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CABLE PROPERTIES AND COMPARTMENTATION IN *ACETABULARIA* *

C. FREUDLING and D. GRADMANN

Institut für Biologie I der Universität Tübingen, Auf der Morgenstelle 1, D-7400, Tübingen (F.R.G.)

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Summary

The electrical cable properties of three different compartmentation types of *Acetabularia* cells have been investigated. These three types were: normal cells, 'stumps' (filled with cytoplasm, no central vacuole) and 'tubes' (cytoplasm depleted vacuoles). The latter two types have been obtained by centrifugation of normal cells. Qualitatively, the characteristic biphasic voltage response upon rectangular current pulses is the same in these three types. Quantitatively, however, the two conductances which can be obtained from the biphasic voltage response as well as the apparent capacity of several $F \cdot m^{-2}$ which derives from the large time constant of the second phase, are drastically increased in stumps and decreased in tubes compared to normal cells. The resting potential is a few mV more negative in stumps, and more positive in tubes, than in normal cells.

Based on the existence of the high resting potential and the apparent large capacity in the non-vacuolated stumps, it is concluded that the electrogenic Cl^- pump of *Acetabularia* is located in the plasmalemma membrane and that the apparent large capacity is not a result of the complicated membraneous organisation of the vacuolar system. Several possibilities are discussed, in relation to the quantitative correlation between intracellular compartmentation and electrical membrane parameters.

Introduction

The steady state electrophysiological properties of the plasmalemma membrane of *Acetabularia* under normal conditions, have been summarized in an

* Dedicated to Noe Higinbotham on his 65th birthday.

analog circuit model [1]. This model has also been used successfully for the interpretation of dynamic electrical membrane effects [2,3]. For this study, it is sufficient to use a simplified model which consists of the passive diffusion system with a relatively low conductance ($g_D \approx 1\ S \cdot m^{-2}$) and an active electrogenic channel (Cl^- pump) in parallel. This pump apparently consists of an electromotive force, E_P of about −190 mV, in series with two conducting elements P1 and P2 ($g_{P1} \approx 100\ S \cdot m^{-2}$, $g_{P2} \approx 10\ S \cdot m^{-2}$), where P2 and E_P are shunted by an apparent capacity, C_P, of some $10\ F \cdot m^{-2}$. E_P represents 1/2 of the free energy of ATP hydrolysis, C_P might reflect the cell's ATP pool, P1 stands for a carrier system of the pump and P2 is in close relationship to the energy metabolism.

It is unusual but not novel to suppose electrically measurable equivalents in vivo for metabolic events [2,4,5]. In particular, a postulated quantitative equivalence between C_P and the ATP pool needs closer examination. One way to confirm this suggestion is to disprove alternate explanations. For instance, the vacuolar compartmentation with its complicated intracellular membraneous system [6] could be a reason for the apparent large time constants in the electrical experiments by which the large C_P values are obtained. In this case, the electrical transfer function should be completely different, if the measurements are carried out on non-vacuolated cells.

Such non-vacuolated cell pieces have already been used [7]. They can easily be obtained by appropriate centrifugation of normal, young cells, without a cap. By this procedure, 'stumps' (without vacuoles) and 'tubes' (with almost no cytoplasm) can be obtained and compared with normal cells. If these non-vacuolated pieces also show large time constants, it would confirm our idea about the metabolic origin of the apparent huge capacity C_P.

Since there is no agreement in the literature as to whether the electrogenic pump of *Acetabularia* is located in the tonoplast [8] or in the plasmalemma [1,9], the resting potential of the non-vacuolated stumps should be crucial in answering this question.

This paper presents full evidence for these two questions. In addition, however, there are quantitative differences in the electrophysiological membrane parameters between the three types of compartmentation. These results will be discussed in relation to various aspects of the interactions between plasmalemma and cytoplasm.

Materials and Methods

Objects

Cells of the giant marine green alga *Acetabularia mediterranea* were cultured in Erdschreiber solution [10,11]. Only young cells, without a cap and thus with approximately cylindrical shape, were used. The diameter of these cells varied from 0.28—0.44 mm, the length from 15—40 mm.

Before preparing non-vacuolated 'stumps' and the remainder cytoplasm depleted 'tubes', the cells were exposed for 30—60 min to approx. $0.2\ mol \cdot m^{-3}$ Neutral red in sea-water. The red stained vacuole (pH 2.5 [1]) and the yellow stained cytoplasm (pH about 7) could easily be distinguished. 5—7 of these stained cells were mounted with the rhizoid on a perspex holder, apex

downwards. After centrifugation (approx. 30 min, 600 g) in sea-water, the cytoplasm accumulated almost quantitatively in the apical part of the cell, occupying about 10% of the total cell volume. The red staining in the remainder indicated that the vacuole was still intact including tonoplast, and, as we shall see, a hardly visible cytoplasmic layer with intact plasmalemma. The small intermediate region between these two parts could be eliminated by two ligatures. Isolated stumps and tubes were obtained by cutting this region off. These parts were used within 4 h for further experiments, before the stumps had developed new vacuolar vesicles which become microscopically visible 24 h after centrifugation.

General conditions

In all experiments, artificial sea-water was used as a bathing medium, containing the following components in mol · m^{-3}: Na^+ 461, K^+ 9.7, Mg^{2+} 53, Ca^{2+} 10, Cl^- 539, SO_4^{2-} 28 and HCO_3^- 2, buffered by 10 mol m^{-3} Tris-HCl at pH 8. The temperature was 24—28° C. The light used in the experiments and in microscopic observations was 100 W · m^{-2} white light from a quartz-iodide lamp (250 W).

Electrical measurements and analysis

Standard glass-microelectrode techniques were used. For voltage recording, difference amplifiers (10×) with high input resistance (>10^{11} Ω) were used as described previously [12]. For injection of rectangular current pulses, a variable voltage source (—120—+120 V) was used with a 10^8 Ω resistor and the injecting electrode (<10^7 Ω) in series. From the bath, the current was conducted by a low resistance silver wire and 1 kΩ resistor to ground. The voltage drop across this resistor was used as a measure of the applied current (1 mV ≙ 1 μA). In general, the current electrode was impaled half way along the length (=2 l) of the cell, and the two voltage recording electrodes at distances 0 (V_0) and x(V_x) from the current electrode. All three parameters (I_0, V_0 and V_x) were recorded on a chart recorder which did not resolve changes in the ms range (rise time ≈0.3 s).

Linear cable theory [13] was used for the analysis of the data. The membrane conductance, g_m (S · m^{-2}) = $1/r_m$ (r_m, membrane resistance in Ω · m^2) was calculated from the equation

$$g_m = (fI_0)/(V_0 2\lambda\pi d) \tag{1}$$

where d is the cell diameter and

$$f = \cosh(1/\lambda)/\sinh(1/\lambda) \tag{2}$$

is the correction factor for finite length. This factor f is about 1.04, if the length constant

$$\lambda = x/\ln(V_0/V_x) \tag{3}$$

is about 50% of l. In some cases, the current electrode and the voltage electrode for V_0 was impaled at the very end of the cell. In these cases, the factor 2 in

Eqn. 1 was omitted. If, as in a few cases, the current electrode was neither in the middle nor at the end of the cell, the mean conductance of the two asymmetric parts was used.

Since in *Acetabularia* membrane conductances are rather sensitive to the actual membrane voltage V_m, we used known correlations [1] to correct the conductance data for a standard resting potential of −170 mV. Similar results were obtained in several experiments at different membrane voltages V_m of the same cell. For these cells $g_m(V_m)$ was calibrated individually in order to obtain the standardized g_m for $V_m = -170$ mV.

Capacity values were obtained from the time course of the voltage (V_0 and V_x) and the conductance values:

$$C = \tau g \tag{4}$$

with the time constant $\tau = a\tau'$,

$$\tau' = t/\ln(V_s/(V_s - V_t)) , \tag{5}$$

where V_s is the saturation value of the voltage changes $V_{0,x}$, V_t the changes of $V_{0,x}$ at the time t and a is a correction factor which takes into account the relative cell length (l/λ) and the location of the electrodes. The appropriate calibration curves for a are given by the linear cable theory [13].

Results

General observations

Each of the three systems (stumps, normal cells and tubes) show the same basic voltage response upon a small rectangular current pulse. First, there is a fast change with a time constant in the range of ms. This change follows a simple exponential time course which has been described for normal cells in previous papers [1,14]. It is not resolved by the chart recordings used in this study. Following this fast change, there is a further, much slower one with a time constant in the range of s. This slow response as well as the fast one can be almost completely associated with the function of the electrogenic pump in *Acetabularia*, since the conductance values calculated from these responses are at least one order of magnitude larger than the passive conductance of the diffusion pathways in this voltage range [1]. Therefore, the total transfer function obtained under normal conditions is a fair approximation for the properties of the electrogenic channel itself.

The typical biphasic response of normal cells has been demonstrated several times [1,12,13]. Fig. 1 shows an example of an original result obtained from a stump. The voltage responses at the distances 0 (V_0) and $x = 1.7$ mm (V_x) from the current electrode are registered. The trace V_0 shows the biphasic response very clearly, with the fast change and the slower one. In the trace V_x, the responses are, of course, smaller. Since the fast response reflects a relatively high membrane conductance, it is clear that the corresponding length constant λ is smaller than for the entire response. Therefore, the fast response is reduced by a larger factor at the distance x from the current electrode.

Since the slow change is unusual for biomembranes, it is not evident whether the calculations of linear cable theory are appropriate, especially because

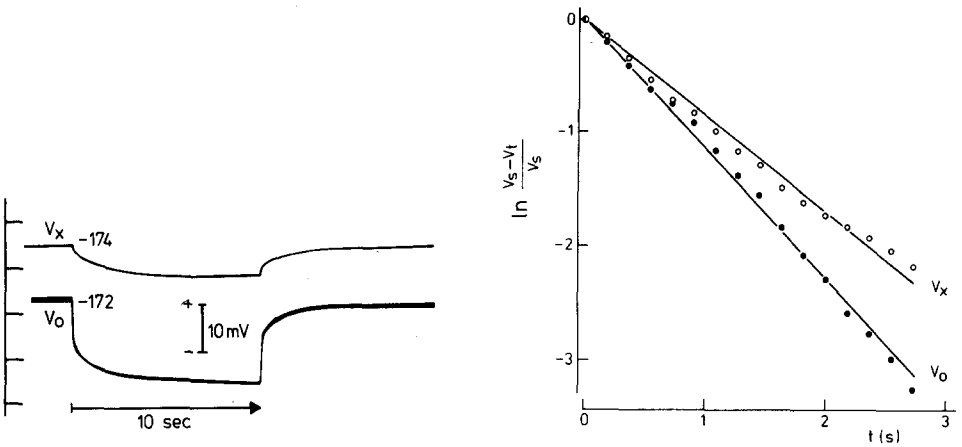

Fig. 1. Example of the typical biphasic responses V_0 and V_x in stumps upon a hyperpolarizing, square-waved current pulse (10 s, 1.2 μA). The current-injecting electrode and voltage electrode V_0 were at the very end of the stump, V_x 1.7 mm away. Apparent resting potentials of V_x and V_0 (difference probably due to electrodes) is marked on the traces in mV.

Fig. 2. Semilogarithmic plot of the slow voltage responses V_0 (filled) and V_x (open) from Fig. 1; dots represent readings in 0.18 s intervals from the continuous original traces. Deviations from linearity within reading error.

capacity values result which are about three orders of magnitude larger than normal membrane capacities. It is important to know whether the formal conditions which are common for conventional cable analysis are fulfilled. Therefore, it is necessary to demonstrate that the time course of the slow change follows a simple exponential function. In Fig. 2, the slow changes from Fig. 1 are plotted in a semilogarithmic scale. The points in Fig. 2 are readings at 0.18 s intervals from the continuous traces of Fig. 1. For both voltage changes, V_0 and V_x, we obtain fair linearity by the semilogarithmic plot $\ln((V_s - V_t)/V_s)$ versus time. From this result, it seems justified to use linear cable theory for the slow response.

Quantitative results

From the magnitudes of the fast response and of the entire voltage changes at the distance 0 and x from the current electrode, V_0 and V_x, two membrane resistances, r_m^0 and r_m^∞ can be calculated ($r_m = g_m^{-1}$) as well as two values for the inner resistivity r_i^0 and r_i^∞ ($r_i = r_m \lambda^{-2}$). The means of these four parameters and of the time constant τ_0^∞ of the slow change of $V_{0,x}$ are listed in Table I for tubes, normal cells and stumps. The r_i values turn out to be about the same whether calculated from the fast response or from the entire response ($r_i^0 \approx r_i^\infty$). This demonstrates that our methods yield reliable results.

However, the differences between the r_i values from stumps and normal cells are obvious. They confirm our suggestion that the tips of the inserted electrodes are located in the cytoplasmic compartment rather than in the central vacuole, which has a smaller resistivity [14]. Unfortunately, we were not able to insert three electrodes properly in the sensitive tubes. Therefore, no r_i values for the tubes could be measured directly; they were assumed to be about the

TABLE I

Cable properties of tubes, normal cells and stumps, obtained from the voltage responses V_0 and V_x upon rectangular current pulses, n: number of measurements (cells); r_m^0 membrane resistance calculated from the fast voltage changes; r_m^∞: membrane resistance calculated from the entire voltage changes (fast plus slow); r_i^0 : resistivity of the cell's interior calculated from the fast voltage changes; r_i^∞: resistivity of the cell's interior calculated from the entire voltage changes; τ^∞: time constant of the slow voltage change, corrected according to linear cable theory; ( ) *: numbers not from cable properties, see text. Means ± S.E.M.

	Tube	Normal	Stump
n	2	6	6
r_m^0 ($m\Omega\ m^2$)	108 ± 17	14 ± 3	2.7 ± 0.5
r_m^∞ ($m\Omega\ m^2$)	252 ± 43	82 ± 21	17 ± 3
r_i^0 ($m\Omega$ m)	(250) *	334 ± 41	552 ± 51
r_i^∞ ($m\Omega$ m)	(250) *	376 ± 84	561 ± 97
τ^∞ (s)	2.41 ± 0.24	2.73 ± 0.28	2.01 ± 0.50

TABLE II

Conductance g_{P1} and g_{P2} of the pump elements P1 and P2, apparent capacity C_P of the tentative ATP pool and resting potential V_m in tubes, normal cells and stumps; g_{P1}, g_{P2} and C_P are related to membrane area. Calculations from the same experiments as in Table I, based on the analog circuit model outlined in the Introduction. Means ± S.E.M.

	Tube	Normal	Stump
g_{P1} ($S\ m^{-2}$)	9.2 ± 1.4	71 ± 15	372 ± 65
g_{P2} ($S\ m^{-2}$)	6 ± 2	13 ± 4	62 ± 15
C_P ($F\ m^{-2}$)	16 ± 6	43 ± 15	148 ± 36
V_m (—mV)	154 ± 4	162 ± 4	170 ± 3

vacuolar resistivity ($\approx 0.25\ \Omega \cdot m$) which was calculated from the ionic content [9] and from conductivity measurements of diluted cell sap [1].

In order to discuss these results on the basis of our knowledge about the analog circuit of the *Acetabularia* membrane [1] as outlined in the Introduction, the data have been rearranged to present relevant information about the particular elements in the membrane in Table II. Since in the investigated state, the conductance of the passive diffusion pathways (shunted to the active channel) is about 1% of the element P1 and 10% of the element P2 in series with P1 ($=g_P$), we obtain g_{P1} from r_m^0, g_P from r_m^∞ and g_{P2} from g_P and g_{P1} by appropriate small corrections. The slow time constant τ^∞ and g_{P2} give the apparent capacity $C_P = \tau^\infty\ g_{P2}$. The mean values ± S.E.M. in Table II are based on calculations for each trial, for statistical reasons.

Discussion

Basic conclusions

Initially, two questions have been answered by our experiments: 1, is the biphasic transfer function an effect of the intracellular compartmentation? and 2, in which membrane is the electrogenic pump located?

1. The biphasic voltage response upon squarewave current pulses, typical of normal vacuolated cells, is also found in stumps (Fig. 1) which are depleted of vacuole and tonoplast. Therefore, the response cannot be due to the particular intracellular membraneous organisation in *Acetabularia*.

2. Since the stumps (without tonoplast) show the same high, or even more negative resting potential as normal cells (Fig. 1, Table II), the responsible electrogenic pump must be located in the plasmalemma membrane, rather than in the tonoplast.

Comparison of quantitative data

If we compare the quantitative results from the three cell types as listed in Table II, we observe a clear increase of g_{P1}, g_{P2} and C_P in stumps, and a clear decrease in tubes, compared to normal cells. On strict statistical grounds, however, the question remains open whether these parameters change by the same factor or not; i.e. whether these parameters represent three properties of only one entity which is accumulated in stumps and diluted in tubes or whether they represent independent functions.

The hypothesis that C_P represents the cell's ATP pool appears to be confirmed, since in stumps we would expect more ATP per membrane area and in tubes less than in normal cells. Similar considerations would hold for P2, which has been suggested to bear a close relationship to the cell's energy metabolism [1,12].

More problems arise in interpreting the changes of P1, because it is supposed to be a 'pure' membrane function (the carrier system of the pump) [1]. Four explanations seem possible, but not satisfying, at present:

1. P1 is a function of ATP. In this case, the ATP concentration at the membrane should be essential, rather than simply the ATP content of the cell. Only if the membrane consumed a large part of the cell's ATP turnover would the ATP concentration be increased at the stump membrane, because in this case the ATP synthesizing power of the cytoplasmic volume would be markedly increased compared to the ATP consumption by the cell membrane. However, in normal cells, the pump only consumes about 5% of the entire ATP turnover [1]. Only for the low value of P1 in tubes would the above consideration be appropriate, because of the very small cytoplasmic content compared to the membrane area in tubes.

2. P1 is accumulated in the stumps by centrifugation. Since (transport-) proteins have a density of about $1.3\ g \cdot ml^{-1}$ and the lipid matrix of the membrane about $0.7\ g \cdot ml^{-1}$, this might, indeed, be possible. However, the relatively gentle centrifugation used (30 min at $600 \times g$) seems to be insufficient for such an effective separation.

3. On centrifugation, the plasma membrane becomes folded so that more transport units appear per cell surface area. In this case, the plasmalemma should already be folded in normal cells and stretched in tubes. However electromicrographs of the normal plasmalemma do not show many invaginations of the plasma membrane [6].

4. The synthesizing sites for P1 (or its precursors) in the cytoplasm are limiting for the density of P1 in the plasmalemma. In this case, the available amount of cytoplasm per membrane area (high in stumps and low in tubes)

would be critical for the density of P1 in the membrane. This mechanism would imply a rather high turnover of P1 in the membrane, because within 1 h from the beginning of the centrifugation the effects are obvious. This explanation could, for instance, be examined by measuring the relaxation time of the changes of P1 upon centrifugation under various conditions which inhibit protein synthesis.

Final remarks

Despite the present lack of a definite interpretation of our quantitative results, our findings demonstrate the important role of cytoplasmic factors in the normal function of the cell membrane. In view of this, it seems essential to investigate biomembranes in vivo, since isolated membranes will probably only show a restricted range of their important biological functions.

The properties of the transfer function in stumps, normal cells and tubes show that the large time constants cannot be due to the intracellular membraneous organisation, because they also exist in non-vacuolated stumps. Our results are consistent with the suggestion that these large time constants are due to an apparent capacity which might be equivalent to the cell's ATP pool.

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References

1 Gradmann, D. (1975) J. Membrane Biol. 25, 183—208
2 Gradmann, D. (1976) J. Membrane Biol. 29, 23—45
3 Gradmann, D. (1978) J. Membrane Biol. 44, 1—24
4 Slayman, C., Long, W. and Lu, C. (1973) J. Membrane Biol. 14, 305—338
5 Gradmann, D. and Slayman, C.L. (1975) J. Membrane Biol. 23, 181—212
6 Franke, W.W., Kartenbeck, J. and Spring, H. (1976) J. Cell Biol. 71, 196—206
7 Saddler, H.D.W. (1970) J. Exp. Bot. 21, 345—359
8 Rogatykh, N.P., Yasinovskii, V.G. and Zubarev, T.N. (1976) Biofizika 12, 656—660
9 Saddler, H.D.W. (1970) J. Gen. Physiol. 55, 802—821
10 Hämmerling, J. (1944) Arch. Protistenk. 97, 7—56
11 Beth, K. (1953) Z. Naturforsch. 8b, 334—342
12 Gradmann, D. (1970) Planta 93, 323—353
13 Hogg, J., Williams, E.J. and Johnston, R.J. (1969) J. Theoret. Biol. 24, 317—334
14 Gradmann, D. and Klemke, W. (1974) in Membrane Transport in Plants (Zimmermann, U. and Dainty, J., eds.), pp. 131—138, Springer Verlag, Berlin