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Properties of three different ion channels in the plasma membrane of the slime mold *Dictyostelium discoideum*

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'Patch-clamp' experiments in the cell-attached configuration have shown the existence of three distinct types of ion channels in the plasma membrane of *Dictyostelium discoideum*. Channels DI (slope conductance 11 pS) and DII (slope conductance 6 pS) promote an outward current at depolarizing voltages. A third ion channel (HI, slope conductance 3 pS) opens preferentially at hyperpolarization and promotes inward current flow. It is suggested that under physiological conditions current through the DI and DII channels is carried by K^+, whereas Ca^{2+} may be the current carrier in the HI channel. The density of these ion channels in the membrane of *D. discoideum* is low: approx. $0.1/\mu m^2$ for the DI and HI channel and $0.02/\mu m^2$ for the DII channel. The gating properties of the ion channels appear to be complicated because openings are grouped into bursts of activity. The probability of the DI channel being in the open state increases with depolarization. The mean channel life-time is about 20 ms and voltage-independent. The burst duration increases with depolarization whereas the interburst time decreases. The minimal kinetic model accounting for the behaviour of the DI channel is a three-state model with two closed and one open state. A detailed analysis of the gating of the DII and the HI channel was prevented by their low rate of occurrence (DII) or fast inactivation (HI). The formation of a seal resistance ≥ 1 GΩ depends critically on the composition of the pipette solution. Examination of a series of monovalent and divalent cations as well as different organic and inorganic anions has shown that 'gigaseals' are formed only in the presence of at least 1 mM Ca^{2+} or Sr^{2+}, whereas Ba^{2+}, Mg^{2+} and monovalent cations (Li^+, Na^+, K^+, Rb^+, Cs^+) do not support the formation of high seal resistances. Anions seem not to affect the seal formation.

Introduction

The slime mold *Dictyostelium discoideum* offers unique opportunities to study chemotaxis and morphogenetic processes. An impressive amount of data has been accumulated on the biochemical aspects of the events leading from an ensemble of single amoebae to a multicellular organism (for review see Refs. 1–3). On the other hand the relevance of electrical membrane phenomena for the signal transduction mechanism controlling the aggregation of *D. discoideum* cells is unknown.

The major agent mediating the information exchange between individual *D. discoideum* cells is cAMP which is released from such cells into the extracellular medium and sensed by cAMP receptors on the external site of the plasma membrane of *D. discoideum*. In aggregation competent cells the binding of cAMP leads to an enhanced uptake of Ca^{2+} [4] and to a release of K^+ [5]. In addition oscillations of Ca^{2+} and K^+ fluxes during the aggregation phase were observed [5,6]. In analogy to excitable cells it has been hypothesized that these ion fluxes reflect changes of the membrane potential [5]. Therefore, it seems possible that time dependent changes of the membrane potential mediated by cyclic nucleotides are part of the signal transduction process like in photoreceptor cells or taste cells [7,8].

Thus it seemed worthwhile to look for ion channels in the plasma membrane of *D. discoideum* which may serve as the basis for electrical phenomena. Because of the small size of the cells reliable long term measurements of the membrane potential using conventional microelectrodes were not yet possible [9]. In a previous report [10] we have described properties of one type of ion channel (DI in the terminology of this paper). In this publication we extend the characterization of this

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ion channel and describe the properties of two other ion channels (DII, HI) occurring in the plasma membrane of *D. discoideum*.

Materials and Methods

Dictyostelium discoideum strain AX-2 kindly provided by Dr. Malchow (Universität Konstanz, F.R.G.) was cultivated as described [11]. To induce differentiation cells were washed three times and finally resuspended in Sørensen phosphate buffer (pH 6) at a cell density of 10^7 cells/ml. Washed cells were shaken at 150 rpm and room temperature until use. The time after the removal of nutrient is designated t_n, n indicating the number of hours after the removal of nutrient.

Single-channel currents were measured by the patch-clamp technique [12].

Current recording was performed with an EPC-5 or EPC-7 amplifier (List Electronic, Darmstadt, F.R.G.). Signals were stored on magnetic tape (Racal store 4 DS) at a bandwidth of 1.25 kHz. For analysis current signals were replayed at reduced speed on a strip chart recorder (Brush, Gould and manually digitized (HP 9111A Graphics tablet).

Appropriate filtering was performed with a Krohn and Hite model 3321, 24 dB/octave.

The patch-electrodes were pulled from hematocrit tubes (CEEBEE capillary tubes, type 101-PS, Bardram Lab. supplies, Birkerod, Denmark). In some experiments the tip of the pipette was coated with sylgard [13]. The resistance of the pipettes was between 10 and 40 MΩ. Using electrodes made of borosilicate glass no 'giga-seals' could be obtained. An agar bridge made from 2% agar in 100 mM KCl was used as reference electrode.

The composition of the electrolytes used to fill the patch pipettes and the bath is given in the text. The solutions were buffered at pH 6.7 to 7.1.

Results

Conditions for single channel recording

In previous experiments high seal resistances (≥ 1 GΩ) could be obtained only if the pipette solution was made from pure Ca^{2+} salts [10]. Therefore, we investigated systematically the magnitude of the seal resistance obtained with pipette solutions containing different monovalent (Na^+, K^+, Rb^+, Cs^+), divalent cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) and anions (acetate, cyclamate, gluconate, glucuronate, methanesulfonate). The seal resistances obtained with the different solutions are compiled in Table I). Solutions containing the divalent cations Ca^{2+} or Sr^{2+} yield seal resistances ≥ 1 GΩ. Seal resistances of this magnitude are also obtained with solutions containing monovalent cations (Li^+, Na^+, K^+, Rb^+) and more than 1 mM Ca^{2+} (Table I). Lower resistances (100–500 MΩ) were observed with solutions containing only monovalent cations or the divalent cations Ba^{2+} or Mg^{2+}. Anions seem not to affect the magnitude of the seal resistance.

In many cells enzymatic pretreatment of the cell membrane enhances the magnitude of the seal resistance and/or the probability of the formation of a high seal resistance [14,15]. Enzymatic treatment of *D. dis-*

TABLE I

Effect of the electrolyte composition on the magnitude of the seal resistance (R_s)

The pH was buffered between 6.7 and 7.1 with 1 mM Tricine or Hepes plus Tris. In addition to the cations indicated all solutions contained at least 1 mM K^+. M^+ indicates the alkaline ions Li^+, Na^+, K^+, Rb^+, Cs^+ and A^- marks the organic anions acetate, cyclamate, gluconate, glucuronate or methanesulphonate. The concentration refers to the cations. 'e' indicates an enzymatic pretreatment of the *D. discoideum* cells (see Methods). Electrolytes marked '$^+$' and '*' contain in addition 0.1 to 1 or 2 to 2.5 mM Ca^{2+}, respectively. The number in parentheses (3rd column) indicates the percentag of experiments yielding the seal resistance indicated.

Electrolyte	Concentration (mmol/l)	No. of patches	R_s (GΩ)	Buffer	Other conditions
M^+Cl^-	10	240	0.1– 0.3	Tris-Hepes	
M^+Cl^-	10	40	0.1– 0.3	Tris-Tricine	
M^+Cl^-	10	890 (55%)	1 –30	Tris-Tricine	*
$BaCl_2$	10	90	0.1– 0.5	Tris-Hepes	
$CaCl_2$	10	2100 (33%)	1 –40	Tris-Hepes	
$CaCl_2$	10	50 (31%)	1 –15	Tris-Tricine	
$CaCl_2$		30 (35%)	1 –10	Tris-Tricine	e
$MgCl_2$	10	70	0.1– 0.3	Tris-Hepes	
$SrCl_2$	10	40 (28%)	1 –15	Tris-Hepes	
$SrCl_2$	10	210 (38%)	1 –18	Tris-Tricine	
SP-buffer		60	0.1– 0.2	Tris-Hepes	
M^+A^-	10	40	0.1– 0.4	Tris-Hepes	
$Ca(A^-)_2$	10	530 (31%)	1 –24	Tris-Tricine	
K^+/Na^+A^-	5/5	240 (48%)	1 –18	Tris-Tricine	*
M^+Cl^-	10	55	0.1– 0.2	Tris-Tricine	e, $^+$

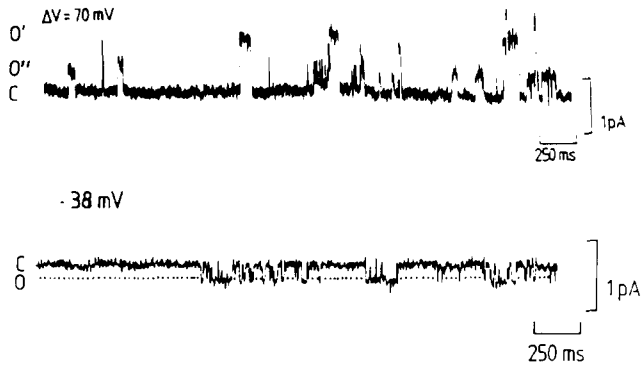

Fig. 1. Current fluctuations recorded from the plasma membrane of *D. discoideum* in the cell-attached mode. The upper trace shows two types of outward current (*O'* and *O''*) fluctuations obtained at 70 mV depolarization ascribed to the channel types DI and DII resp. The lower trace shows inward current fluctuations ascribed to channel HI obtained from a different cell at a 38 mV hyperpolarization. Recording conditions were: 15 mM NaCl, 1 mM KCl, 2 mM $CaCl_2$, pH 6.9, stage t_5 (upper trace) and 15 mM KCl, 2 mM $CaCl_2$, pH 7.0, stage $t_{6.5}$ (lower trace).

coideum cells with a mixture of trypsin, hyaluronidase and mixed glycosidase (0.5 mg/ml) each) for 15 to 30 min did not yield higher seal resistances or increase the frequency of the formation of a 'giga-seal'.

The number of successful seal formations seems to depend on the stage of development after the removal of nutrient. It increases from approx. 40% at stage t_0 to 70% at stages t_5 through t_7. Finally it should be mentioned that the life time of a seal often did not exceed 5 min in the cell-attached configuration. This is probably caused by the mobility of the *D. discoideum* cell.

Overview on the different channel types

In this paragraph a brief account of the three most prominent channel types in the plasma membrane of *D. discoideum* is given to facilitate the further description of the results. The arguments leading to the distinction of at least three channel types are discussed more extensively below.

Because ion channels could not be observed reproducibly in excised membrane patches (see also Ref. 10) this report is restricted to the description of ion channels observed in the cell-attached mode. Two obvious disadvantages of this approach are: (i) the ion concentration on the cytoplasmic side of the membrane cannot be changed and (ii) the voltage across the membrane patch is not known because it is the sum of the unknown membrane potential of the cell plus the applied pipette potential. In the following $\Delta V > 0$ means depolarization of the membrane and $\Delta V < 0$ a hyperpolarization.

In Fig. 1 the upper trace shows a current record obtained at a depolarization by +70 mV. The step-like current fluctuations show two distinct amplitudes of 1 and 0.4 pA. These current fluctuations are tentatively attributed to the opening and closing of two distinct types of ion channels called DI and DII. The current flow through these ion channels is outward at depolarizing membrane potentials and increases with depolarization. Smaller current fluctuations (approx. 0.25 pA at $\Delta V = -38$ mV) are observed at hyperpolarizing membrane potentials (Fig. 1, lower trace) and are attributed to channel HI. Current flow through this channel is inward at potentials more negative than the resting potential.

DI channel: conductance and gating characteristics

Fig. 2A shows otuward current fluctuations attributed to the opening and closing of a DI channel at different depolarizing membrane potentials. The amplitude of the single-channel current and the open probability increase with depolarization. Openings appear in groups separated by silent periods (see also inset Fig. 4 and Ref. 16).

Fig. 2B shows the linear relation between the channel amplitude and applied voltage for a variety of electrolytes (see legend for details). In the voltage range $\Delta V = 0$ mV to $\Delta V = +120$ mV, the slope conductance is about 11 pS. The average slope conductance was 11.4 ± 0.4 pS ($\bar{x} \pm$ S.E., $n = 76$). The slope conductances obtained with different pipette solutions are listed in Table II. Anions seem not to affect the slope conductance. In the presence of high concentrations of Ca^{2+} the slope conductance is slightly reduced.

Openings of the DI channel were not observed at $\Delta V < 0$ mV. Thus, the reversal potential of the current voltage relation could not be determined. The extrapolated reversal potential is near to the 'resting' or zero-current potential of the cell. It is independent from the type of cation or anion in the pipette (Fig. 2B). It is possible, however, that the resting potential is different in different electrolytes.

The distribution of open and closed times at $\Delta V = 62$ mV is shown in Fig. 3. The duration of the short openings within a burst (open-time or channel lifetime) can be described by an exponential distribution with a mean open time of 21 ms. The distribution of closed times between bursts is described by an exponential

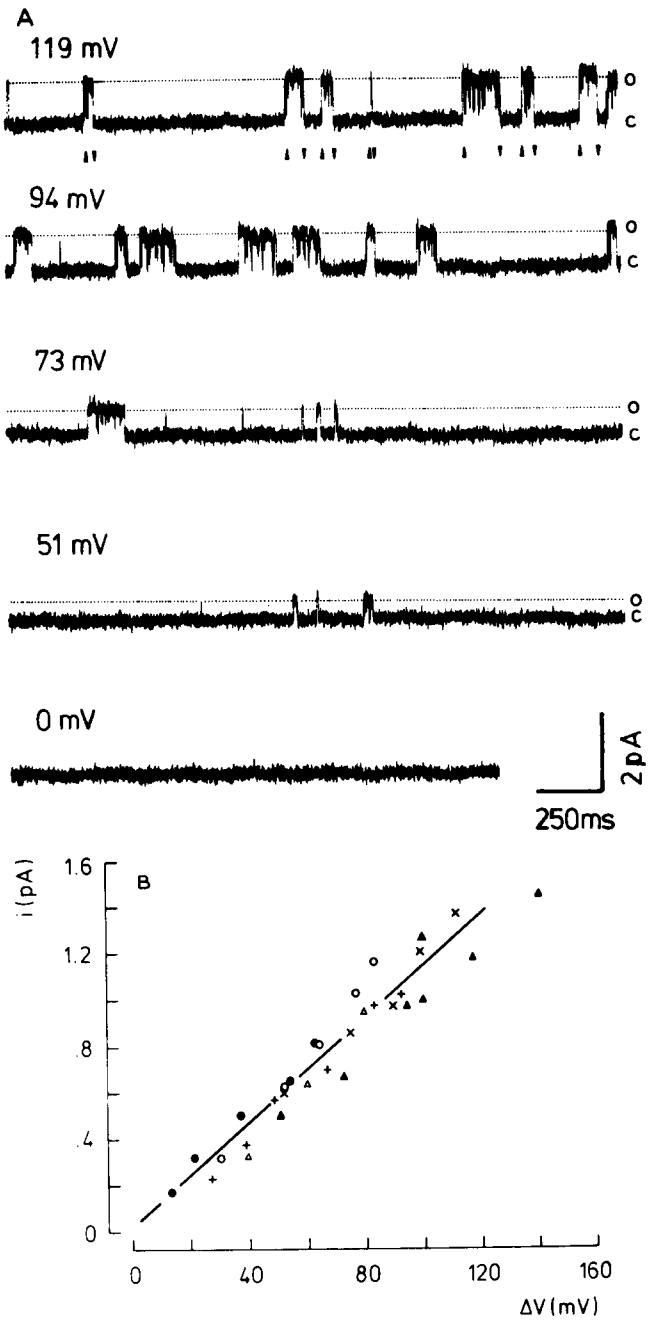

Fig. 2. (A) Fluctuations of the DI channel at different levels of depolarization. Channel openings are grouped into bursts as indicated by the arrowheads in the uppermost trace. (B) Current voltage relation of the DI channel obtained in different electrolytes (pH 7.0). 15 mM Na-gluconate (○), 15 mM KCl (+), 20 mM CsCl (●), 10 mM Na-cyclamate (△), 30 mM CsCl (▲), 5 mM KCl plus 5 mM NaCl (×). All solutions contained 2.5 mM $CaCl_2$ and at least 1 mM KCl. The line indicates a slope conductance of 11 pS.

function with a time constant of 35 ms at $\Delta V = +62$ mV. The closed time between bursts is voltage dependent and decreases with depolarization (Fig. 4). The burst duration is 35 ms at $\Delta V = +40$ mV and increases with depolarization (Fig. 4). The short closures within a burst last for less than 10 ms and cannot be resolved because of the limited time resolution of the measuring circuit.

The voltage dependence of the parameters describing the kinetic behaviour of the DI channel is shown in Fig. 4.

The probability of the DI channel to be in the open state increases with depolarizing membrane potentials (Fig. 4). This is largely due to a reduction of the interburst time and to a prolongation of bursts (Fig. 4). The lifetime of the channel opening, however, is not voltage dependent.

Properties of the DII channel

In 4% of the experiments ($n = 850$) outward current fluctuations were observed with an amplitude approx. 50% lower than that of the DI channel (Fig. 1). These current fluctuations were assigned to a different channel (DII, see Discussion).

Fig. 5A shows the distribution of current fluctuations from a membrane patch containing both channel types. Current-voltage relations of the DI and DII channels are shown in Fig. 5B. The slope conductance of the DII channel is 7 pS compared to 16 pS for the DI channel. The extrapolated reversal potential is identical for both channels at $\Delta V = +20$ mV. On the average the slope conductance of the DII channel is 6.2 ± 0.5 pS ($\bar{x} \pm$ S.E.,

TABLE II

Magnitude of the slope conductance of the DI channel in various electrolytes

All solutions contain at least 2 mM Ca^{2+} and 1 mM K^+.

Electrolyte	Concentration (mM)	Slope conductance (pS) ($\bar{x} \pm$ S.E.)	No. of expts.
$CaCl_2$	5	11.5±2.4	2
$CaCl_2$	10	10.6±0.5	21
$CaCl_2$	25	9.5±1.1	5
Ca-acetate	10	7.1±1.4	2
Ca-cyclamate	10	9.2±1.4	3
Ca-methanesulfonate	10	12.4	1
$SrCl_2$	10	12.2±0.6	8
LiCl	10	12.8±1.3	3
Na-gluconate	1.5	8.3±0.3	2
Na-cyclamate	10	10.3±0.7	2
NaCl	15	12.7±0.7	17
Na-gluconate	15	15	1
KCl	10	15.8±2.8	2
K-gluconate	20	17.0±4.6	2
CsCl	20	12.5±1.1	3
CsCl	30	14.2±0.7	2
Organ. anions		11.6±1.2	13
chloride		11.3±0.5	63
Monovalent cations		12.6±0.6	34
Divalent cations		10.6±0.3	42
Total		11.4±0.4	76

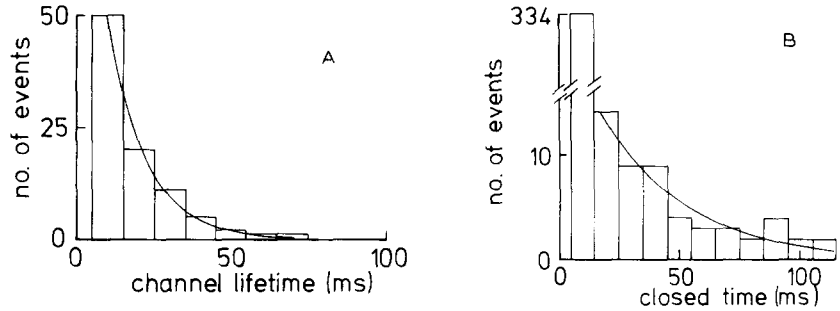

Fig. 3. Distribution of open and closed times of the DI channel at $\Delta V = 62$ mV. (A) The channel lifetime can be fitted by a single-exponential function with a time constant of 21 ms (continuous line). (B) The distribution of closed times shows an excess of events at times < 10 ms corresponding to the brief closures within a burst (see inset Fig. 4). The remainder can be described by an exponential function with a time constant of 45 ms (continuous line). Experimental conditions: 10 mM Na-cyclamate, 2 mM Ca-cyclamate$_2$, 1 mM KCl, pH 6.8, stage t_4.

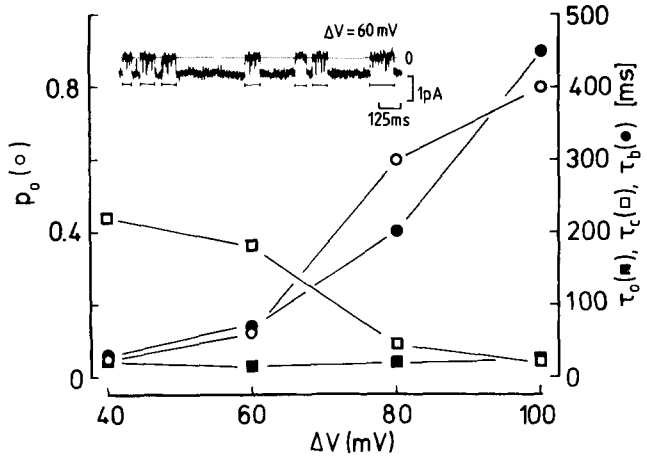

Fig. 4. Voltage-dependent parameters of the DI channel: the open probability (○), the channel life-time (■), the burst duration (●) and the interburst time (□). Electrolyte (bath and pipette): 20 mM CsCl, 2.5 mM $CaCl_2$, 1 mM KCl, pH 7, stage t_7. The inset shows a recording of current fluctuations to illustrate the definition of a burst. Bursts are marked by bars.

$n = 7$) compared to 11 ± 0.4 ($\bar{x} \pm$ S.E., $n = 76$) under similar conditions. So far the DII channel was mostly observed in chloride solutions. Because of its rare occurrence and its low activity a detailed analysis of the gating kinetics could not be performed. The mean open time was 10 ms at $\Delta V = +100$ mV. The closed times range from 0.1 to 1s.

Preliminary experiments indicate that cAMP increases the rate of occurrence of the DII channel. In the absence of cAMP it was observed in 0.3% of the experiments. In the presence of cAMP (1 nM to 1 μM) the probability of occurrence increased to 10%.

Characteristics of the HI channel

A third type of ion channel (HI) was observed in 17% of the experiments ($n = 850$) at membrane potentials equal or more negative than the resting potential ($\Delta V \leqq 0$ mV). The insert of Fig. 6 shows fluctuations of the HI channel at $\Delta V = -33$ mV. At this potential the current flow through the open channel is inward. No openings were observed at voltages more positive than the resting potential ($\Delta V > 0$ mV). Fig. 6 also shows that the current voltage relationship between $\Delta V = 0$ and $\Delta V = -80$ mV is linear with a slope conductance of 2.5 pS. On the average the slope conductance of the HI channel was 2.8 ± 0.2 pS ($\bar{x} \pm$ S.E., $n = 21$). The

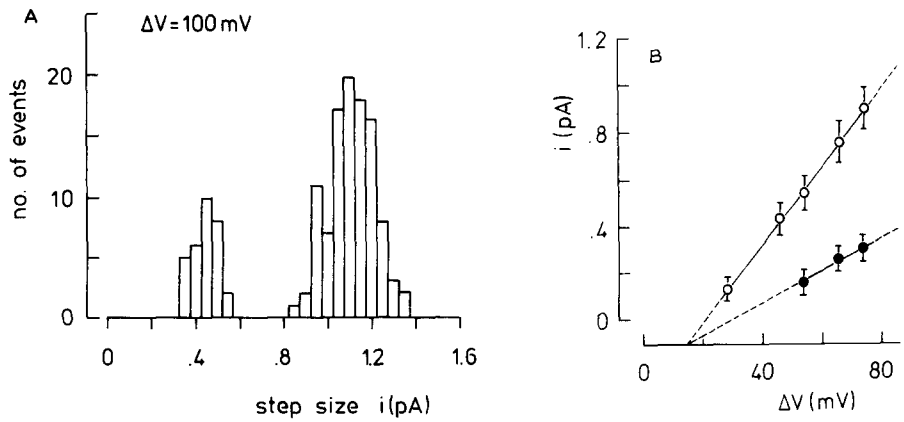

Fig. 5. (A) Distribution of current fluctuations recorded at $\Delta V = +100$ mV from a membrane patch containing the DI and DII channel. Electrolyte: see (B). Stage $t_{4.5}$. (B) Current-voltage relation of the two classes of current fluctuations. Different experiment. The slope conductances are 6.7 and 16 pS, respectively. Electrolyte (bath and pipette): 15 mM NaCl, 2.5 mM $CaCl_2$, 1 mM KCl, 1 mM Tricine-Tris, pH 7.0. Stage t_7.

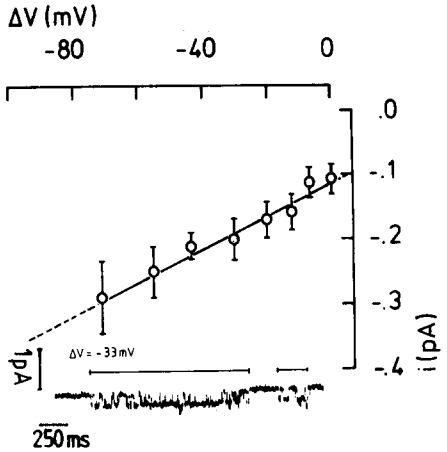

Fig. 6. Current voltage relation of the HI channel. The bars indicate the standard deviation of at least 10 channel openings. Electrolyte (bath and pipette): 10 mM KCl, 2 mM $CaCl_2$, pH 7.0. Stage $t_{7.5}$. The inset at the bottom shows current fluctuations at $\Delta V = -33$ mV. Current flow is inward. The bars indicate the duration of the burst. Electrolyte (bath and pipette): 15 mM NaCl, 2.5 mM $CaCl_2$, pH 7.0. Stage t_1.

slope conductance was determined in $CaCl_2$, NaCl, KCl and CsCl (Table III). No significant effect on the conductance was observed. The extrapolated reversal potential is 56 mV more positive than the resting potential ($\Delta V = +56$ mV). On the average it was 49 ± 2.7 mV ($\bar{x} \pm$ S.E. $n = 6$) and independent from the pipette solution used (see above). The HI channel was very rarely observed if the pipette solution contained organic anions instead of chloride. Its lifetime distribution can be described by an exponential function with a mean open time of 12 ms (Fig. 7A). The mean channel lifetime appears not to be voltage dependent (Fig. 8). The distribution of closed times can be described by a sum of two exponential functions with time constants of 5 and 111 ms (Fig. 7B). The slow time constant seems not to be voltage dependent (Fig. 8). The fast time constant of closed times cannot be resolved sufficiently. Although this channel occurred nearly as frequently as the DI channel, it is difficult to describe quantitatively because its activity decreases rapidly after the seal formation.

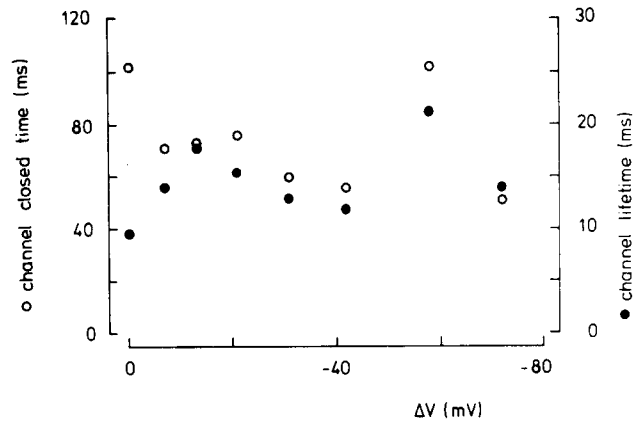

Fig. 8. Voltage dependence of the channel lifetime (●) and the closed time between burst (○). Same experiment as Fig. 6.

Discussion

Are the DI, DII and HI channel distinct entities?

In the foregoing sections the properties of three kinds of channels in the membrane of *D. discoideum* were

TABLE III

Magnitude of the slope conductance of the HI channel in different electrolytes (see also Table II)

Because of the small number of available current voltage relations the single-channel current at $\Delta V = 0$ mV is also included.

Electrolyte	Concentration (mM)	Slope conductance (pS) ($\bar{x} \pm$S.E.)	n	Elementary current at $\Delta V = 0$ (pA) ($\bar{x} \pm$S.E.)	n
$CaCl_2$	5	4.5	1		
$CaCl_2$	10	2.3 ± 0.2	4	0.16 ± 0.025	4
CsCl	20	4.5	1	0.11 ± 0.005	2
CsCl	30	2.1 ± 0.1	2	0.12 ± 0.02	2
KCl	10	3.1 ± 0.5	3	0.10 ± 0.004	7
KCl	15	2.5 ± 0.3	4	0.12 ± 0.02	3
KCl	20	1.6	1	0.08	1
KCl	30	–		0.13 ± 0.012	3
NaCl	10	–		0.16	1
NaCl	15	2.7 ± 0.2	4	0.15 ± 0.006	13
Total		2.7 ± 0.17	20	0.13 ± 0.005	36

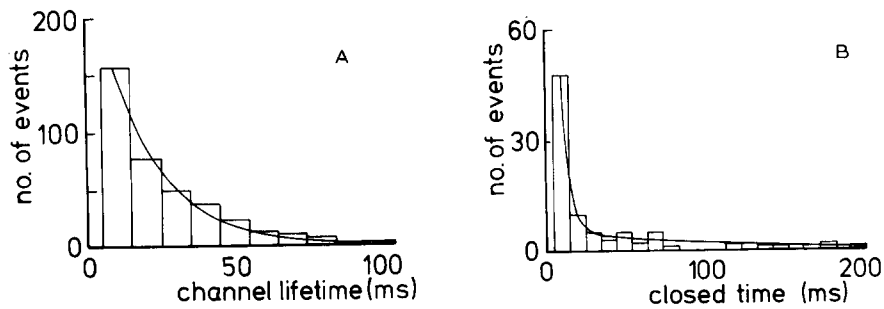

Fig. 7. Distribution of open and closed times of the HI channel. (A) The distribution of the channel lifetime can be described by an exponential function with a time constant of 12 ms (continuous line). (B) The distribution of closed times can be described by a sum of two exponential functions with time constants of 5 and 110 ms. Electrolyte: 10 mM KCl, 2 mM $CaCl_2$, pH 7.0. Stage $t_{7.5}$.

described. This classification was made for the sake of clarity and it seems appropriate to discuss the arguments favouring this idea.

Channel DII can be observed in the same patch together with channel DI as a separate and distinct conductance level. Thus it seems not very probable that DII represents a small DI channel. Another possibility is that DII represents another open state of DI with a lower conductance (a so called sub-state). Although this cannot be completely excluded on the basis of the data available we prefer the idea that both conductances are due to different channel molecules because: (i) channel DI was mostly observe in the absence of DII and (ii) the definition of substates (e.g., Ref. 17) implies direct transitions between two open states of the same channel. Such direct transitions between the DI and DII levels are observed very rarely. In most cases openings of DI occurred separated in time from openings of the DII channel.

Channel HI, however, is clearly distinct from DI and DII because both channels can be observed in one patch at the same membrane potential, HI promoting current inflow and DI current outflow. Because of the very different reversal potentials it seems unlikely that HI is related to DI or DII.

Ion selectivity

The current flow through channel DI is outward. Thus it may be carried by an outflow of cations or an inflow of anions. Measurements with a variety of organic and inorganic anions in the pipette solution have shown that the conductance of the DI channel is independent from external anions. Thus it seems reasonable to assume that the outward current through the DI channels is carried by an outflow of cations. Since K^+ is the only cation found in the intracellular space in significant concentrations [5,18] we assume in agreement with our previous report [10] that under cell-attached conditions the current through the DI channel is carried by K^+.

The DII channel was observed only in experiments with chloride solutions in the pipette. Because of the small number of observations of the DII channel we do not know is this happened by chance or if it indicates an inflow of anions through this channel.

The current flow through the HI channel is inward with a reversal potential more positive than the resting potential. This would suggest that the current is due to an influx of cations because the reversal potential for Cl^- is mostly near to the resting potential. If the inward current is carried by cations Ca^{2+} would be a candidate because the slope conductance of HI is independent from the type of monovalent cations in the pipette. However, the Ca^{2+} concentration was always 2.5 mM in these experiments. Furthermore, the low conductance of HI which is comparable to that of known Ca^{2+} channels ($\leq$ 10 pS at physiological Ca^{2+} concentrations [19]) agrees with this idea.

Neither the direction of the current through the DI and DII channels nor that through the HI channel could be reversed. With respect to the DI channel the reason is probably that the open probability becomes very low at the resting potential or more negative potentials. A similar explanation may hold for the HI channel but cannot be verified because the activity of this channel is so low that the open probability could not be estimated. The alternative explanation that HI is strongly rectifying seems not very convincing because the resting potential, the most positive potential where the HI channel could be observed, is still far from the extrapolated reversal potential.

Voltage dependence of the DI channel

The steady state probability of the DI channel to be in the open state increases with depolarization. The voltage dependence can be described by the Boltzmann distribution [20,21]:

$$p_o/p_c = A \exp(-z\delta FE/RT)$$

p_o/p_c is the ratio between open and closed channels, F is the Faraday constant, E the electrical field, R the gas constant and T the absolute temperature. The number of charges translocated during the open closed transition is given by z and the relative distance over which these charges are transferred within the electric field is given by δ. The number of charges z and the relative distance δ are usually taken together as the 'effective' charge [21]. Fig. 9 shows that the voltage dependence of the gating of the DI channel can be described by the Boltzmann relation with 1.4 effective gating charges. This is much less than the effective gating charge of voltage dependent Na^+ and K^+ channels in nerve membranes (6 and 4.5, respectively [20]), but compares with the voltage dependence of the K^+ channel from sarcoplasmic reticulum [22].

A kinetic model with two closed (C_1, C_2) and one open state (O) is the minimum which accounts for the

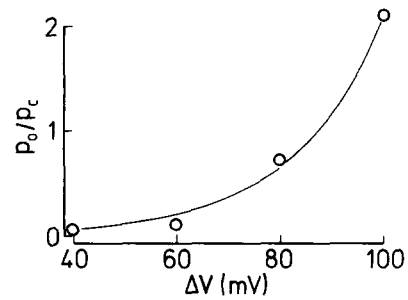

Fig. 9. Plot of the ratio between open and closed DI channels (p_o/p_c) vs. membrane potential. The voltage dependence of this ratio can be described by the Boltzmann distribution (continuous line, see text for details). Same experiment as Fig. 4.

observation that the distribution of open times is described by a single-exponential function whereas the distribution of closed times needs at least two. Two different arrangements are possible:

$$C_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} O \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C_2$$

$$C_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_2 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} O$$

Sakmann and Trube [23] have shown that it is almost impossible to distinguish between these possibilities on the basis of data obtained from the measurement of stationary fluctuations. Model II seems, however, the more realistic alternative because of the voltage independence of the channel lifetime τ_0. If model II holds, τ_0 is given by $1/k_{-2}$ i.e., one of the rate constants is voltage independent. Within the framework of model I the channel lifetime is determined by $1/(k_2 + k_{-2})$. Voltage independence of τ_0 might be obtained by voltage independence of both rate constants or by opposite voltage dependencies which cancel each other.

Thus only model II is considered. For this model the rate constants k_1, k_{-1}, k_2 and k_{-2} are obtained from the following relations [16,23].

$$\tau_0 = 1/k_{-2} \tag{1}$$

$$\alpha = k_2/k_{-1} \tag{2}$$

$$\beta = 1/(k_{-1} + k_2) \tag{3}$$

$$\tau_b = 1/k_1 \tag{4}$$

β is the duration of closures within a burst, α is the number of openings per burst and τ_b is the mean interval between bursts. Fig. 10 shows that rate constants k_2 and k_1 increases with depolarization whereas k_{-1} decreases, k_{-2} is voltage independent as determined experimentally (Fig. 4).

The steady-state open probability is determined by the rate constants

$$p_0 = \frac{k_1 \cdot k_2}{k_1 \cdot k_{-2} + k_{-1} \cdot k_{-2} + k_1 \cdot k_2} \tag{5}$$

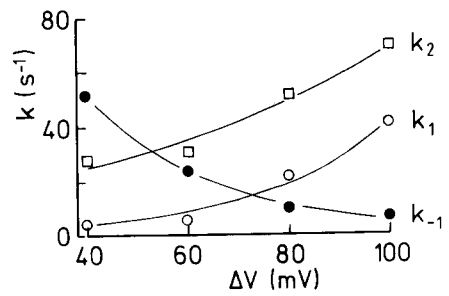

Fig. 10. Plot of the rate constants k_1, k_{-1}, and k_2 calculated for the DI channel according to model II vs. membrane potential. Same experiment as Fig. 4.

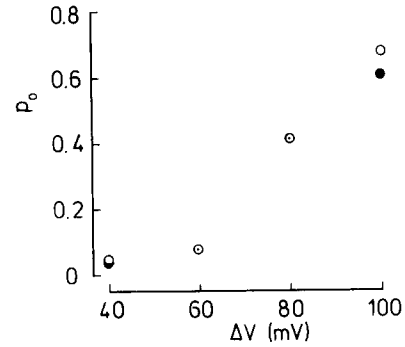

Fig. 11. Comparison between the experimentally determined open probability (●) of the DI channel and the open probability calculated from Eqn. 5 (○). Experimental data from Fig. 4.

Fig. 11 compares the open probability determined experimentally with that calculated from the rate constants shown in Fig. 10 and the experimentally determined channel lifetime.

Properties of the DI channel in Ca^{2+} solutions

Previously we have reported properties of an ion channel in the presence of Ca^{2+} saline [10]. This ion channel shares many properties with the DI channel (slope conductance, voltage dependence of the open probability). Thus we suggest that it is identical with the DI channel. On the other hand some properties are different from those seen in the presence of monovalent cations. In the absence of monovalent cations the current-voltage relation becomes rectifying near the resting potential and the single channel conductance is sligthly smaller.

At present we have no explanation for the strong rectification observed in Ca^{2+} salines.

Physiological relevance

The ion channels described in this report may form the molecular basis for the K^+ [5] and Ca^{2+} fluxes [4,6,23] observed in *D. discoideum*. It has been hypothesized that these oscillatory K^+ and Ca^{2+} fluxes which occur in strong correlation with the oscillations of cAMP release [5,6] may act together to establish an electrical oscillator as it is found, e.g., in the pacemaker cells of *Aplysia* [6]. It is also possible that Ca^{2+} acts directly as a second messenger on intracellular enzymatic systems like the adenylate cyclase [25,28].

In addition K^+ and Ca^{2+} fluxes can be induced by exogenous cAMP [5,6].

The magnitude of these K^+ and Ca^{2+} fluxes is low (between 10^7 and 10^8 ions/cell per min) compared to the transport capacity of a single open ion channel like DI or HI (10^8 ions/min). Thus a small number of open ion channels may easily account for the observed ion fluxes.

If the DI and the HI channels would mediate the oscillatory and cAMP dependent ion fluxes then one

would expect that the activity of these channels is increased by the addition of cAMP. No effect of cAMP was observed on the DI and HI channels. On the other hand, some evidence was obtained that the observation rate of the DII channel was increased by the addition of cAMP. More experiments are, however, necessary to establish this point and to elucidate how cAMP affects this channel. A possible explanation for the lack of cAMP sensitivity of the DI (DII) channel is the loss of cAMP sensitivity of K^+ fluxes in the presence of Ca^{2+} activities > 10 μM [26]. It is reminded that Ca^{2+} concentrations > 1 mM were necessary in the experiments described here.

In conclusion it seems possible that the DI (and DII) and the HI channel form the basis for the ion fluxes observed by others but more experiments are necessary to clarify this point.

At present one would not expect that the HI and the DI or DII channels act together as an electrical oscillator. The DI channel opens preferentially at membrane potentials more positive than the resting potential and promotes an outward current. Thus this channels would be suited to drive the membrane potential back to the resting potential after a foregoing depolarization. There is, however, no evidence that the HI channel is able to depolarize the membrane. The current flow through the HI channel is inward and may be carried by Ca^{2+} but no activity of this channel at depolarizing membrane potentials was observed. Thus the function of the HI channel may be to impede a hyperpolarization of the membrane rather than to depolarize it.

We can not exclude the possibility however that the HI channel inactivates rapidly at positive membrane potentials and therefore escaped detection at membrane potentials more positive than the resting potential.

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