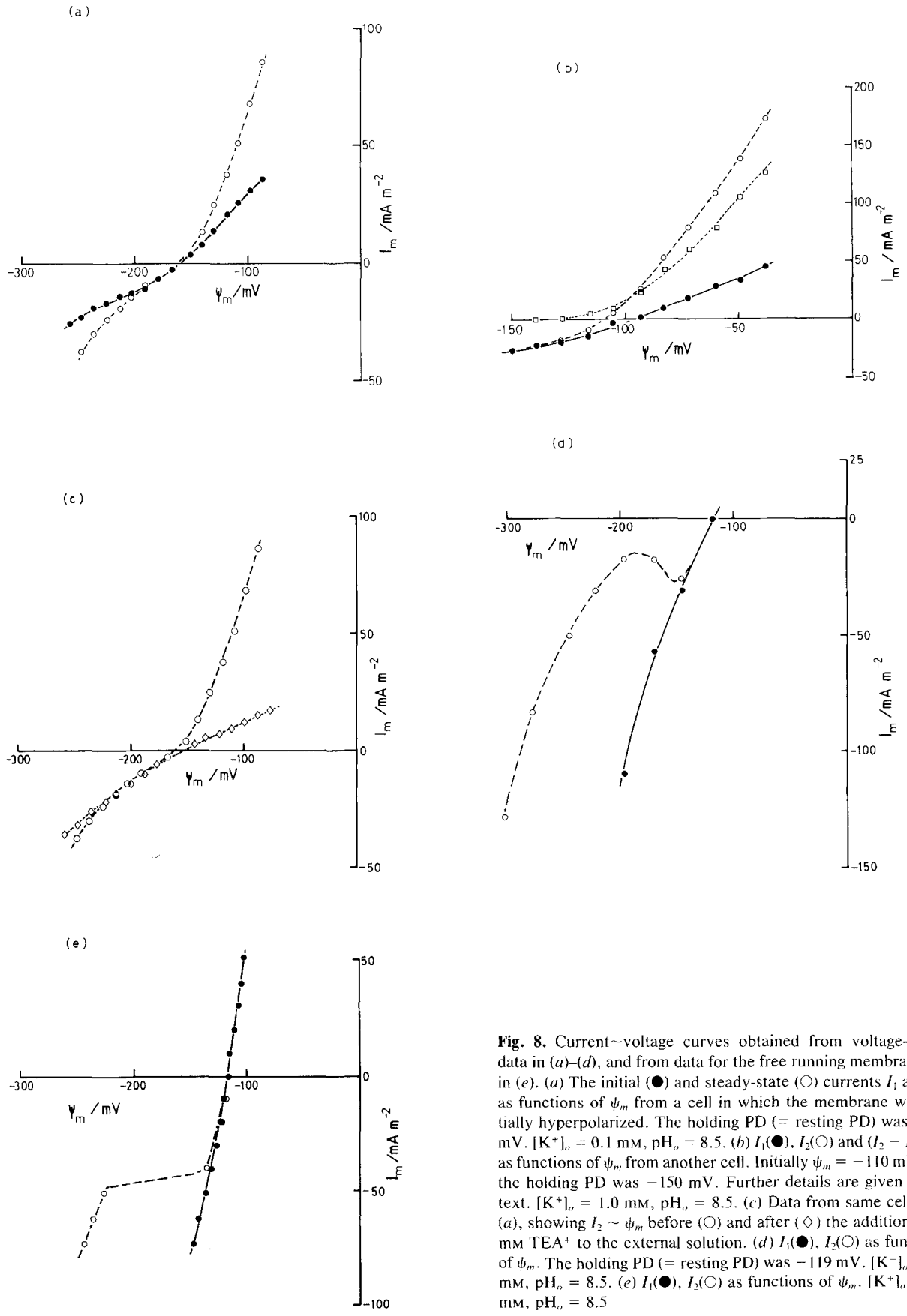

Fig. 8. Current–voltage curves obtained from voltage-clamp data in (*a*)–(*d*), and from data for the free running membrane PD in (*e*). (*a*) The initial (●) and steady-state (○) currents I_1 and I_2, as functions of ψ_m from a cell in which the membrane was initially hyperpolarized. The holding PD (= resting PD) was −161 mV. $[K^+]_o$ = 0.1 mM, pH_o = 8.5. (*b*) I_1(●), I_2(○) and ($I_2 - I_1$) (□) as functions of ψ_m from another cell. Initially ψ_m = −110 mV, but the holding PD was −150 mV. Further details are given in the text. $[K^+]_o$ = 1.0 mM, pH_o = 8.5. (*c*) Data from same cell as in (*a*), showing $I_2 \sim \psi_m$ before (○) and after (◇) the addition of 10 mM TEA^+ to the external solution. (*d*) I_1(●), I_2(○) as functions of ψ_m. The holding PD (= resting PD) was −119 mV. $[K^+]_o$ = 1.0 mM, pH_o = 8.5. (*e*) I_1(●), I_2(○) as functions of ψ_m. $[K^+]_o$ = 1.0 mM, pH_o = 8.5

Table. Electrical parameters of the *Chara inflata* membrane

$[K^+]_o$ (mM)	pH_o	PD (mV)		Conductances (S m^{-2})	
		$\psi_m(I_1 = 0)$	ψ_K[a]	$\partial I_1/\partial\psi_m\|_{I_1=0}$	$\partial(I_2 - I_1)/\partial\psi_m(\psi_m = -100$ mV)
			K^+ channels "closed"		
0.1	6.5	−142 ± 7(3)[b]	−166	—	—
0.1	7.0	−196 ± 8(9)	−166	—	—
0.1	8.5	−163 ± 6(29)	−166	—	—
0.1	8.5	−152 ± 9(7)	−166	0.31 ± 0.03(7)	1.28 ± 0.49(7)
1.0	8.5	−165 ± 10(4)	−108	—	—
1.0	8.5	−112(1)	−108	0.61(1)	2.94(1)
3.0	7.0	−211 ± 7(6)	−79	—	—
			K^+ channels "open"		
1.0	8.5	−96 ± 7(8)	−108	—	—
1.0	8.5	−93 ± 6(11)	−108	1.41 ± 0.18(11)	—
3.0	7.0	−83 ± 5(6)	−79	—	—
3.0	8.5	−62(1)	−79	—	—

[a] Nernst PD for K^+; *see* text for details.
[b] Mean value ± standard error of the mean. Sample size shown in parentheses.

upon a step in ψ_m, but after a time that allowed the decay of the purely capacitative current ($I_c = C\,\partial\psi_m/\partial t$ where C is the capacitance of the membrane), and (b) I_2, the "steady" current, usually taken several seconds after the start of the pulse, when the rate of change of current was small. Figure 8*a* shows curves of $I_1, I_2 \sim \psi_m$ for a cell in which the membrane was hyperpolarized, and in which the current showed clear voltage- and time-dependent behavior for positive steps in ψ_m. Figure 8*b* shows I_1, $I_2 \sim \psi_m$ curves plotted from the data shown in Fig. 2*c*. When the time-dependent behavior of the current was eliminated by TEA^+ (*see* Fig. 2*b*) the $I_2 \sim \psi_m$ curve was as shown in Fig. 8*c*. $I_1, I_2 \sim \psi_m$ curves for a cell where the resting membrane PD was close to ψ_K are shown in Fig. 8*d*; here the $I_2 \sim \psi_m$ curve shows a region of negative slope.

Figure 8*e* shows I_1, $I_2 \sim \psi_m$ curves obtained from the response of the free running membrane PD to pulses of constant current. The steady curve follows the instantaneous curve until for a particular value of ψ_m, about −120 mV in the figure, it diverges to a new position by an approximately horizontal transition, with the region of negative slope being absent.

The following quantities have been obtained from experiments on a total of 29 cells: ψ_m ($I_2 = 0$), $[\partial I_1/\partial\psi_m]_{(I_1=0)}$ and $[\partial(I_2 - I_1)/\partial\psi_m](\psi_m + -100$ mV) and are shown in the Table. The data is classified according to whether (a) positive voltage-clamp steps produced the increasing membrane currents shown in Figs. 2*a,c*—that is, K^+ channels are closed (*see* Discussion) or (b) negative voltage-clamp steps produced the decreasing membrane currents shown in Figs. 4 and 5—that is, K^+ channels are open. Data from cells where pH was other than 8.5, and from cells in the dark are also included in the Table.

Discussion

Identity and Location of Ion Channels

In a number of plant membranes ions move through specific channels. These channels which at present are only inferred, have characteristic kinetics of opening and closing. For example, in the charophyte *Chara corallina,* there are specific Cl^-, Ca^{2+} and K^+ voltage- and time-dependent channels involved in the action potential (Beilby & Coster, 1979) and in *Hydrodictyon africanum,* there are voltage- and time-dependent K^+ channels (Findlay & Coleman, 1983). Our experiments with *Chara inflata* show that there are both inward and outward voltage- and time-dependent membrane currents that flow in response to voltage-clamped steps in the membrane PD. The evidence suggests that in the *Chara inflata* membrane there are at least two types of channels, K^+ channels which open with sigmoid kinetics for positive steps in ψ_m, and close with complex exponential kinetics for negative steps in ψ_m, and Cl^- channels which open for negative steps in ψ_m, although the evidence for Cl^- channels is far from conclusive. The major part of this paper deals with K^+ channels.

The identification of the K^+ channels rests mainly on the observed effects of TEA^+ (a known blocker of K^+ channels in animal membranes; Arm-

strong, 1975) in (a) preventing the time-dependent current flow in response to positive or negative steps in ψ_m (*see* particularly Fig. 2) and in (b) causing g_m to fall and ψ_m to become more negative when it is applied to cells where the channels are initially open. Other evidence is the dependence of ψ_m, when the K^+ channels are open, on $[K^+]_o$ as shown in the Table and in Fig. 8. The evidence, on its own, is not entirely convincing because although ψ_m becomes less negative as $[K^+]_o$ increases, the expected fit to the Nernst equation is not good. However, the evidence as a whole, and the close similarities between the clamp currents in *Chara inflata* and *Hydrodictyon africanum* (where the identity of the K^+ channels is clearer) support the identification of K^+ channels in *Chara inflata*.

If the electrode recording the PD is in the vacuole, then ψ_m measures in fact the PD across a composite membrane consisting of plasmalemma and tonoplast in series. Thus in principle there is a problem in ascribing the channel behavior to either of the membranes separately. However, if in *Chara inflata,* the overall conductance of the tonoplast is much higher than the plasmalemma conductance, as it is in *Chara corallina* (Findlay & Hope, 1964), then the major part of any appreciable change in conductance, measured across the composite membrane, for instance, a change of about 4× when the K^+ channels open (*see* Table), must occur across the plasmalemma. Large changes in conductance of the composite membrane also occur for hyperpolarizing steps in ψ_m (Fig. 8). We therefore conclude that the voltage- and time-dependent K^+ and Cl^- channels in *Chara inflata* are most probably located in the plasmalemma.

An interesting feature of the membrane of *Chara inflata* is that K^+ channels did not always open for positive steps in ψ_K. In some cells, the K^+ channels only opened after the transient ionic current associated with the action potential had occurred. In other cells, there appeared to be no channel opening. There do not seem to be any obvious reasons for these differences. The behavior of the membrane of *Chara inflata* with regard to the opening of K^+ channels, lies between that of *Hydrodictyon* (Findlay & Coleman, 1983) where the channels opened consistently when the membrane was depolarized, and that of *Chara corallina* (Keifer & Lucas, 1982) where the K^+ channels only opened after the Ca^{2+} and Cl^- currents of the action potential had been initiated.

Opening and Closing of K^+ Channels

For positive steps in ψ_m, I_m (an outward current) rises from an initial level with a characteristic time course as the K^+ channels open (*see* Figs. 2*a*, 2*c*). The opening of K^+ channels can be blocked when TEA^+ is applied to the outside of the cell. The remaining current (Fig. 2*b*), which we assume to be current flow through other diffusive pathways and the H^+ efflux pump conductance, shows a rapid rise at the beginning of the pulse, but little variation with time during the pulse. Provided this current is not affected by TEA^+, and also remains constant when TEA^+ is absent and the K^+ channels are opening, it may be subtracted from the total current, to yield the current I_K carried by K^+. This current, which reaches 90% of its maximum change in 2 to 4 sec, has a time course very much slower than that observed in excitable membranes of animals such as the squid (Hodgkin & Huxley, 1952) but is similar to the time course for the K^+ current flowing across the membrane in cells of the plant, *Hydrodictyon africanum* (Findlay & Coleman, 1983).

A good demonstration of the opening of K^+ channels is also shown in Fig. 1*a*. After the initial hyperpolarization at light-off, ψ_m became less negative but g_m initially decreased, probably as a result of a decrease in the conductance of the H^+ efflux pump (*see* Findlay, 1982, for a discussion of similar behavior in *Hydrodictyon africanum*). When ψ_m moved above a threshold level, g_m increased and ψ_m moved at an increasing rate towards ψ_K. This increase in g_m is due to the opening of K^+ channels. The figure also shows a slow change in ψ_m to a value less negative than ψ_K. This change could possibly be caused by the opening of other types of channel, possibly Cl^-, whose identity remains to be determined.

The decay of I_K, as the K^+ channels close, has been observed in two types of experiment. Where the channels are initially open, negative steps in ψ_m cause closing of the channels, with the rate of closing, as shown by the rate of decline of I_K (an inward current), a function of ψ_m (*see* Figs. 4 and 5). One obvious feature of these decaying currents is the variability of their time course from cell to cell, particularly where, as in Fig. 4, the rate of decay of I_K increases with time, with the production of a sharp "elbow" in the curves. Alternatively the K^+ channels, if initially closed, can be opened by positive steps in ψ_m and then closed again when ψ_m is returned to the holding level. This is shown in Fig. 2*c*. The bunching-up of the tail currents indicates that g_K ($= \partial I_K/\partial \psi_m$) is tending to a maximum value. The data shown in Fig. 2*c* is also interesting, because ψ_m was initially at −100 mV, with the K^+ channels open. Setting the holding PD to −150 mV caused the channels to close, and then positive steps in ψ_m caused the reopening of the channels.

The blocking of K^+ channels by TEA^+ and the consequent decrease in g_m is well illustrated in Fig.

1*b*. Initially, the K^+ channels were open, and ψ_m was near ψ_K. The addition of TEA^+ blocked the channels, and the characteristic increase in ψ_m, and decrease in g_m occurred. The time course of change in ψ_m is similar to that for steps of inward current applied to the free running membrane PD, but slower, indicating perhaps, the time course of blocking of the channels by TEA^+.

Other Inward Current

The K^+ current will be inward or outward depending on whether ψ_m is more, or less negative than ψ_K. There are also at least two other components of the inward current which can be distinguished from I_K by their time courses. One, associated with the action potential is initiated when ψ_m is less negative than about −50 mV, and shows inactivation (Fig. 3). If this current is similar to that in *Chara corallina*, then it will be predominantly a Cl^- current (Hope & Findlay, 1964). The other inward current is initiated when ψ_m is made more negative than about −200 mV, and increases to a maximum value with a time scale of seconds (Fig. 5).

Current-Voltage Curves

K^+ Channels Initially Closed

When the K^+ channels are closed, the $I_1 \sim \psi_m$ curve for the membrane will represent the current-voltage curve for the pump and diffusive pathways. In Fig. 8*b* the $I_1 \sim \psi_m$ curve was obtained at a short time after the start of the clamp step, before the K^+ channels opened. Where the K^+ channels opened, the $(I_2 - I_1) \sim \psi_m$ curve should give the current-voltage curve for the K^+ channels alone. This curve shows the extent of opening of the K^+ channels in the steady state, as a function of ψ_m. The value of $g_K (= \partial(I_2 - I_1)/\partial\psi_m)$ of 1.28 S m^{-2}, from the Table, is about three times higher than the corresponding value for *Hydrodictyon* (Findlay & Coleman, 1983).

K^+ Channels Initially Open

For cells where $\psi_m \simeq \psi_K$ and where K^+ channels were open, the $I_1 \sim \psi_m$ curve gives the current-voltage curve for the open channels, together with a component from the pump, and remaining diffusive pathways. For $[K^+]_o$ = 1.0 mM, g_m = 1.41 S m^{-1}, about twice the value obtained with the K^+ channels initially closed. The $I_2 \sim \psi_m$ curve diverges from the $I_1 \sim \psi_m$ curve when ψ_m is taken below the threshold for closing of the K^+ channels, and shows a region of negative slope. If only K^+ channels open as ψ_m is made more negative than the threshold, we would expect the $I_2 \sim \psi_m$ curve to diverge approximately towards that for pump and diffusive components. However, as ψ_m becomes more negative, the inward current, as shown in Fig. 5, appears, and its steady magnitude increases. The combined effect of the closing of the K^+ channels, and the appearance of the inward current produced the hump in the $I_2 \sim \psi_m$ curve; after the K^+ channels have closed, the $I_2 \sim \psi_m$ curve is that for the time-dependent inward current alone, together with the pump and diffusive components.

The current-voltage curves for the free running membrane are similar to those for the voltage-clamped membrane, except that the hump in the $I_2 \sim \psi_m$ curve is replaced by a horizontal segment. This occurs because of the positive feedback control of the closing of the K^+ channels, as discussed below, and shown in Fig. 6. Similar current-voltage curves have been observed in *Chara corallina* (Ohkawa & Kishimoto, 1977). Coster (1969) has shown that in *Chara corallina* there is a large increase in Cl^- efflux when the membrane PD is made very negative, and he attributed this to an instantaneous reversible breakdown of the membrane, or "punch-through." Ohkawa and Kishimoto (1977), from electrical measurements, also suggest that the inward current is carried by Cl^-. Recently, Beilby and Beilby (1983) have demonstrated a time-dependent, noninactivating, inward current in *Chara corallina* during negative voltage-clamp steps. It seems likely, therefore, that both in *Chara corallina* and *Chara inflata* (Findlay & Tyerman, 1984), there may be a predominantly inward Cl^- current flowing through channels activated by negative voltage steps, rather than as a result of "punch-through."

Free Running Membrane PD

The time course of the free running membrane PD, as shown in Figs. 6 and 7, can be explained from our knowledge of the voltage- and time-dependence of I_K and other membrane currents. Figure 6 shows data from a cell where the membrane potential was initially near ψ_K and a major fraction of the K^+ channels were open, as indicated by the small, time-independent changes in the membrane PD in response to constant current steps of either polarity. For negative steps of sufficient amplitude, the membrane PD reaches a value more negative than the threshold value for the closing of the K^+ channels. As a greater number of K^+ channels close, the current~voltage curve for the membrane shifts towards that for pump and diffusive components (*see*

Fig. 8*a*). This means that ψ_m becomes more negative, which in turn tends to close an even greater number of K^+ channels. Because of this positive feedback, once the threshold value is reached during negative current steps, ψ_m becomes more negative very rapidly. However, as ψ_m becomes more negative, the other voltage-dependent inward current is activated, and at times of 5 sec or so, it reaches its maximum. Thus ψ_m at long times after the start of the inward current pulse will lie on the $I_2 \sim \psi_m$ curve. This curve lies between the current ~voltage curves for K^+ channels open and K^+ channels closed. Consequently, the excursion of ψ_m during the inward current pulse will be from the $I_1 \sim \psi_m$ curve, towards the curve for pump and diffusive components, and then back towards the $I_2 \sim \psi_m$ curve. The extent of the excursion of the current ~voltage curve towards the curve for pump and diffusive component will be determined by the relative rates of decay of I_K and appearance of the other inward current. Figure 6*a* shows data where the response of ψ_m showed a definite peak, while Fig. 6*b* shows ψ_m remaining at a plateau. In the first case the ratio of rate of decay of I_K to rate of rise of other inward current, would be less than for the second case. When TEA^+ blocked the K^+ channels the time course of ψ_m during the negative step did not show the characteristic increase in $\partial\psi_m/\partial t$ of the initial rising phase, and ψ_m changed more directly to a steady value, determined by the $I_2 \sim \psi_m$ curve.

There have been many reports in the literature of hyperpolarizing responses of the *Chara* membrane similar to those shown in Fig. 6 for *Chara inflata* [*see*, for example, Hope (1965), Kishimoto (1966), Ohkawa and Kishimoto (1977) and Tazawa and Shimmen (1980)]. Various explanations have been proposed to account for these responses; most are in terms of two states of the membrane. These hyperpolarizing responses can now be explained in terms primarily of the behavior of voltage-dependent K^+ channels in the membrane, whose rate of closing increases as the membrane PD is made more negative, together with the opening of other types of channels, probably Cl^-, carrying inward current, as discussed by Findlay and Tyerman (1984).

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References

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

Beilby, M.J., Beilby, B.N. 1983. Potential dependence of the admittance of *Chara* plasmalemma. *J. Membrane Biol.* **74**:229–245

Beilby, M.J., Coster, H.G.L. 1979. The action potential in *Chara corallina*. II. Two activation-inactivation transients in voltage clamps of the plasmalemma. *Aust. J. Plant Physiol.* **6**:323–335

Coster, H.G.L. 1969. The role of pH in the punch-through effect in the electrical characteristics of *Chara australis*. *Aust. J. Biol. Sci.* **22**:365–374

Findlay, G.P. 1982. Electrogenic and diffusive components of the membrane of *Hydrodictyon africanum*. *J. Membrane Biol.* **68**:179–189

Findlay, G.P., Coleman, H.A. 1983. Potassium channels in the membrane of *Hydrodictyon africanum*. *J. Membrane Biol.* **75**:241–251

Findlay, G.P., Hope, A.B. 1964. Ionic relations of cells of *Chara australis*. VII. The separate electrical characteristics of the plasmalemma and tonoplast. *Aust. J. Biol. Sci.* **17**:62–77

Findlay, G.P., Hope, A.B. 1976. Electrical properties of plant cells: Methods and findings. *In:* Encyclopedia of Plant Physiology. New Series, Vol. 2, Part A. Transport in Plants II. U. Lüttge and M.G. Pitman, editors. pp. 53–92. Springer, Berlin-Heidelberg-New York

Findlay, G.P., Tyerman, S.D. 1984. Voltage-dependent inward current in the membrane of *Chara inflata*. *In:* Membrane Permeability: Experiments and Models. A. Bretag, editor. pp. 39–42. Techsearch, Adelaide

Hodgkin, A.L., Huxley, A.F. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.* (*London*) **116**:449–472

Hope, A.B. 1965. Ionic relations of cells of *Chara australis*. X. Effects of bicarbonate ions on electrical properties. *Aust. J. Biol. Sci.* **18**:789–801

Hope, A.B., Findlay, G.P. 1964. The action potential in *Chara*. *Plant Cell Physiol.* **5**:377–379

Keifer, D.W., Lucas, W.J. 1982. Potassium channels in *Chara corallina*. *Plant Physiol.* **69**:781–788

Kishimoto, U. 1966. Hyperpolarising response in *Nitella* internodes. *Plant Cell Physiol.* **7**:429–439

Latorre, R., Coronado, R., Vergara, C. 1984. K^+ channels gated by voltage and ions. *Annu. Rev. Physiol.* **46**:485–495

Ohkawa, T., Kishimoto, U. 1977. Breakdown phenomena in the *Chara* membrane. *Plant Cell Physiol.* **18**:67–80

Smith, P.T., Walker, N.A. 1981. Studies on the perfused plasmalemma of *Chara corallina:* I. Current-voltage curves: ATP and potassium dependence. *J. Membrane Biol.* **60**:223–236

Tazawa, M., Shimmen, T. 1980. Action potential in characeae: Some characteristics revealed by internal perfusion studies. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas and J. Dainty, editors. pp. 349–362. Elsevier-North Holland Biomedical, Amsterdam

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