Biochimica et Biophysica Acta 857 (1986) 287–290
Elsevier

BBA 70305

BBA Report

Single ion channels in the slime mold *Dictyostelium discoideum*

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(Received December 24th, 1985)

Key words: Ion channel; Patch clamp; (*D. discoideum*)

Single ion channels with different conductances and gating characteristics were observed in the plasma membrane of the slime mold *Dictyostelium discoideum* by means of the patch-clamp technique in the cell-attached mode. The predominant channel type shows outward current flow, probably carried by K^+ ions. The slope conductance of this channel is 9 pS and its probability to be open increases with depolarization of the membrane. The channel is observed from 1 to 8 h after the beginning of starvation.

In animal cells, ion channels of the plasma membrane play a vital role in a variety of processes like nervous excitation, sensory transduction, excitation-contraction coupling and excitation-secretion coupling [1]. Very little is known, however, about the presence and function of ion channels outside the animal kingdom. The cellular slime mold, *Dictyostelium discoideum*, which lives as unicellular amoebae during its reproductive phase is regarded as an unique model to study morphogenesis and differentiation processes for its characteristic feature to form a multicellular stage in the absence of nutrients [2]. The aggregation reaction of *Dictyostelium* cells involves oscillatory behavior [3–15] and it is suggested that ion fluxes across the plasma membrane are intrinsic components of this oscillator system [12–14]. We have applied patch-clamp techniques [16] to analyze the role and the mechanism of ion transport in the plasma membrane of *Dictyostelium*. Predominantly a voltage-sensitive outward rectifying ion channel was observed, which has a slope conductance of about 9 pS in the cell-attached mode. The current flow is outward at membrane potentials more positive than the resting potential and it is proposed that it is carried by potassium ions.

After the formation of a giga-seal between the recording pipette and a *Dictyostelium* cell very often spontaneous current fluctuations like that shown in Fig. 1 were observed which are interpreted as opening and closing of single ion channels. The current flow is outward and the amplitude and the frequence of the current fluctuations increase if the membrane is depolarized (Fig. 1). Even with prolonged depolarization, this channel is not desensitized or inactivated. The results reported here were obtained in the 'cell-attached' configuration with solutions of Ca^{2+}-salts in the pipette and in the bath, which leads to seal resistances of 10–40 GΩ. No seals of this magnitude were achieved with K^+ or Na^+ as electrolytes. Channel activity disappeared after disruption of the patch from the cell. Fig. 2 shows the relation between the channel amplitude and the applied potential. It should be noted that the absolute membrane potential is not known. The current-voltage relation is non-linear with a slope conductance of 15 pS measured in the linear part of the curve. With Cl^- as the anion, the mean slope conductance is 9 ± 1.9 pS ($\bar{x} \pm$ S.D., $n = 22$). Within experimental error it is independent from

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the anion in the pipette: it is 8 ± 1.8 pS ($n = 3$) and 7 ± 2 pS ($n = 2$) with cyclamate$^-$ and acetate$^-$, respectively. As the slope conductance is largely independent of the anion in the pipette, it seems likely that the outward current is carried by an efflux of cations out of the cell and not by an influx of anions into the cell. The most abundant cation in the *Dictyostelium* cell is K^+ [14,17]. Therefore we suggest tentatively that the current is carried by K^+ ions. Reversal of the current flow of this channel type was not observed, possibly because there was no K^+ in the pipette. The

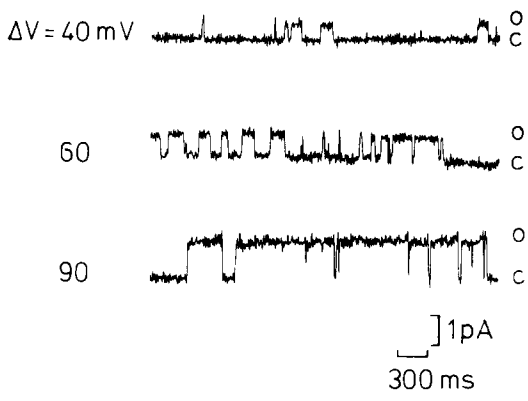

Fig. 1. Stationary patch-clamp recordings of discrete current fluctuations (ion channels) from a *Dictyostelium* cell in the cell-attached mode at different membrane potentials. The current flow is outward and the amplitude rises with increasing depolarization as well as the probability to be in the open state (o, open; c, closed). Bandwidth 150 Hz; solution 10 mM $CaCl_2$. The deviation from the unknown resting potential is indicated for each trace. A positive sign indicates a depolarization. The patch contained 1 channel; $t_{4.5}$ (see below). Methods: *Dictyostelium* strain Ax-2 was cultivated as described before [10]. To induce differentiation the cells were washed and resuspended in Sørensen phosphate buffer pH 6.0 (density about 10^7 cells per ml) and shaken at room temperature. The stage of differentiation is defined as t_n; n, number of hours after the removal of nutrient. For measurements, 20 μl of the cell suspension were diluted in 1 ml test solution. Identical salt solutions were used in the bath and in the pipette. Solutions: 10 mM $CaCl_2$ or 10 mM calcium-acetate$_2$ or 10 mM calcium (cyclamate)$_2$ plus 1 mM Hepes (pH 7.0). Temperature 21 to 22°C. The pipettes were made from hematocrit capillary tubes. In the evaluated experiments, the seals remained stable for 10 to 30 min. Currents were recorded with an EPC-7 amplifier (List Electronic, Darmstadt, F.R.G.) and stored on magnetic tape (Racal Store 4D). For analysis the records were replayed at reduced speed on a strip chart recorder and digitized (HP9111 digitizer). Low-pass filtering was performed with a KROHN-HITE model 3321, 24 dB/octave.

determination of the slope conductance suffers from the non-linearity of the current-voltage relation and the unknown membrane potential. The membrane potential of a cell may change during an experiment, caused either by degradation of the cell or by an oscillating membrane potential related to oscillatory K^+ fluxes [14]. Preliminary evidence for oscillatory behavior was obtained in some experiments which showed a slow decline and later increase of the amplitude of the single-channel current.

The distribution of open times of this ion channel type can be described by a single exponential function (Fig. 3a). The amplitude distribution of the current fluctuations is shown in the inset of Fig. 3a. The distribution of the closed times needs at least two exponential functions to be fitted (Fig. 3b). This indicates a channel with at least two closed states. The lifetime of the open channel as well as its probability to be open increase with depolarization. Fig. 4 shows the voltage-dependence of the channel lifetime and of the open probability. The channel lifetime increases from 41 ms at $\Delta V = 0$ to 156 ms at $\Delta V = +80$ mV, while the open probability increases from 0.07 to 0.95. This indicates that besides the closing rate constant transitions between closed states are voltage-dependent. The density of active channels is low: a rough estimate gives about 1 channel per μm^2. In 70% of the experiments, no channel activ-

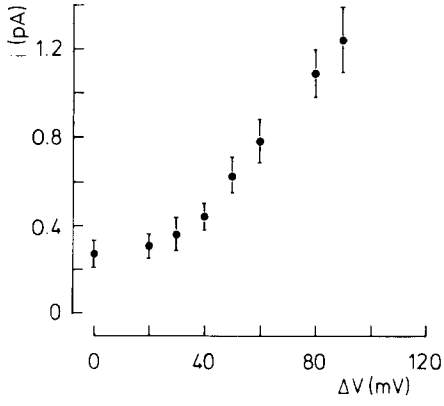

Fig. 2. Voltage-dependence of single-channel current. The linear part of the current-voltage relation has a slope conductance of 15 pS. The bars indicate the standard deviations. The number of events were between 73 and 235. Same experiment as Fig. 1.

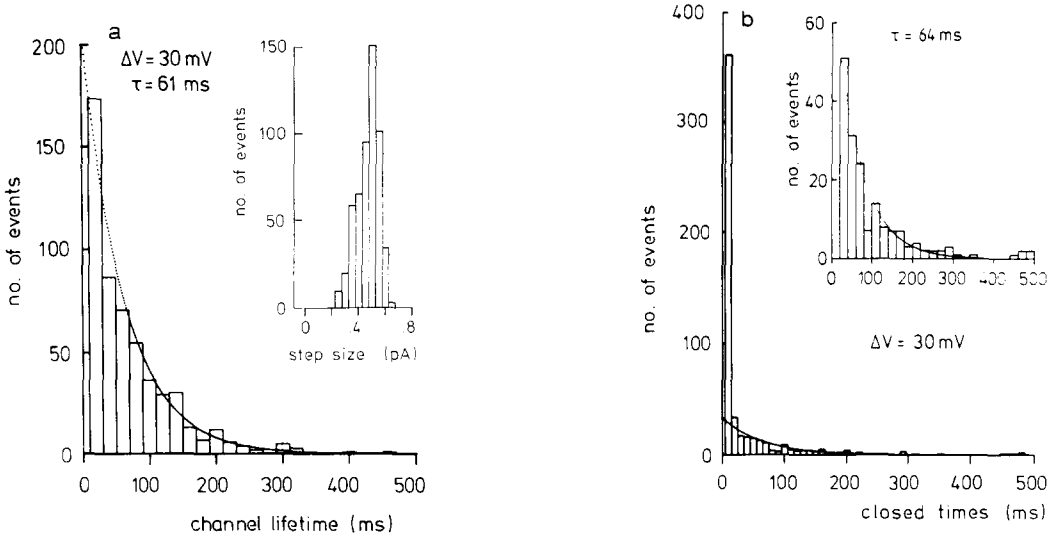

Fig. 3. Channel lifetime and closed time. (a) Distribution of the channel lifetime at $\Delta V = +30$ mV. The dotted line indicates an exponential function with a time constant of 61 ms. The inset shows the amplitude distribution at $\Delta V = +30$ mV. The patch contained one channel. Solution 10 mM $CaCl_2$, bandwidth 200 Hz, 21°C, $t_{3.5}$. (b). Distribution of closed times. The closed times can be subdivided into two classes. One with a time constant of less than 10 ms and a second with a time constant of 64 ms. The inset shows the distribution used to fit this second exponential function. For the fit the closed times shorter than 10 ms were omitted. Same experiment as Fig. 3a.

ity was observed after successful formation of a seal, and only one active channel was observed in 60% of the remaining experiments. The physiological function of the described ion channel has to be clarified. It has been observed at all stages of aggregation between 1 and 8 h after the beginning of starvation and the present data do not indicate a correlation between one of the channel parameters and the stage of differentiation. A net release of about 10^9 K^+ ions per cell per min was observed after stimulation with cAMP at stages t_6 or later [14]. Up to now we did not obtain unequivocal evidence for any effect of cAMP on the channel activity, but a K^+ release of this magnitude could be easily achieved by a small increase of the open probability of the ion channel described. Two other types of current fluctuation were observed occasionally: inward current fluctuations activated by negative membrane-potentials, which were seen in the cell-attached mode, and a small ion channel occurring in excised patches with symmetric $CaCl_2$-solutions.

The conductance of other K^+ channels examined by the patch-clamp method varies between 5 and several hundreds of pS [18]. Taking into account the different ionic conditions of measurements the *Dictyostelium* channel is in the range of the 'small' type of K^+ channels [18] and comparable to K^+ channels described in plant cells [19]. Recently, we found a basic, cAMP-inde-

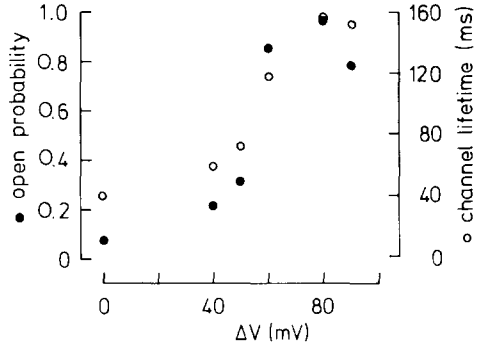

Fig. 4. Voltage-dependence of the channel lifetime and the probability to be in the open state. The channel lifetime increases from 40 ms at $\Delta V = 0$ to a saturating value of about 160 ms at $\Delta V = +80$ mV. Closures shorter than 3 ms were not taken into account. The probability to be open increases from about 0.07 at $\Delta V = 0$ to 0.95 at $\Delta V = +80$ mV and decreases at $\Delta V = +90$ mV to 0.78. The open probability in a patch with one active channel was calculated by the ratio of the time which the channel spent in the open state to the total time of the measurement. Same experiment as Fig. 1.

pendent Ca^{2+} oscillation in *Dictyostelium* (Bumann, J., Malchow, D. and Wurster, B., unpublished data). Ca^{2+} oscillations may arise due to voltage-gated Ca^{2+} influx and subsequent Ca^{2+}-activated K^+ efflux as has been suggested for example for pacemaker neurons of *Aplysia* [20]. The voltage-dependent K^+ channel described here, as well as the occurrence of K^+ oscillations in *Dictyostelium* [14], raise the possibility that a similar membrane oscillator exists in *Dictyostelium*.

We thank Dr. E. Bamberg for helpful criticism of the manuscript. D.M. is supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 138.

References

1 Hille, B. (1984) Ionic Channels of Excitable Membranes, Sinauer, Sunderland
2 Loomis, W.F. (1982) The Development of *Dictyostelium discoideum*, Academic Press, New York
3 Shaffer, B.M. (1962) Adv. Morphog. 2, 109–182
4 Gerisch, G. (1968) Curr. Top. Dev. Biol. 3, 157–197
5 Durston, A. (1974) Dev. Biol. 37, 225–235
6 Alcantara, F. and Monk, M. (1974) J. Gen. Microbiol. 85, 321–324
7 Gerisch, G. and Hess, B. (1974) Proc. Natl. Acad. Sci. USA 71, 2118–2122
8 Gerisch, G. and Wick, U. (1975) Biochem. Biophys. Res. Commun. 65, 364–370
9 Wurster, B., Schubiger, K., Wick, U. and Gerisch, G. (1977) FEBS Lett. 76, 141–144
10 Malchow, D., Nanjundiah, V., and Gerisch, G. (1978) J. Cell Sci 30, 319–330
11 Gerisch, G., Malchow, D., Roos, W. and Wick, U. (1979) J. Exp. Biol. 81, 33–47
12 Malchow, D., Boehme, R. and Gras, U. (1982) Biophys. Struct. Mech. 9, 131–136
13 Bumann, J., Wurster, B. and Malchow, D. (1984) J. Cell Biol. 98, 173–178
14 Aeckerle, S., Wurster, B. and Malchow, D. (1985) EMBO J. 4, 39–43
15 Klein, P., Theibert, A., Fontana, D. and Devreotes, P.N. (1985) J. Biol. Chem. 260, 1757–1764
16 Sakmann, B. and Neher, E. (1983) Single-Channel Recording, Plenum Press, New York
17 Marin, F.T. and Rothman, F.G. (1980) J. Cell Biol. 87, 823–827
18 Latorre, R. and Miller, C. (1983) J. Membrane Biol. 71, 11–30
19 Schroeder, J.I., Hedrich, R. and Fernandez, J.M. (1985) Nature 312, 361–362
20 Gorman, A.L.F., Hermann, A. and Thomas, M.V. (1982) J. Physiol. 327, 185–217