Monitoring electropermeabilization in the plasma membrane of adherent mammalian cells

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ABSTRACT When an electrical potential of order one volt is induced across a cell membrane for a fraction of a second, temporary breakdown of ordinary membrane functions may occur. One result of such a breakdown is that molecules normally excluded by the membrane can now enter the cells. This phenomenon, generally referred to as electropermeabilization, is known as electroporation when actual pores form in the membrane. This paper presents a unique approach to the measurement of pore formation and closure in anchored mammalian cells. The cells are cultured on small gold electrodes, and by constantly monitoring the impedance of the electrode with a low-amplitude AC signal, small changes in cell morphology, cell motion, and membrane resistance can be detected. Because the active electrode is small, the application of a few volts across the cell-covered electrode causes pore formation in the cell membrane. In addition, the heat transfer is very efficient, and the cells can be porated in their regular growth medium. By this method, the formation and resealing of pores due to applied electric fields can be followed in real time for anchorage-dependent cells.

INTRODUCTION

Electropermeabilization is defined as the phenomenon of a normally selective biological membrane suddenly becoming permeable to a wide variety of ions and molecules under influence of a strong electric field. A special case of electropermeabilization is electroporation, when small transient pores are formed in the membrane. Pore formation occurs when the transmembrane potential, which is normally about 60–110 mV, is raised to about 1V (1–4). Under these conditions, the pores reseal soon after the high field is removed. If the transmembrane potential is raised further, irreversible membrane damage takes place, culminating in cell death (1, 2). Electropermeabilization of cell membranes is commonly used to insert foreign material into the cell. Macromolecules, which are normally excluded by the plasma membrane, can be induced to enter the cells through the membrane pores (1–3, 5). Recently, however, some speculation has been made concerning whether large molecules like DNA enter cells through pores, or by endocytosis induced by the electrical field (5).

Electropermeabilization has been studied in several ways, including rapid-freezing electron microscopy (6), detection of macromolecule entry into the cells (3, 5), and a relaxation in the membrane resistance (7–11). The last method is probably the one most suited for investigating the mechanism of pore formation and resealing, because the cells studied this way are not exposed to foreign material which might influence the results. Relaxation of membrane resistance has been measured by patch clamping (7, 8), fluorescent imaging (9) and current–voltage measurements (10, 11). In most cases reported in the literature, current–voltage measurements have been conducted on suspended cells placed between two closely spaced electrodes in a specially constructed "electroporation" chamber (10, 11). In such studies, a high voltage (10^2–10^3 V) was applied for a very short duration (10^{-5}–10^{-4} sec), and electropermeability was detected as a change in conductivity (10) or a deformation of the applied square wave pulse (11). Such high voltages were necessary in order to cause the required potential drop across the cell membrane in the cell suspension, where the two electrodes were placed a few millimeters away from each other (10). The use of such high voltages caused unwanted Joule heating (4), and therefore required the use of special high resistivity solutions. It has been suggested that some mammalian cells are "unstable" in very high resistivity media (12). A different approach is to use cells attached to a substrate where they are in a monolayer. In the few reported cases of electropermeabilization using attached cells (13, 14), however, the electrode geometry used was such that the main voltage drop occurred across the bulk of the electrolyte and not the cell layer. In these situations high voltage, and consequently, high resistivity media were required.

In this paper we report the use of current–voltage measurements to detect pore formation and monitor pore resealing in the plasma membrane of adherent mammalian fibroblastic cells. The method for measuring cell impedance used in this work was developed by Giaever and Keese, and has been explained in greater detail elsewhere (15, 16). The same system has now been used for electropermeabilization experiments. The cells are grown on small gold electrodes (area $\sim 10^{-3}$ cm^2) evaporated on the bottom of tissue culture dishes. A small alternating current (1 μA) at a frequency of 4,000 Hz is passed between this electrode and a counter electrode (area ~ 2 cm^2) placed some distance away (see Fig. 1 for circuit details). The electrolyte used is the regular tissue culture medium, which has a resistivity of $\sim$54 Ω-cm. The potential drop across the small electrode is much larger than the potential drop across the solution or the large electrode; therefore, the impedance of the small electrode dominates the system (17). As cell membranes have a very high impedance, the presence of cells on this

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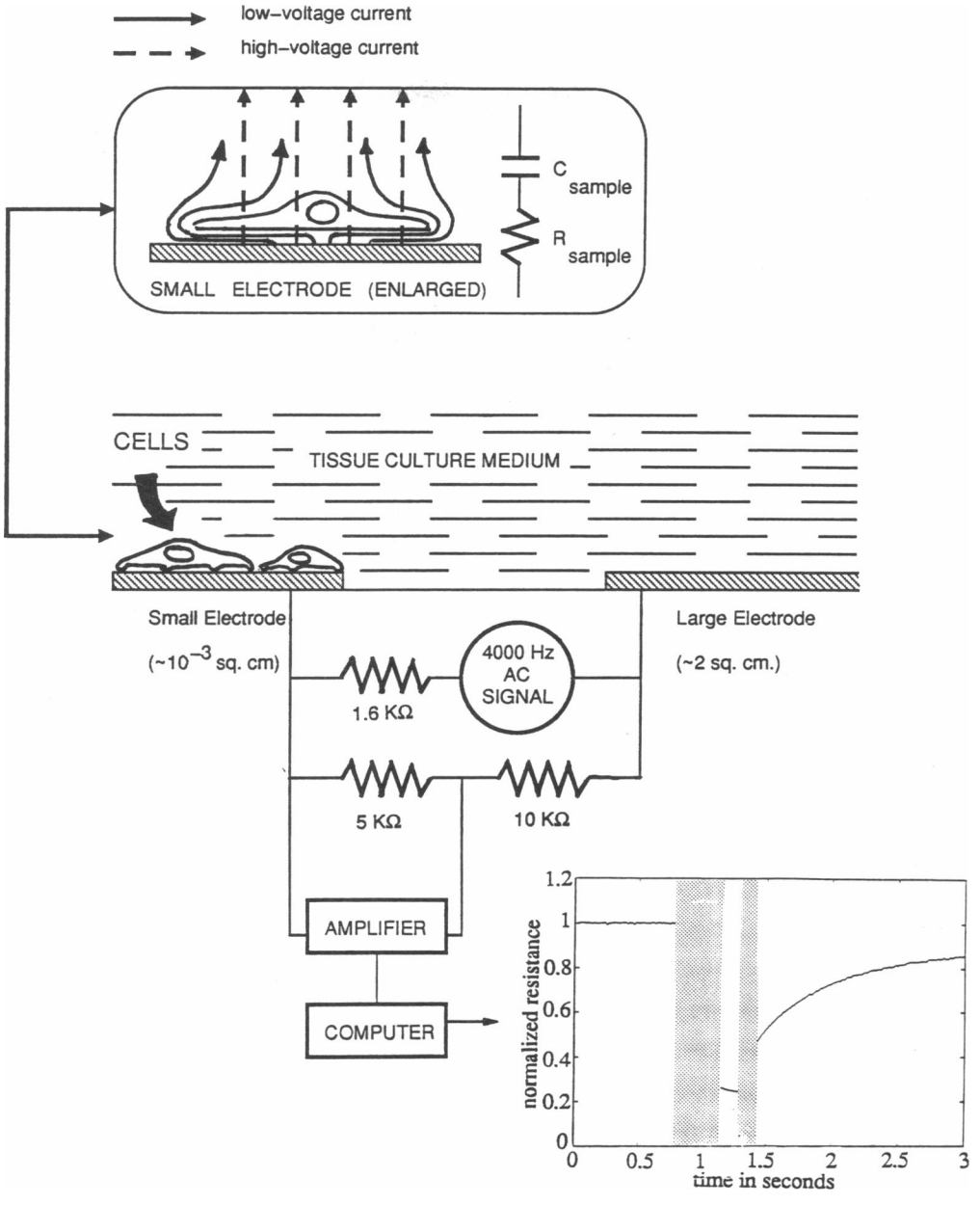

FIGURE 1 The basic setup used in the experiments. The voltage across the sample is too large to be measured by the amplifier; therefore, a voltage divider is used, and the amplifier measures only the voltage across the 5 KΩ resistor. This must be taken into consideration whenever the voltage across the sample itself is to be calculated—this voltage is roughly three times the measured voltage. The sample is treated as a simple RC series circuit, as shown in the inset. The position of the large electrode is not important because the impedance is dominated by the small electrode.

electrode greatly affects the measured impedance, and the resistance may go up by a factor of 4–8 for a confluent cell layer. Under these circumstances, any small change in impedance of the cell layer, either due to cell motion, morphological changes, or a change in membrane resistance, is easily detected. As will be shown later in this report, a 200 ms, low voltage (1V–5V) pulse will be sufficient to permeabilize the cell membrane. Because the Joule heating is constrained to a small volume close to the electrode, it can be shown that the rate of heat dissipation is sufficient to keep the temperature under control even with such low resistivity media. Pores in the cells are detected as a sudden drop in the impedance of the cell layer on the electrode, which can be constantly monitored in this setup using a much lower applied voltage (~1mV). The lower voltage mode has been found to be non-invasive (18), hence the cell layer can be monitored for long periods of time with no additional damage to the cells under study.

MATERIALS AND METHODS

Cells and cell culture. Transformed human lung fibroblasts WI-38/VA-13 (referred hereafter as VA13 cells), were used in all experiments. The cells were cultured in Dulbecco's Modified Eagle Medium

(DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.05 mg/ml gentamicin (all from GIBCO laboratories, Grand Island, NY) at 37°C in a humidified atmosphere containing 5% CO_2.

Circuit and electrode setup. Gold electrodes were evaporated on the bottom of a tissue culture dish as described in earlier work (15). The dish containing the electrodes was placed in an incubator and connected to a lock-in amplifier interfaced with a computer as shown in Fig. 1. The amplifier measures the in- and out-of-phase (real and imaginary) voltages across the small electrode and these are converted by the computer into a resistance and capacitance in series taking the external circuit into consideration. The amplifier can take readings as fast as once every 5 milliseconds, and the setup can be used to monitor the impedance of the sample. The electrolyte used is the cell growth medium (DMEM + 10% FBS).

Procedure. The impedance of the cell-free electrode is first measured using a current of approximately 1 μA at 4,000 Hz. Under these circumstances, the measured resistance is about 1,000 Ω, of which about 10 Ω is due to the large electrode (area ~ 2 cm^2), 600 Ω to the solution resistance, and the rest is due to the small electrode (area ~ 10^{-3} cm^2). Since impedance of the gold electrode is frequency dependent (16, 19), the frequency of the oscillator was maintained throughout at 4,000 Hz, even during the application of the high voltage pulse (pulsation). The measured capacitance of the small electrode is about 10 nF. VA13 cells were seeded in the dish at a concentration of 10^5 cells/cm^2 and reached confluence in about 24 hours. During this time, the measured resistance is observed to increase to about 6,000 Ω, and the capacitance decreases to about 8.5 nF. The increased resistance is due to the cells on the small electrode, as the solution resistance and the resistance of the large electrode do not change significantly. It has been seen that cell response to pulsation varied with the degree of confluence of the cell layer; therefore, to obtain consistent results, the voltage pulse was applied only after the ratio of resistance of the cell-covered electrode to that of the empty electrode reached 6.

A short time prior to pulsation, the amplifier was set to take data once every 10 milliseconds, and the impedance was followed with a 3.5 mV signal applied by the oscillator. Next, the amplifier settings were changed to avoid overload, and the high voltage (1 V–5 V) pulse was applied for 200 milliseconds. The voltage was then dropped back to 3.5 mV, and the amplifier settings were changed back to normal. Because of these amplifier adjustments the impedance of the cell layer for a short time before and after the pulse could not be measured.

The peroxidase assay. Electropermeabilization of the plasma membrane during high field application was determined by the entry of the enzyme horseradish peroxidase (HRP, Donor: hydrogen peroxideoxydoreductase; EC1.11.1.7; Sigma Chemical Co., St. Louis, MO) into the cells. Six different electrodes with an equally confluent layer of VA13 cells on each of them were used for this experiment. The HRP was added to the extracellular medium at a concentration of 1 mg/ml, and an electric pulse was applied across the cell-covered electrode as described above. Each electrode was pulsed with a different voltage ranging between 0 and 5 volts. Next, the cells were thoroughly washed with phosphate buffered saline (PBS) to remove enzyme that had not entered the cells and then were fixed with 10% formalin in PBS. For detection of HRP inside the cells, the formalin solution was first replaced by washing three times with PBS, and the cell layer was flooded with a mixture of 6 ml BIStris ([Bis-(2-hydroxyethyl)imino]tris-(hydroxymethyl)methane), 0.1M, pH 7.6; 120 μl 4-Chloro-1-Napthol (1% in C_2H_5OH) and 50 μl H_2O_2 (0.3% in H_2O). The substrates are converted to a blue precipitate when exposed to active HRP and reveal those cells that received the enzyme upon pulsation. The entire experiment was repeated three times to confirm the reproducibility of the results.

RESULTS

Fig. 2 illustrates the effect of a strong electric field of duration 200 ms on the resistance of the cell-covered electrode. From the figure, we see that the resistance drops considerably during the pulse; the higher the voltage applied, the larger the drop. It may be noted that after the 1V pulse the resistance immediately returns to its original value; however, when higher voltages were applied, the original resistance is regained more slowly, over the period of several seconds. In all four cases, at the end of the experiment, the resistance returned to about 96% of the original value. These results were consistent and varied only slightly from experiment to experiment. This is illustrated in Fig. 3, where we have plotted the response of four different cell layers upon pulsation. In (*a*) two layers were subjected to 2 volts for 200 milliseconds, and in (*b*) two other layers were pulsed with 3 volts for 200 milliseconds. The results for identical pulses are very similar; the small difference in response observed will be discussed later.

Fig. 4, *a* and *b* show a pulse of 1V and 2V, respectively, applied across an electrode without cells under similar conditions. The return after pulsation is now instantaneous, and although the resistance drops during the high voltage pulse, this drop is much smaller than that seen with cells on the electrode. Note that the scales are different in the two figures, with the resistance of the cell-covered electrode being many times larger than the resistance of the cell-free electrode. For comparison, in Fig. 4*c* is shown the result of Figs. 2*a* and 4*a* on the same scale. Again, in Fig. 4*a* and *b*, there is a small bump in the resistance seen soon after the pulse. This is due to the resetting of the amplifier parameters, and can be seen even if a pure resistor is used as the sample (data not shown). This bump is also seen in Fig. 2*a* but it is masked by the much larger resistance changes in Fig. 2*b*, *c*, and *d*.

An easy way to check for membrane permeability is the introduction of a foreign molecule into the cell. In a series of experiments, we added the enzyme horseradish peroxidase to the extracellular fluid and then pulsed the cells. Horseradish peroxidase can enter the VA13 cells by endocytosis even without the application of the pulse, but under our experimental conditions, the amount entering the cells this way has been found to be too small to be detected, unless the exposure time is more than half an hour. To eliminate the possibility of enzyme entry into the cells by endocytosis, we have applied the pulse less than one minute after enzyme addition. Immediately after the pulse, the cells were washed and fixed, and then tested for enzymatic activity. As described in the previous section, 0–5 V were applied to six different cell-covered electrodes with HRP present in the medium. It was found that an applied voltage of 1 or 2 V was too small to cause notable entry of HRP into the cells; whereas cells on the electrodes pulsed with 3 V or more had taken up the enzyme. Only a few cells were found to be stained with the blue dye on the electrode pulsed with 3 volts, while many more blue-stained cells were found in the case of the 4V and 5V pulsed electrodes. Also, the

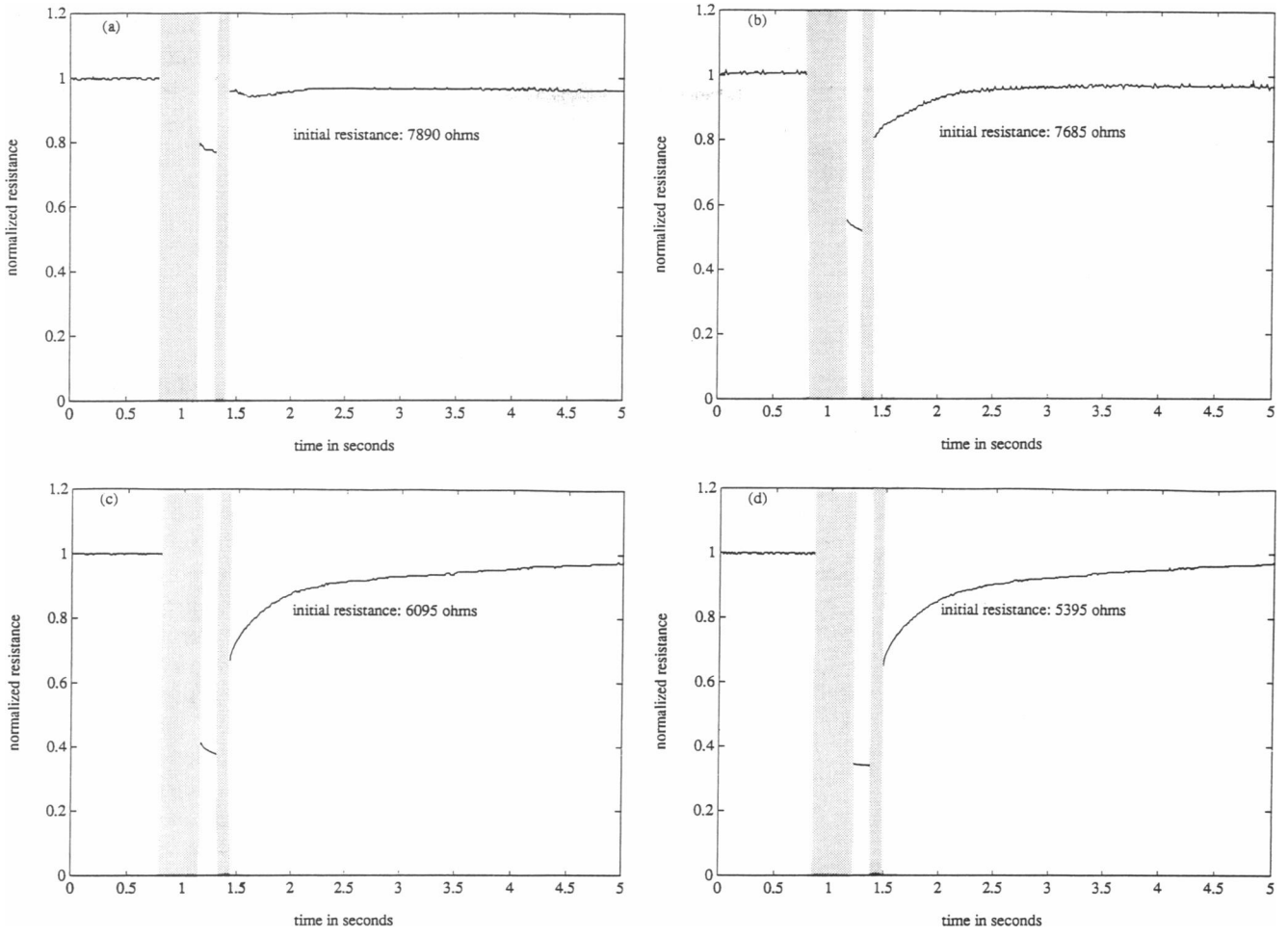

FIGURE 2 Observed changes in the measured resistance of cell-covered electrodes due to application of (*a*) 1 V, (*b*) 2 V, (*c*) 3 V, and (*d*) 4 V. The resistance plotted in the *y*-axis has been normalized to the initial resistance of the cell-covered electrode measured before the application of the pulse. These initial resistances are mentioned in the inset in each figure. A brief period immediately before and after the high voltage pulse cannot be monitored in the present setup due to the time required to change the amplifier settings. These periods are stippled in the above figures. The periods between these stippled areas represent the data taken with the high voltage, whereas those outside these areas represent data taken with the 3.5 mV applied potential.

intensity of the dye in the cells pulsed with 5 V was greater than that of those pulsed with 4 V. The above results have been compared in Table 1.

DISCUSSION

The changes in resistance recorded in Fig. 2 can, in principle, be due to the following factors: (1) the electrochemical effect of the small gold electrode, (2) cell motion and/or morphological changes in the cells in response to the electric shock, or (3) a lowering of membrane resistance caused by enhanced permeability. All these different effects are considered below.

The voltage appearing across the cell membrane can be estimated by analysis of the circuit shown in Fig. 1. The average voltage across the cell layer is given by:

$$V_c = V_a[Z_C - Z_N]/[R_s + (1 + R_s/R_p)Z_C] \qquad (1)$$

where V_a is the externally applied voltage, Z_C and Z_N are the total impedances in ohms of the cell-covered and cell-free electrodes, respectively, and the circuit resistors are R_s = 1.6 KΩ, and R_p = 5 KΩ + 10 KΩ. For typical values of the cell-covered electrode, V_c is ~65% of the applied voltage, the rest being distributed over the electrode interface, the solution resistance, and the external resistors. As an example, we shall discuss the case shown in Fig. 2*d* when a 4V signal is applied across a cell-covered electrode. From Eq. 1 it can be shown that roughly 2.6 V is across the cell layer, 0.8 V is across the electrode, and the rest of the voltage drop occurs across the solution resistance and the resistor R_s. The voltage drop across the electrode (0.8 V) is very similar to that obtained across an empty electrode if a 2V pulse is applied as shown in Fig. 4*b*. Thus the voltage drop across the gold electrode is much smaller than that across the cell layer, and should not influence the results significantly. Fig. 4*c* compares the results of Figs. 2*a* and 4*a* to show that the changes in resistance seen in the cell-covered electrode are mostly due to the presence of cells. Hence it is clear

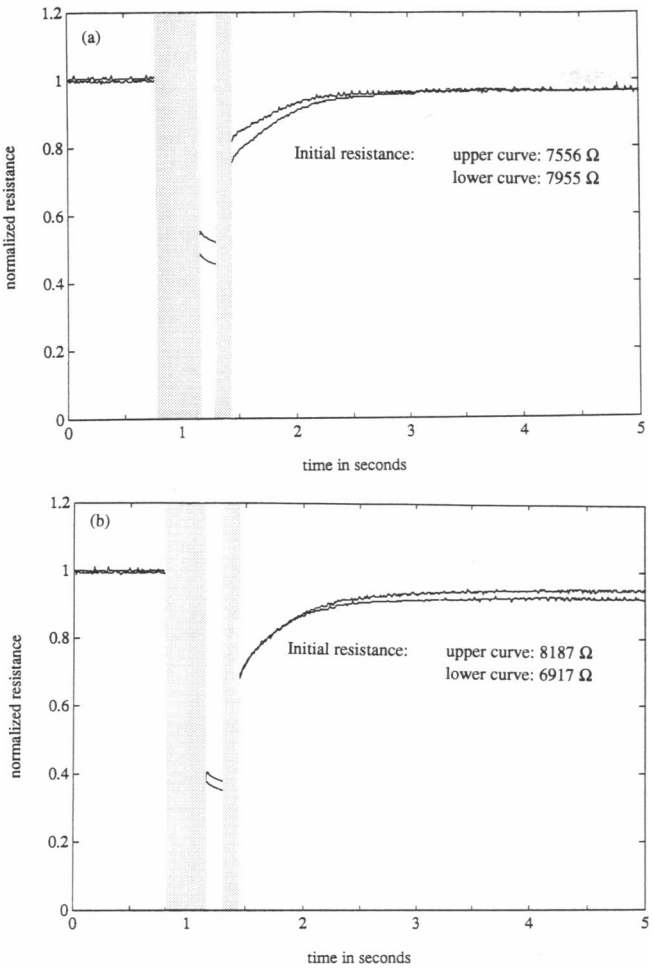

FIGURE 3 (*a*) Two different electrodes have been pulsed with a 2 V, 200 ms pulse each, and the responses of the two cell layers have been traced on the same plot. The upper curve represents a cell layer with a resistance ratio (ratio of resistance of the cell-covered electrode to that of the empty electrode) 5.00, while the lower curve represents a cell layer with a resistance ratio 6.00. Both these electrodes are of comparable surface area. (*b*) This shows the results of plotting two electrodes with very different surface areas but the same resistance ratio (6.1), and each pulsed with a 3 V, 200 ms pulse.

that a change in Z_N due to the field cannot explain the large resistance changes during pulsation.

To look at the impedance changes due to the other factors in more detail we shall use a model developed by Giaever and Keese (20). According to this model, at 4,000 Hz, the current from the electrode normally flows in spaces under the cells and between the cells, with only a small capacitively coupled current passing through the intact plasma membrane (see inset in Fig. 1 for details). Only if the membrane is punctured will a large amount of current flow through the cell itself. Eq. 2, based on this model, relates the specific impedance of the cell-covered electrode, Z_c (Ω-cm^2), to the specific impedance of the empty electrode, Z_n (Ω-cm^2), the specific impedance of the membrane itself Z_m (Ω-cm^2), the specific resistivity between the cells, R_b (Ω-cm^2), and a parameter $\alpha = r_c(\rho/h)^{0.5}$ ($\Omega^{1/2}$-cm), where r_c is the radius of the disk-shaped cell, ρ is the resistivity of the electrolyte, and h is the height of the space between the ventral side of the cell and the substratum:

$$\frac{1}{Z_c} = \frac{1}{Z_n} \times \left(\frac{Z_n}{Z_n + Z_m} + \frac{\dfrac{Z_m}{Z_n + Z_m}}{\dfrac{\gamma r_c}{2}\dfrac{I_0(\gamma r_c)}{I_1(\gamma r_c)} + R_b\left(\dfrac{1}{Z_n} + \dfrac{1}{Z_m}\right)} \right). \quad (2)$$

Here, I_0 and I_1 are the modified Bessel functions of the first kind of order 0 and 1, respectively, and

$$\gamma r_c = \alpha[(1/Z_n) + (1/Z_m)]^{0.5}. \quad (3)$$

For the VA13 cells under consideration, the standard values found are $R_b = 2.2$ Ω-cm^2 and $\alpha = 7$ (r_c has been measured at 11×10^{-4} cm) [20]. The normal membrane resistivity of an intact membrane is of the order of 10^3 Ω-cm^2 while the capacitance of the membrane is of the order of 10^{-6} Farads/cm^2. Eq. 2 shows that Z_c, the parameter actually measured, will change if either of Z_m, Z_n, α, or R_b changes. A change in Z_n can be brought about only by an electrochemical reaction of the small gold electrode in contact with the electrolyte which, as we have already seen, is negligible compared to the other changes. Cellular motion, or a change in cell morphology, can result in either a change in α, or a change in R_b, whereas membrane permeability will cause a change in Z_m.

When a cell layer is shocked with an applied electric pulse, the cells may respond by movement or a change in morphology (21, 22), and these would alter both α and R_b. If only α and R_b were to change, a very large decrease in both these parameters, arising from major alterations in cell morphology, would be required to explained the results. However, no large-scale change in cellular morphology has been observed by simultaneous time-lapse video-micrography in the 5-second time ranges recorded in Fig. 2. In addition, any change in impedance due to cellular motion or morphological changes would be much slower than the changes seen. Even if the cells could break away from the electrode in the span of milliseconds due to the applied field, a fast recovery seems unlikely, since even small movements normally require several minutes (23).

The explanation, therefore, is that the membrane impedance, Z_m, changed drastically during the pulse, accounting for the large drop in impedance observed in Fig. 2. By using Eq. 2 it is possible to calculate the change in Z_m necessary to explain the observed resistance changes. In Fig. 5, we have used this equation to plot changes in electrode resistance against corresponding changes in membrane resistivity. The width of the curve represents variations in parameters such as electrode area, α and R_b, as the actual resistance of the cell-covered

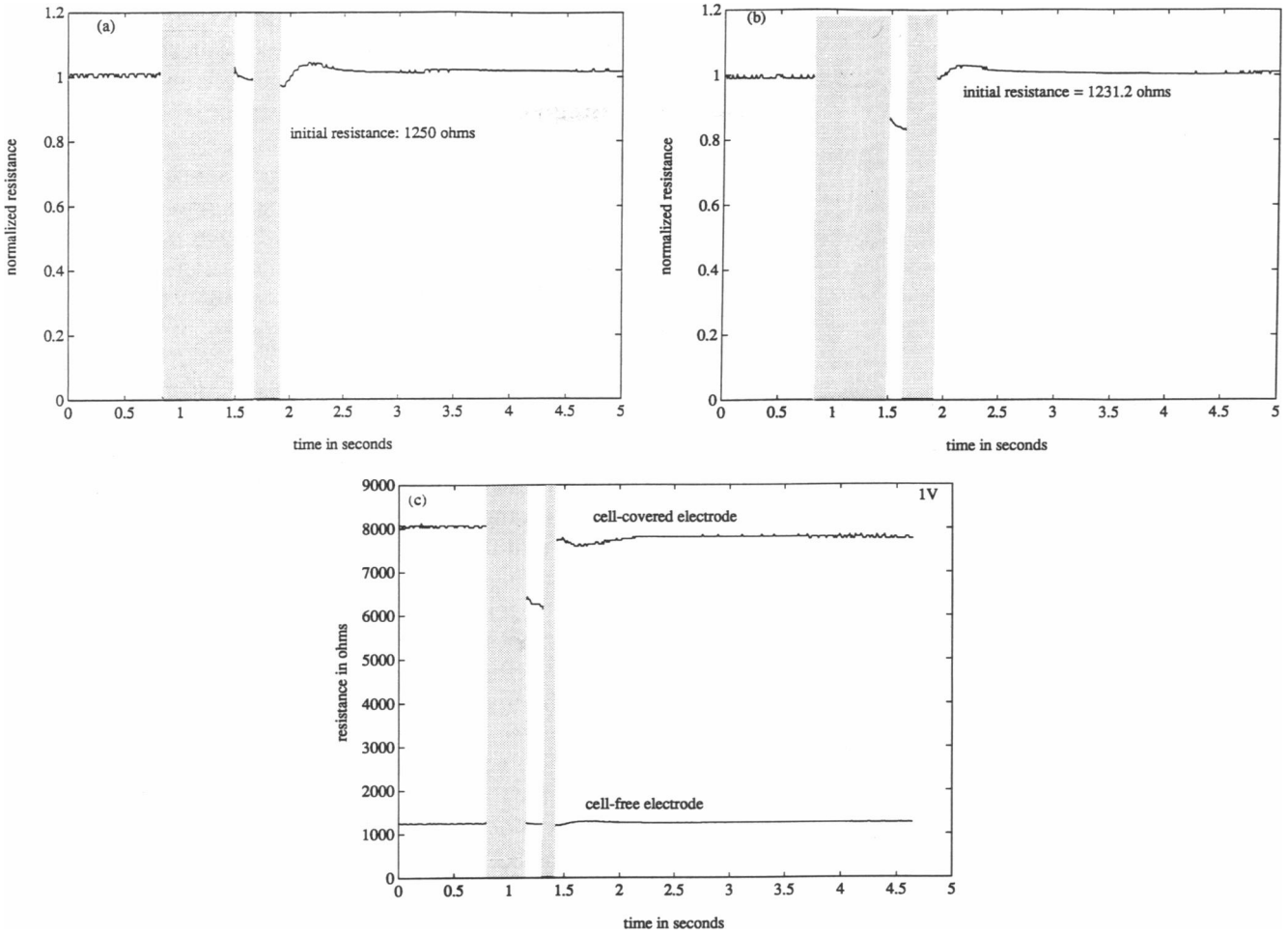

FIGURE 4 Observed changes in the measured resistance of a cell-free electrode due to application of (*a*) 1 V and (*b*) 2 V. We have not shown higher voltages because such high voltages never appear across the electrode when it is covered with cells. In (*b*) the resistance falls slightly during the pulse. This is due to the non-ohmic behavior of the gold electrode in contact with the electrolyte. In (*c*), we show the results from Figs. 2*a* and 4*a* on the same scale (not normalized). It is clear from this figure that compared to the contribution of the cells on the resistance changes, the contribution of the electrode is negligible. The effect of only the cells can be obtained by subtracting out the lower curve from the upper one.

electrode would depend on these as well. We can use this figure to calculate the fall in membrane resistivity that would cause the measured drop in resistance. As an example, we shall consider the steep resistance drop shown in Fig. 2*b*, where 2 V was applied to the electrode. Using Eq. 1, we can show that this is equivalent to 1.3 V across the cell layer or about 0.65 V across each membrane. We have considered the entire voltage drop to be across the basal and apical membranes of the cells because the cytoplasm in between has very low resistivity. From Fig. 2*b* we can see that the resistance dropped to 55% of its original value during the high voltage pulse. Using Fig. 5, we can estimate that the membrane resistivity, R_m, has dropped from about 1,000 Ω-cm^2 to about 2 Ω-cm^2 as a result of the pulse. Such a large change in membrane resistivity can only be explained by pore formation.

Although our system does not allow us to distinguish between a small population of large pores from a large population of small pores, the measurement of the drop in membrane resistivity as obtained from Fig. 5 allows us to estimate the average diameter of the pores formed, if we make an assumption about the number of pores

TABLE 1 Response of cells to pulsation

Applied voltage across cell-covered electrode	Intensity of dye	Percentage of cells stained as seen on the electrode
0 volts	—	0%
1 volt	—	0%
2 volts	—	0%
3 volts	+	<20%
4 volts	++	>50%
5 volts	+++	>50%

Increase in number of cells and intensity of blue stain inside cells with increased pulsing voltage, as measured by eye estimation from photographs of the blue-stained cells taken soon after they had been pulsed in the presence of the enzyme horseradish peroxidase. No blue stained cells were seen upon application of 0–2 volts, after which the amount of stain on each electrode increased rapidly.

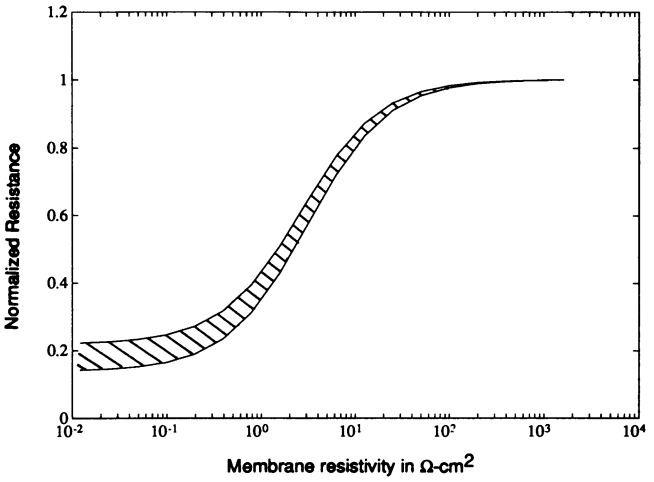

FIGURE 5 The theoretical relationship between the cell membrane resistivity and the normalized electrode resistance obtained using Eq. 2. To first order, no change in the membrane capacitance occurs with the formation of pores. The width of the curve represents variations between the electrodes in parameters such as electrode area, α and R_b (see text for details).

formed per cell. This estimate will give us an idea of the amount of damage we are doing to the cell membrane and may be useful in controlling the factors affecting the cell response. If we assume n pores of fixed size per cm^2 on each side of the cell layer, the pore radius can be calculated from the relationship:

$$R_m = [\rho t_m/(\pi r_p^2) + \rho/(2r_p)] \times 2/n \qquad (4)$$

where ρ (54 Ω-cm^2) is the resistivity of the tissue culture medium, t_m (8 nm) the membrane thickness, and r_p is the pore radius. The first term in the expression is the resistance of a single pore, and the second term is the sum of the constriction resistance on each side of that pore (17). The factor of 2 outside the bracket is due to the fact that the current goes through two membranes. If $n = 1$, the pore diameter would be roughly 1,000 nm for an application of a 2 volt pulse, and if $n = 1{,}000$, the diameter would be 3 nm. Since the enzyme horseradish peroxidase (diameter 5.34 nm) did not enter the cells at this voltage, the latter seems more probable. Data presented by others on erythrocytes (6, 24) also suggest that for cells of the size used, a thousand pores per cell would be a reasonable estimate. For example, this number is in agreement with the observations of Chang and Reese (6), who have found less than seven pores per μm^2, which, coupled with the fact that the radius of the cells we use is 11 μm, and therefore a cell area of about 380 μm^2, gives approximately 2,000 pores per cell. This number also agrees with the estimates of Dimitrov and Sowers (24), but exceeds somewhat the estimate of Kinosita and Tsong (3).

The observations made in Fig. 2 can now be explained in the light of the above discussion. When a voltage pulse is applied, the permeability of the membrane increases, because of pore formation. These pores start resealing as soon as the high voltage is turned off, and therefore, recovery of initial membrane resistance is observed. This is in accordance with the observations of others (6, 9, 10) who have determined that the pores form within 100 μs after the application of the pulse and start resealing within a few milliseconds. It has been proposed that pore resealing takes place in three stages (25)—a rapid initial decrease in pore size, a slower secondary decrease, and a complete resealing over a long period of time. The data presented in Fig. 2 are in accordance with these observations—we see that the slope of the resistance decreases over time. It is significant that although the drop in resistance during the pulse is proportional to the magnitude of the pulse, at the end of the experiment in all four cases, the resistance is 96% of the initial resistance. This may mean that the VA13 cells used have a characteristic resealing time, independent of the magnitude of the pulse, unless a threshold is crossed, when irreversible membrane rupture takes place. More detailed studies will show whether the 4% drop in resistance seen at the end of the experiments presented in Fig. 2 results from the presence of very small pores, or changes in cell morphology. It may be noted that data reported in the literature have found the time taken for complete resealing of the pores to vary widely—from a few seconds to a few hours (25).

It has been observed that the response of the cells to pulsation varied with the degree of confluency of the cell layer on the electrode. This is demonstrated by the results reported in Fig. 3. In Fig. 3*a* we have plotted the response of two different cell layers, both pulsed with 2 volt, 200 millisecond pulses. The upper curve represents a cell layer of resistance ratio 5.00, while the lower curve represents a cell layer of resistance ratio 6.00. Using Eq. 1, and taking into consideration both the resistive and reactive parts of the impedance of the cell-covered and cell-free electrode, the potential across the cell layer on application of the pulse is found to be about 1.1 V in the first case, while it is about 1.2 V for the second. The difference in voltage caused a slight difference in the response of the cell layer even though the voltage applied across the cell-covered electrode in both the cases is the same. If the degree of confluency of two cell layers is the same, their response to pulsation is similar. This is illustrated in Fig. 3*b*, where two electrodes of widely different surface areas were used, but the resistance ratio in both cases was approximately the same. Both electrodes were pulsed with 3 volt, 200 millisecond pulses, and in this case, the potential across the cell layer was similar. The slight difference in cell recovery may be due to the fact that while the cell layer represented by the upper curve was two days old, that represented by the lower curve was only one day old. The difference in cell response according to the age of the cell layer has also been observed by Raptis and Firth (14).

For clarity, we have only talked about the measured changes in resistance due to pore formation. As is evident from Eq. 2, very similar changes in the measured reactance take place due to formation of pores. During these experiments, we have tracked the changes in reactance as well, and these measurements support and confirm the conclusions drawn in this paper. Finally, the method can be improved by using an amplifier that has a faster response time and can take data more frequently. This will allow us to look at further details in the formation and resealing of the membrane pores.

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REFERENCES

1. Neumann, E., A. E. Sowers, and C. A. Jordan. 1989. Electroporation and Electrofusion in Cell Biology. Plenum Press, New York. 436 pp.
2. Chang, D. C., B. M. Chassey, J. A. Saunders, and A. E. Sowers. 1992. Guide to Electroporation and Electrofusion. Academic Press, San Diego, CA. 581 pp.
3. Tsong, T. Y. 1991. Electroporation of cell membranes. *Biophys. J.* 60:297–306.
4. Weaver, J. C. 1991. Electroporation. *In* Interfacial Phenomena in Biological systems. M. Bender, editor. Marcel Dekker, Inc., New York. 253–277.
5. Chernomordik, L. A., A. V. Sokolov, and V. G. Budker. 1990. Electrostimulated uptake of DNA by liposomes. *Biochim. Biophys. Acta.* 1024:179–183.
6. Chang, D. C., and T. S. Reese. 1990. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophys. J.* 58:1–12.
7. Chernomordik, L. V., S. I. Sukharev, S. V. Popov, V. F. Pastushenko, A. V. Sokirko, I. G. Abidor, and Y. A. Chizmadzhev. 1987. The electrical breakdown of cell and lipid membranes: the similarity of phenomenologies. *Biochim. Biophys. Acta.* 902:360–373.
8. O'Neill, R. J., and L. Tung. 1991. Cell-attached patch-clamp study of the electropermeabilization of amphibian cardiac cells. *Biophys. J.* 59:1028–1030.
9. Hibino, M., M. Shigemori, H. Itoh, K. Nagayama, and K. Kinosita. 1991. Membrane conductance of an electroporated cell analyzed by submicrosecond imaging of transmembrane potential. *Biophys. J.* 59:209–220.
10. Kinosita, K., and T. Y. Tsong. 1979. Voltage induced conductance in human erythrocyte membranes. *Biochim. Biophys. Acta.* 554:479–497.
11. Pliquett, F., and S. Wunderlich. 1980. Relationship between cell parameters and pulse deformation due to these cells as well as its change after electrically induced membrane breakdown. *Bioelectrochem. Bioenerg.* 10:467–475.
12. Marszalek, P., D.-S. Liu, and T. Y. Tsong. 1990. Schwan equation and transmembrane potential induced by alternating electric field. *Biophys. J.* 58:1053–1058.
13. Zheng, Q., and D. C. Chang. 1991. High-efficiency gene transfection by in-situ electroporation of cultured cells. *Biochim. Biophys. Acta.* 1088:104–110.
14. Raptis, L., and K. L. Firth. 1990. Electroporation of adherent cells in-situ. *DNA and Cell Biology.* 9:615–621.
15. Giaever, I., and C. R. Keese. 1984. Monitoring fibroblast behavior in tissue culture with an applied electric field. *Proc. Natl. Acad. Sci. USA.* 81:3761–3764.
16. Giaever, I., and C. R. Keese. 1986. Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture. *IEEE Trans. on Biomed. Eng.* BME-33:242–247.
17. Holm, R. 1985. Electrical Contacts Handbook. Springer-Verlag, Berlin, Germany. 20–25.
18. Mitra, P., C. R. Keese, and I. Giaever. 1991. Electric measurements can be used to monitor the attachment and spreading of cells in tissue culture. *Biotechniques.* 11:504–510.
19. DeRosa, J. F., and R. B. Beard. 1977. Linear AC electrode polarization impedance at smooth noble metal interfaces. *IEEE Trans. Biomed. Eng.* BME-24:260–268.
20. Giaever, I., and C. R. Keese. 1991. Micromotion of mammalian cells measured electrically. *Proc. Natl. Acad. Sci. USA.* 88:7896–7900.
21. Escande-Geraud, M. L., M. P. Rols, M. A. Dupont, N. Gas, and J. Teissie. 1988. Reversible plasma membrane ultrastructural changes correlated with electropermeabilization in Chinese hamster ovary cells. *Biochim. Biophys. Acta.* 939:247–259.
22. Gaas, G. V., and L. V. Chernomordik. 1990. Reversible large scale deformations in the membranes of electrically treated cells: Electroinduced bleb formation. *Biochim. Biophys. Acta.* 1023:1–11.
23. Giaever, I., and C. R. Keese. 1989. Fractal motion of mammalian cells. *Physica D.* 38:128–133.
24. Dimitrov, D. S., and A. E. Sowers. 1990. Membrane electroporation—fast molecular exchange by electroosmosis. *Biochim. Biophys. Acta.* 1022:381–392.
25. Saulis, G., M. S. Venslaukas, and J. Naktinis. 1991. Kinetics in pore resealing in cell membranes after electroporation. *Bioelectrochem. Bioenerg.* 26:1–13.