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 La³⁺. 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 Ca²⁺ or 5 mM tetraethylammonium (TEA). The K⁺ channels are therefore blocked by TEA, closed by low [K⁺]o or high [Ca²⁺]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 [Ca²⁺]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 La³⁺ 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 CaCl₂, 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 O and after • application of 0.1 mM LaCl₃. (LaCl₃ 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 Δ, 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 Ca²⁺ 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 LaCl₃ for ~1 hr (Beilby, 1984). In the work on K⁺ channels the La³⁺ was applied with some misgivings, as previous studies indicate some influence of La³⁺ 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 LaCl₃. 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 LaCl₃. In the present work the LaCl₃ 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⁺]₀

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