J. Membrane Biol. 99, 41–49 (1987)

The Journal of
Membrane Biology

Current-Voltage Relationships of Potassium Channels in the Plasmalemma of *Acetabularia*

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Summary. The outer membrane of mechanically prepared protoplasmic droplets from *Acetabularia mediterranea* has been investigated by patch-clamp techniques. These membranes are shown to consist of physiologically intact plasmalemma. With the Cl^- pump inhibited, microscopic currents through K^+-selective channels were studied. These currents compare well with macroscopic K^+ currents as previously determined by standard microelectrode techniques and tracer flux measurements. There is about one K^+ channel per μm^2 in the plasmalemma. The current-voltage relationship (*I-V* curve) of the main open channel (channel A) is sigmoid over a voltage range between about −100 and +100 mV with saturation currents of about ±10 pA. A second species (or different state of channel A) of K^+-selective channels (channel B) differs from channel A by smaller saturation currents (about ±7 pA) and a much smaller open probability. The open probability of channel A increases from almost zero at large negative voltages to about ½ at large positive voltages. Taking the closed times into account, the mean steady-state *I-V* curve of channel A displays outward rectification about the equilibrium voltage for K^+ and negative slope conductance at larger negative voltages. The open channel *I-V* curves of the channels A and B, the changes of the *I-V* curve of the open channel A upon variation of the external K^+ concentration, as well as the mean steady-state *I-V* curves of channel A are described by simple reaction kinetic models, the parameters of which are determined to fit the experimental data. The results are discussed with respect to data from other K^+ channels in plants and with respect to regulation of the cytoplasmic K^+ concentration in *Acetabularia*.

Key Words open channel · voltage gating · outward rectification · reaction kinetic model · uniport

Introduction

One of the most important advantages of patch-clamp techniques is the possibility of investigating ion transport mechanisms across biological membranes on a molecular, microscopic level (for a collection of fundamental contributions in this field, *see* Sakmann & Neher, 1983), in order to understand the macroscopic transport properties of the investigated membrane. As far as plant membranes are concerned, the macroscopic electrical properties are rather well investigated for the plasmalemma of intact giant algal cells of *Characeae* and of *Acetabularia* (for reviews, *see* Hope & Walker, 1975, Gradmann, 1984; Beilby, 1985). It is, therefore, of particular interest to study the role of microscopic channel currents in the electrical behavior of the plasmalemma in these cells.

This study is a first attempt to do this for the case of *Acetabularia*. The plasmalemma of these cells exhibits a striking outward rectification of K^+ currents (Gradmann, 1970, 1975; Gradmann & Bentrup, 1970; Mummert & Gradmann, 1976). However, this phenomenon was poorly understood on physicochemical grounds. For the solution of this problem, considerable theoretical and experimental progress has been achieved during the past years. Theoretically, by the reaction kinetic interpretation of electrical membrane phenomena in plants (Hansen et al., 1981; Gradmann, Klieber & Hansen, 1987) and experimentally by successful extension of patch-clamp techniques to plant membranes (Coleman & Walker, 1984; Schroeder, Hedrich & Fernandez, 1984; Coleman, 1986; Hedrich, Flügge & Fernandez, 1986; Lühring, 1986).

For direct access of the measuring micropipette to the plasmalemma we used mechanically prepared protoplasmic droplets. From these preparations microscopic currents through single, K^+-selective channels have been recorded.

The experimental results are explicitly described by simple reaction kinetic models for K^+ channels. Furthermore, these microscopic K^+ currents are discussed with respect to macroscopic K^+ currents (Gradmann, 1975) and macroscopic K^+ fluxes (Mummert & Gradmann, 1976) and their particular impact for the regulation of cytoplasmic K^+.

Materials and Methods

Plant Material

Acetabularia mediterranea was cultured in artificial seawater under a 12/12 hr light-dark period as described by Lüttke and Grawe (1984). For patch-clamp experiments only the caps of this marine alga were used.

Isolation of Protoplasmic Droplets

For the investigation of K^+ diffusion, the standard test medium was designed to eliminate the electrogenic Cl^- pump by using low Cl^- concentrations compared to normal 550 mM Cl^- in seawater. This test medium contained 130 mM KCl or KI, 1 mM NaCl or NaI, 5.5 mM $CaCl_2$, 5.5 mM $MgCl_2$ and (initially) about 500 mM sorbitol for osmotic adjustment. Immediately before use, the medium was filtered (Schleicher & Schuell, 0.2 μM).

For preparation of protoplasmic droplets a circle segment was cut from the cap of a cell in artificial seawater. Spherical droplets of 100–200 μm diameter formed spontaneously from the cell content flowing out of each chamber of the cap. These protoplasmic droplets were collected with a Pasteur pipette, washed in test medium and transferred into the same medium in the experimental chamber. These droplets show a thin peripheric layer with chloroplasts in which cytoplasmic streaming takes place. Surprisingly, the protoplasmic droplets maintained their apparent healthy state (cytoplasmic streaming) over many hours when sorbitol was omitted from the test medium. Actually, most measurements have been performed without osmotic adjustment, because in these media with low buoyant density the droplets sedimented much better to the bottom of the experimental chamber.

Patch-Clamp Experiments

Patch-clamp experiments were carried out according to the methods described by Hamill et al. (1981) with a commercial patch-clamp amplifier (E/M-EPC7, List, Darmstadt, FRG).

Glass capillaries with solid inner filament (Hilgenberg, FRG), providing easy and quick filling of the pipette, were pulled in two steps on a modified vertical electrode puller (700 C, DKI, USA). The tips of these pipettes were fire polished under microscopic control using a platinum-iridium heating wire (Ø 100 μm). In all experiments the pipette solution was the same as the external test solution. Normally, the tip resistance of these pipettes was in the range of 10 MΩ.

For monitoring the formation of a seal between the pipette and the membrane, rectangular voltage steps were applied to the pipette and the current to ground could be observed on an oscilloscope. After placing the micropipette at the membrane surface by means of a hydraulic micromanipulator (MO-103, Narishige, Japan), the current flow through the micropipette decreased, indicating seal resistances in the range of about 100 MΩ. Seal resistances between the patch electrode and the membrane surface of greater than 1 GΩ (up to 50 GΩ) were attainable by applying a negative pressure to the pipette. All single-channel currents were recorded in this "cell-attached" mode.

When a giga-seal was formed the membrane patch could be broken by additional suction. In this "whole-cell" recording mode membrane voltages as well as changes thereof were measured in order to identify the membrane investigated here (plasmalemma or tonoplast) and to compare the electrical properties of the protoplasmic droplets with those of the intact whole *Acetabularia* cell.

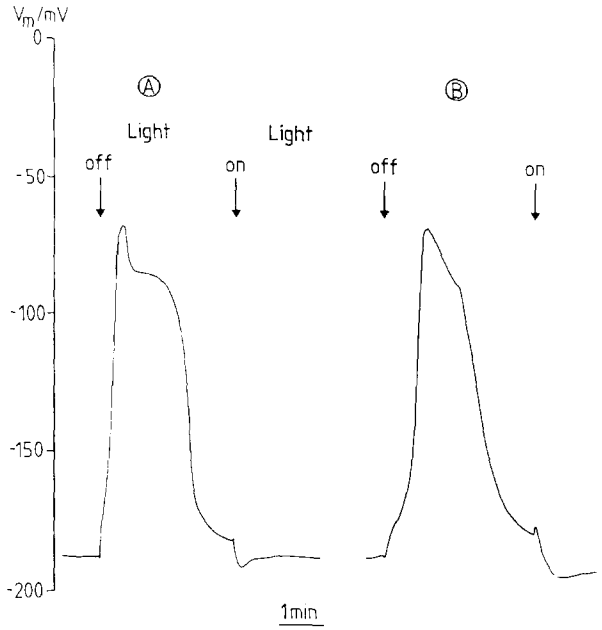

Fig. 1. Examples for equivalent electrical behavior (light-induced voltage changes and especially light-off-triggered action potentials) of (*A*) membrane of protoplasmic droplets recorded by the "whole-cell" patch-clamp mode and (*B*) plasmalemma of intact cells recorded by standard microelectrode techniques (*B*) Redrawn from Gradmann, 1976); external medium: artificial seawater in both experiments

In the "cell-attached" patch-clamp configuration, single-channel currents could be measured in the voltage-clamp mode. These signals due to channel activity were digitized by a modified digital audio processor (PCM-501ES, Sony, Japan) and stored on a video recorder (VS220 RC, Grundig, FRG).

For data analysis, the signals were played back, low-pass filtered with 400 Hz (8 pole 902LPF, Frequency Devices, USA), to a digital oscilloscope (204-A, Nicolet, USA) and copied with an *x-y* plotter (Gila 2000, Laumann, FRG). Current amplitudes as well as open and closed times were evaluated manually.

Results

Membrane Identification

In the "whole-cell" recording mode the membrane of the mechanically isolated protoplasmic droplets showed electrical properties equivalent to those of the plasmalemma of whole intact *Acetabularia* cells recorded by standard microelectrode techniques (for review, *see* Gradmann, 1984). In artificial seawater, membrane voltages up to −190 mV were recorded from the protoplasmic droplets. Light/dark-

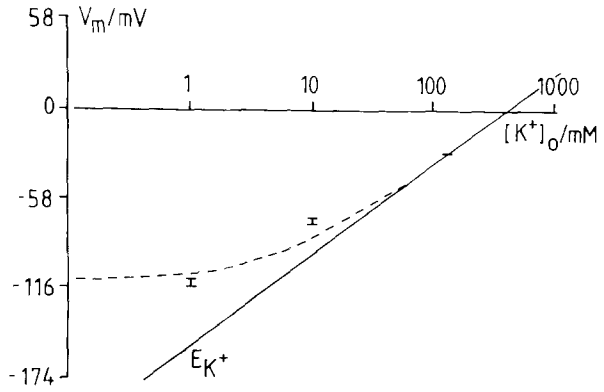

Fig. 2. $[K^+]_o$ sensitivity of the resting membrane voltage of protoplasmic droplets under inhibition of the electrogenic Cl^- pump using low Cl^- medium: 5.5 mM $CaCl_2$, 5.5 mM $MgCl_2$, 131 mM (K + Na) I; "whole-cell" recordings; measurements (bars comprise entire scatter) from recordings when the voltage did not change by more than 2 mV within about 100 sec; solid (straight) line: theoretical locus for the Nernst equilibrium voltage of K^+ at $[K^+]_i$ = 400 mM; dashed curve: fit of the Goldman equation for K^+ and Na^+ to all individual recordings; resulting permeability ratio P_{Na^+}/P_{K^+} = 0.037; ordinate scaled in "Nernst units" for temperature of the experiments between 18 and 21°C

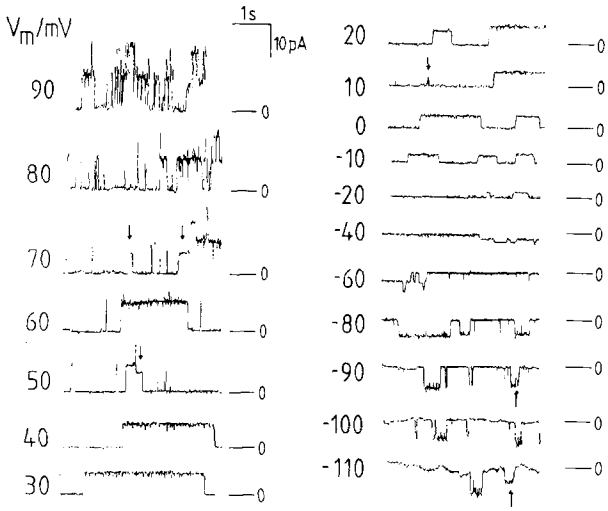

Fig. 3. Examples of original single-channel currents at various membrane voltages V_m (cell interior *vs.* pipette voltage), recorded in the cell-attached mode. —0 to the right of each record mark the actual "baseline" (patch-slang), i.e., the current through the seal (about 8 GΩ in this example), only with all channels closed. Signals were low-pass filtered with 400 Hz. K^+ concentrations: 400 mM inside the drop, 130 mM in bath solution and in pipette. Exceptionally small current amplitudes are marked by arrows. These examples are not representative with respect to the opening frequency over longer time

induced voltage changes as well as action potentials from these droplets are remarkably similar to those from the plasmalemma of the whole intact cell. Examples for this coincidence are shown by Fig. 1. The actual differences between these two traces are random.

These findings demonstrate that the membrane around the protoplasmic droplets consists of intact plasmalemma. Voltage recordings from isolated vacuoles (Bentrup et al., 1986; Hedrich et al., 1986) or from cytoplasmic droplets which are enclosed by a membrane of tonoplast origin (Lühring, 1986) show the opposite sign and much smaller values.

Reducing the activity of the electrogenic Cl^- pump by replacement of Cl^- by I^+ leads to voltages close to the equilibrium voltage for potassium (*see* Fig. 2). The approximate cytoplasmic concentrations for the major ions are known: K^+, 400 mM; Na^+, 70 mM (Saddler, 1970*a*; Mummert, 1979); Cl^-, 500 mM (Mummert, 1979), and Ca^{2+}, assumed 1 μM. Fitting the Goldman equation for K^+ and Na^+ to the data (curve in Fig. 2) yields a permeability ratio P_{Na^+}/P_{K^+} of 0.037. Since the actual equilibrium voltages of Cl^- (E_{Cl^-} = +80 mV) and Ca^{2+} ($E_{Ca^{2+}}$ = +195 mV) differ very much from the reported voltages, the diffusion permeabilities for Cl^- and for Ca^{2+} can be ignored in this context. These results already suggest that in this state the electrical membrane properties of the protoplasmic droplets are mainly controlled by K^+ diffusion.

Single-Channel Current Recordings

Figure 3 shows tracings of openings and closings of the most striking channel at different clamped voltages recorded in the cell-attached patch-clamp configuration. In all figures the given voltages represent the voltage difference across the membrane patch, i.e., cell-interior to pipette voltage, assuming constant internal voltage (*see* Fig. 2) during the experimental time course. Simple inspection of the examples of original recordings (Fig. 3) reveals several characteristics of the channels.

Identification

The channels under investigation are K^+ channels because the open channel currents reverse their sign at about −30 mV, which is the equilibrium voltage for potassium under these particular conditions (130 mM K^+ outside) when the internal K^+ concentration of the droplets is about the same (ca. 400 mM) as in intact cells (Saddler, 1970*a*).

Current-Voltage Curve of the Open Channels

The current-voltage characteristics are about linear (ohmic) for moderate voltage displacements (about

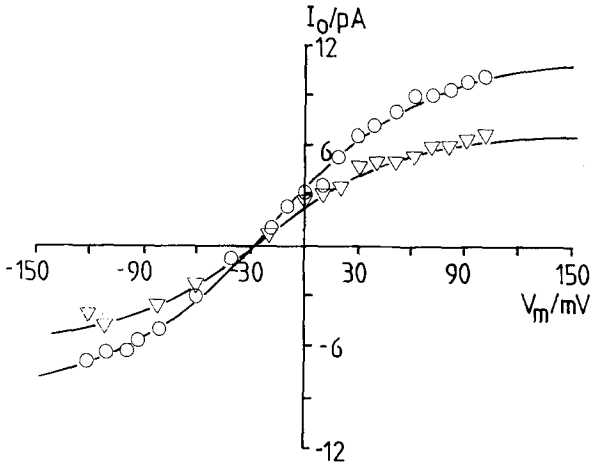

Fig. 4. Current-voltage relationships of the open channels. K^+ concentrations: ca. 400 mM inside the droplet, 130 mM in bath solution and in pipette. Circles: currents of "main" channel (A), triangles: small amplitude currents as marked by arrows in Fig. 3

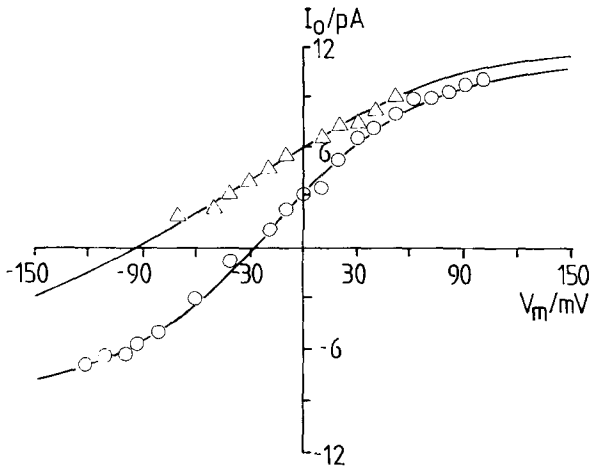

Fig. 5. Current-voltage relationship of the open channel as function of external K^+ concentration. Circles: experimental data with 130 mM K^+ in bath solution and in pipette; triangles: experimental data with 10 mM K^+ in bath solution and in pipette; curves: fits of Eq. (2) to the data with the model parameters in Table 2

50 mV either sign) from the equilibrium but tend to saturate at about 10 pA for large voltage displacements. These sigmoid current-voltage characteristics of the main open channel are represented in more detail by the circle symbols in Fig. 4.

It can be seen from the given examples of original tracings in Fig. 3, that some channel currents (marked by arrows) exhibit a smaller current amplitude compared to the most frequent events. The voltage dependence of these smaller channel currents (triangle symbols in Fig. 4) is (qualitatively) similar to the *I-V* characteristic of the main channel currents (circle symbols in Fig. 4). These low-conductance channel currents also reverse the sign at −30 mV and tend to saturate at high voltage displacements as the main channel currents do. However, the amplitude of the small channel currents (including the saturation currents) is only about 66% of the current amplitude of the most frequent events. These findings indicate that the low-conductance channels carry K^+ as well, even though with a lower rate. In some long term (>1,000 sec) recordings with regular activity of the main channel, the low-conductance channel currents were not observed.

The current-voltage relationship of the main channel changes upon variation of the external K^+ concentration. The data for low (10 mM) potassium outside (not represented in Fig. 3) in Fig. 5 (triangle symbols) display a more negative reversal voltage (extrapolatet −90 mV, as expected for a K^+-selective channel), a somehow flattened shape and a slightly increased positive saturation current. Data for inward currents are not available from this experiment. The data from the -O—O- curve in Fig. 4 have been reproduced as the high-Cl^- control in Fig. 5.

Open-Times

In the examples of Fig. 3 the open "events" appear to be very brief and frequent at large positive voltages and rather long and more seldom at more negative voltages. A preliminary analysis of the complete data, as sampled over a time period of about 30 sec for each voltage, shows that the open-time histogram (*not shown*) consists of two exponentials pointing to two different open states, which cannot be distinguished by different current amplitudes: one state with a mean life time of about 5 msec and another one with a mean life time of about 50 msec. Over longer periods of observation, the two open states contribute similar portions to the overall conductance and these portions do not seem to depend on the voltage. A more detailed treatment of these characteristics will be subject of a separate study.

Steady-State Current-Voltage Relationship of the K^+ Channel

In general, macroscopic K^+ currents in plant plasma membranes investigated by standard microelectrode techniques show an obvious outward rectification about the equilibrium voltage for potassium (Gradmann, 1970; Felle, 1981; Beilby, 1985).

This seems to be inconsistent with the symmetric saturation characteristic of the K^+ channel shown in Figs. 4 and 5. However, these sigmoid *I-V* curves reflect the current-voltage relationship of the open channel taking only the current amplitude into consideration and therefore cannot be compared with the macroscopic K^+ currents, which are steady-state currents.

The data in Fig. 6 are the mean steady-state currents of an individual channel as determined by averaging the single-channel currents (zero currents during the closed times included) over a period of about 25 sec at each voltage (data taken from the same experiments as the examples in Fig. 3). The frequent openings at positive voltages affect this mean steady-state current by a relatively large factor, and the infrequent openings at negative voltages by a relative small one. These resulting mean steady-state currents show an obvious outward rectification about the equilibrium voltage for potassium and a negative slope conductance at large negative voltages. The steep branch of this steady-state *I-V* curve of an individual channel has a slope conductance of about 20 pS within the voltage range between −20 and +100 mV.

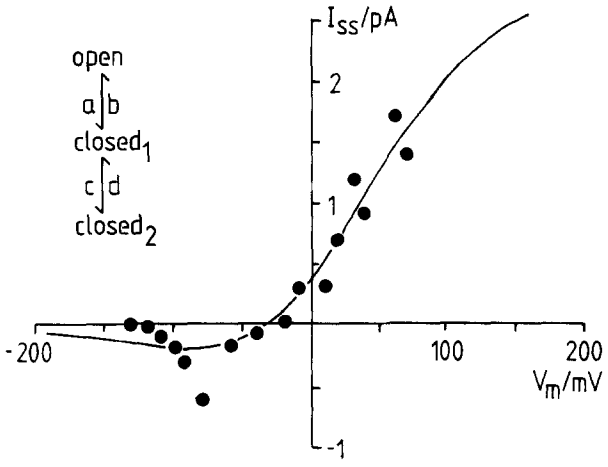

Fig. 6. Steady-state current-voltage relationship of the channel as determined by averaging the single-channel currents from experiment to Fig. 3 over a period of 30 sec. Point: experimental data; curve: fit of Eq. (4) to the experimental data with the parameters $K_1 = 1.3$, $K_2^0 = 5.3$, $z_g = 0.34$ and 2-state parameters in Table 1(A). *Inset:* hypothetical reaction scheme for voltage-dependent activation (inactivation); equilibrium constants: $K_1 = a/b$, $K_2 = c/d = K_2^0 \exp(-z_g eV/kT)$, with z_g being the charge number for voltage sensitivity of gating and $K_2^0 = K_2$ at $V = 0$ mV

Discussion

Membrane Identification

Patch clamp investigations cannot be performed on intact plant cells with a cell wall. Therefore, preparations without a cell wall (e.g. protoplasts or cytoplasmic droplets) or even without cytoplasm (in the case of isolated vacuoles) are used for patch-clamp studies on plants. For a discussion of the results with respect to their possible physiological significance, it is, therefore, important to demonstrate in how far the particular properties of the investigated channel species are correlated with the transport properties of the membrane under physiological conditions.

In the present study, the membrane of the protoplasmic droplet consisted of a physiologically intact plasmalemma. This conclusion is based on the close similarity between the electrical properties recorded from the enclosing membrane of the protoplasmic droplets (membrane voltages up to −190 mV, light/dark-induced voltage changes and action potentials, *see* Fig. 1*A*) and those of the plasmalemma of intact *Acetabularia* cells (Fig. 1*B*).

Furthermore, under inhibition of the electrogenic Cl^- pump, which was achieved by withdrawal of the substrate of the electrogenic pump (Cl^-), the electrical membrane properties are mainly controlled by K^+ diffusion (with a rather small permeability ratio P_{Na^+}/P_{K^+} of about 0.04 (*see* Fig. 2). An almost identical permeability ratio was found in intact *Acetabularia* cells, when the electrogenic pump was inhibited by cold (Saddler, 1970*b*).

These electrophysiological similarities of *Acetabularia* membranes from intact cells and from protoplasmic droplets (without turgor) suggest that turgor pressure is no critical parameter for the electrophysiological properties of the *Acetabularia* membrane. However, Wendler, Zimmermann and Bentrup (1983) reached a different conclusion from intracellular pressure measurements on intact *Acetabularia* cells.

Channel Identification

The data in Fig. 2 suggest that the electrical properties of the membrane under investigation are dominated by the passive permeation of K^+. The reversal voltages for the open channel currents in Figs. 3, 4 and 5 are close to the Nernst equilibrium potentials for potassium as well. These observations consistently indicate that under the given conditions (inhibition of the electrogenic pump), the dominating ion transport system in the membrane is passive diffusion of K^+ through K^+-selective channels.

Alternate State or Alternate Channel

Apart from the most frequent current levels of the main channel, some current levels with a significantly smaller amplitude were also observed (marked by arrows in Fig. 3). Similar observations are described by several authors as different conductance states of the investigated channel (Hamill & Sakmann, 1981; Latorre & Alvarez, 1981; for an overview, *see* Sachs, 1983). The given examples (in particular 50 and 70 mV tracings in Fig. 3) provide some information to the question whether these smaller currents are due to a different conductance state of the main channel or due to a separate channel species. The main open state has the conductance g. If the small conductance (g') would reflect a different state of the main channel, which has to be passed for transitions between the closed state(s) and the full opening, transitions with the amplitude g-g' should occur about as frequent as transitions with the amplitude g'. However, only transitions with the amplitude g or g' can be found and none with the amplitude g-g'. From this point of view, the low amplitude currents do not reflect another conducting state of the main channel. They rather represent a different type of channel.

On the other hand, the open channel A might apparently become channel B by passing a closed state. In this case no transitions with the amplitude g-g' could be observed, as well. In fact, the identical equilibrium voltage of the apparent channels A and B (Fig. 4) and their reaction kinetic similarities (Table 1) indicate functional relationships between these apparently different channels, which may point to a common structural entity. However, there is no strict coupling between the high and the low-conductance channels. Since, in some long term experiments, channel A was observed exclusively, the existence of a different channel species (B) can be concluded, which was absent in the particular patches.

Current-Voltage Relationship of the Open Channel

The channel currents presented in Fig. 4 show an obvious saturation tendency at high voltage displacements from the equilibrium voltage. Such saturation characteristics may be a common feature of K^+ channels in plants (*compare* Fisahn, Hansen & Gradmann, 1986; Schroeder et al., 1984; Lühring, 1986). This kind of *I-V* curve is characteristic for a cyclic charge transporting system with one voltage-sensitive reaction step (Class-I transporter, Hansen et al., 1981). The genuine model for the description of an individual Class-I *I-V* curve is a 2-state model (Hansen et al., 1981) providing two pairs of reaction constants, one of which is voltage sensitive (*see* 2-state model in Fig. 7). The current-voltage relationship of this 2-state model for the current I_o through the open channel is:

$$I_o(V) = ze \frac{\alpha\gamma - \beta\delta}{\alpha + \beta + \gamma + \delta} \quad (1)$$

with $\alpha = \alpha^0 \exp(zeV/2kT)$, $\beta = \beta^0 \exp(-zeV/2kT)$, where α^0 and β^0 are the values for α and β at $V = 0$ mV, z is 1 (for K^+), e, k and T have their usual thermodynamic meanings and the factor 2 stays for the assumption of a symmetric Eyring barrier. The four parameters α^0, β^0, γ and δ were fitted by Eq. (1) to the experimental data in Fig. 4, once for the main, high conductance channel (A) and once for the rare, low conductance channel (B). The curves in Fig. 4 are a graphical representation of the fits. The numerical results of the fits are given by the values in Table 1. These numbers suggest that the two channel species (A and B) mainly differ in the voltage-independent part of the reaction system (γ and δ), whereas the voltage-dependent reaction step (α and β) appears to be similar.

Changing the external substrate concentration, as done in the experiments to Fig. 4, yields two different *I-V* curves, which can be used to determine a 3-state model with three pairs of reaction constants, one of which is voltage sensitive (Gradmann et al., 1987). This 3-state model and its symbols are given in Fig. 7. The current-voltage relationship of this 3-state model for the current I_o of the open channel reads

Table 1. Parameters of the 2-state model (Fig. 7) for the current-voltage relationship of the open channel for the "main" high-conductance K^+ channel (A) and for "rare" low-conductance channel (B)[a]

Reaction	Symbol	A	B
Reorientation ($i \rightarrow o$) of charged complex at $V = 0$	α^0	128	138
Reorientation ($o \rightarrow i$) of charged complex at $V = 0$	β^0	51	53
Gross reaction for V-insensitive steps ($o \rightarrow i$)	γ	72	45
Gross reaction for V-insensitive steps ($i \rightarrow o$)	δ	56	36
	SD	1.2	1.6

[a] Data in 10^6 sec^{-1}; result of fits of Eq. (1) to experimental data in Fig. 4.

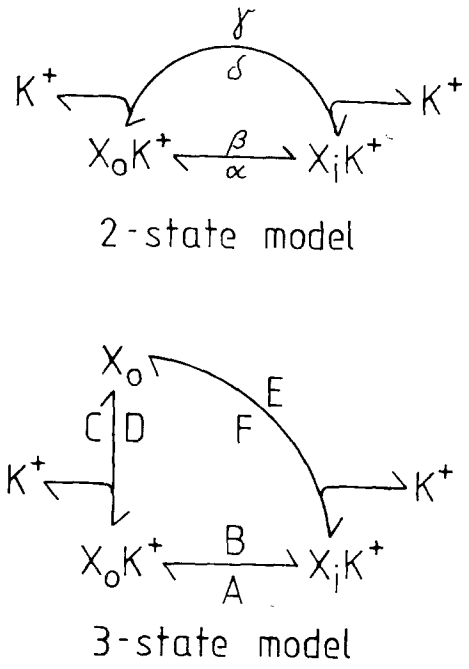

Fig. 7. Reaction kinetic models and nomenclature; $U = \exp(zeV/2kT)$, $z = 1$ (for K^+); 2-state model: $\alpha = \alpha^0 U$, $\beta = \beta^0/U$ with α^0 and β^0 being α and β at $V = 0$ mV; 3-state model: $A = A^0 U$, $B = B^0/U$ and $D = D^0[K^+]_o$, with A^0 and B^0 being A and B at $V = 0$ mV and D^0 being D at an external K^+ concentration of 1 M

$$I_o(V) = ze\frac{ACE - BDF}{A(C + D + E) + B(D + E + F) + C(E + F) + DF} \quad (2)$$

with $A = A^0 \exp(zeV/2kT)$, $B = B^0 \exp(-zeV/2kT)$, $D = D^0[K^+]_o$, where D^0 is the rate constant for K^+ association outside at the standard potassium concentration of 1 M, A^0 and B^0 are the voltage-sensitive 3-state rate-constants A and B at $V = 0$ mV. The drawn curves in Fig. 5 are the *I-V* curves as fitted by Eq. (2).

The resulting numerical values for the six rate constants are listed in Table 2 together with the results of an equivalent analysis (Table VII in Gradmann et al., 1987) on current-voltage data from a K^+-selective channel in the plasmalemma of guard cell protoplasts from *Vicia faba* (Schroeder et al., 1984). Although every rate constant turns out to be smaller for the K^+ channel in *Vicia* compared to *Acetabularia*, the qualitative relation between the individual rate constants ($F < E < A^0 < B^0 < D^0 < C$) appears to be identical for the two K^+ channels from such completely different plant species as *Acetabularia* (a unicellular alga) and *Vicia faba* (a higher plant).

Table 2. Parameters of 3-state model (Fig. 7) for the current-voltage relationship of the main open K^+ channel, and its dependence on the external K^+ concentration[a]

Reaction	Symbol	Values	
		Acetabularia	Vicia
Reorientation ($i \rightarrow o$) of charged complex at $V = 0$	A^0	146	33
Reorientation ($o \rightarrow i$) of charged complex at $V = 0$	B^0	804	167
Debinding of K^+ outside	C	53,306	10,220
Binding of K^+ outside	D^0	30,408	9,080
Gross reaction for reorientation ($o \rightarrow i$) of neutral binding site and K^+ binding inside	E	75	29
Gross reaction with K^+ debinding inside and reorientation ($i \rightarrow o$) of neutral binding site	F	55	25
	SD	1.6	

[a] Data in 10^6 sec^{-1} (except for D^0: 10^6 sec^{-1} M^{-1}); result of fit by Eq. (2) to experimental data in Fig. 3 for *Acetabularia*; values from a K^+-selective channel in *Vicia* (Gradmann et al., 1987) are added for comparison.

Steady-State Current-Voltage Relationship of the Main K^+ Channel

The data in Fig. 6 show an obvious outward rectification about the equilibrium voltage, which is typical for the macroscopic K^+ currents in *Acetabularia* as determined by conventional electrophysiology (Gradmann, 1975) or by tracer flux measurements (Mummert & Gradmann, 1976). The steep branch of the macroscopic K^+ outward current has a slope conductance of about 15 S m^{-2} (Gradmann, 1975). From the corresponding slope of the microscopic mean steady-state *I-V* curve of an individual channel (about 20 pS in Fig. 6), a density of about one channel per μm^2 can be estimated for the normal plasmalemma. This number compares well with the observation that about 3 channels are found in a patch of a few μm^2 membrane area. This coincidence further supports the conclusion that the membrane of the used protoplasmic droplets resembles physiologically intact plasmalemma.

The reaction kinetic interpretation of the apparent negative slope conductance of the mean steady-state *I-V* curve of an individual channel (Fig. 6) at large negative voltages requires the introduction of a second voltage-sensitive step in the entire reaction system, since reaction systems with only one voltage sensitive step have positive slope conductances throughout (Hansen et al., 1981). It is ob-

served that the mean open probability (p_o) of the K^+ channel increases from virtually zero at very negative voltages to larger values at positive voltages. Since the mean open-time of the channel does not appear to be voltage dependent, the immediate reaction step between the open state and the (primary) closed state seems to be voltage independent; voltage independence can also be expected for the reverse reaction (provided the Eyring barrier is not extremely asymmetric). The voltage sensitivity of p_o requires, therefore, an extra (voltage-sensitive and reversible) reaction step.

This requirement is fulfilled by postulating a series of two inactive (closed) states which equilibrate with the open state (*see* inset in Fig. 6) *via* the equilibrium constants $K_1 = a/b$ and $K_2 = c/d$. The latter one is now voltage sensitive: $K_2 = K_2^0 \exp(-z_g eV/kT)$, where K_2^0 is K_2 at $V = 0$ mV and z_g reflects the voltage-sensitivity of the activation/inactivation (gating) mechanism of the channel. In this model, the mean probability for the open state is

$$p_o = \frac{bd}{ac + ad + bd} = \frac{1}{1 + K_1(1 + K_2)}. \qquad (3a,b)$$

The mean steady-state current-voltage relationship of an individual channel, $I_{ss}(V)$, can now be expressed as the product between the current-voltage relationship of the open channel (Eq. (1)) and its mean open probability (Eq. (3)):

$$I_{ss}(V) = I_o(V)p_o. \qquad (4)$$

With the known characteristics of I_o (parameters of channel A in Table 1), the parameters K_1, K_2^0, and z_g in Eq. (3) were fitted to the data in Fig. 6 to be described by Eq. (4). The curve in Fig. 6 is a graphical representation of this fit with the parameters $K_1 = 1.3$, $K_2^0 = 5.3$ and $z_g = 0.34$. As a first approach, Eq. (4) seems to satisfy the qualitative features of the data in Fig. 6.

Physiological Significance

As pointed out by Mummert and Gradmann (1976), rectification of K^+ currents plays an essential role in regulation of the cytoplasmic K^+ concentration in *Acetabularia*. Briefly, at normal 10 mM K^+ in seawater, the large resting voltage in *Acetabularia* would drive net uptake of K^+ up to a Nernst equilibrium of about 10 M (!) K^+ inside. However, during (spontaneous) action potentials, the membrane temporarily depolarizes to voltages more positive than the actual equilibrium potential of K^+ (E_K: ca. −90 mV). Now, during this period and in this voltage range, the K^+ conductance is dramatically increased and causes a substantial loss of internal K^+. Thus K^+ net influx and efflux appear to be balanced in the long range by a certain frequency of action potentials and by an efficient mechanism of outward rectification of K^+ currents. This mechanism has been shown here to consist of voltage-gated activation/inactivation of K^+-selective channels.

This work was supported by the Deutsche Forschungsgemeinschaft (Gr 409/9-5). We thank Mr. H.G. Klieber for valuable discussions.

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Received 25 March 1987; revised 2 June 1987