A Simple Method for Continuous Measurement of Cell Height during a Volume Change in a Single A6 Cell

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Summary A simple electrophysiological method for continuous monitoring of the height of a single renal cell in culture is described. The procedure consists of placing a patch-electrode on the apical cell surface and monitoring the current deflections during osmotic challenge. The mean increase in cell height by 78% hypotonic solution was 2.0 ± 0.2 μm.

Key words: A6 cell, hypotonic cell swelling, patch-clamp equipment.

It is generally accepted that cell volume regulation is an important process to maintain the cell integrity in all animal cells [1, 2]. Volume in living cells can be estimated by microscopy with differential interference contrast optics for attached cells (gallbladder) [3] and cylindrical renal tubules [4, 5]. Volume for single cells, such as lymphocytes [6], Ehrlich ascites tumor cells [7] and isolated epithelial cells in suspension [8, 9], can be measured by a Coulter counter. Measurement of the cross-sectional area of cells [10], cell height [11], or renal tubule diameter [12] is also useful as an index for the cell volume change. However, with phase-contrast microscopy, it is not feasible to accurately focus on the cell surface of a single and monolayer cell in culture. Roy and Sauvé [13] detected the top and bottom of the cell surface by a microelectrode for measuring the cell height, and Crowe and Wills [11] used fluorescent microbeads as landmarks for the apical and basal cell surfaces. Further, O'Connor et al. [14] measured electrical resistance changes in a chamber which reflects cell height changes during regulatory volume decrease. These methods are useful for measuring cell height changes in a monolayer, however, may not be useful for single cells attached to the culture substrate [11, 13].

In this paper, we describe a simple method for continuous measurement of changes in cell height on a single renal cell in culture using the same equipment as for the patch-clamp technique [15].

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Methods

Cell culture. Cells from the A6 line derived from *Xenopus* kidney were grown according to method previously described by Kawahara and Matsuzaki [16]. Briefly, cells were plated on glass coverslips coated with rat tail collagen type I (Sigma, St. Louis, MO, U.S.A.). The culture medium was Dulbecco’s Modified Eagle Medium diluted 15% (by volume) with filtered water to amphibian osmolality (250 mosmol/kg H$_2$O). The single cells 1–3 d after plating were used for experiments.

Solutions. The isotonic bathing solutions contained (in mM): 90 and 60 NaCl, 3 KCl, 0.5 CaCl$_2$, 1 MgCl$_2$, 10 HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid), 5.5 d-glucose, 1 Na-pyruvate, and 54 and 102 mannitol, respectively (pH was adjusted to 7.60±0.02 by NaOH). The hypotonic solutions had an identical composition except that mannitol was omitted. Osmolality of 90 and 60 mM NaCl solutions was 196 and 138 mosmol/kg H$_2$O, respectively. The hypotonic solution was superfused from a beveled glass micropipette (about 20 μm in diameter) near the cell. As described previously [17], a solution surrounding the cell can be completely replaced with the perfusate.

Patch-clamp technique. Patch-pipettes were fabricated from hematocrit tubes (1.5 mm OD; Terumo, Tokyo) by means of a two-stage puller (pp-83, Narishige, Tokyo) according to the method of Hamill *et al.* [15]. When patch-pipettes were filled with a control bathing solution (90 mM NaCl), their electrical resistances were 12–18 MΩ. Rectangular pulses (10 mV, 2.5 Hz) were injected to the electrode through a patch amplifier (CEZ2200, Nihon Kohden, Tokyo), and the corresponding current deflections were monitored. No polarization was observed when the tip of the electrode was either on or above the cell in the bathing solution. The angle of the patch-electrode was 45°.

Cell height measurement. Using the patch-clamp recording system, it was possible to measure cell height in an isotonic solution and the time-dependence of the cell height changes during hypotonic cell swelling. Measurement of cell height in the isotonic bathing solution was basically identical to the microelectrode measurement described by Roy and Sauvé [13]. Briefly, when a patch-electrode touched the cell surface under phase-contrast microscopy (top), a small change in current deflections was observed. After noting the vertical position of the micro-manipulator (MO303, Narishige, Tokyo), the electrode was stepped aside and lowered beside the cell until it reached the coverslip surface (bottom). The vertical difference between the top and bottom position gave the cell height. Cell height changes during hypotonic cell swelling was measured in the following two ways.

Approach A: When the patch-electrode was further lowered on the cell, a small white dimple appeared under phase-contrast microscopy and position-dependent changes in current deflections were observed. When the electrode was lowered within 3–4 μm from the cell surface, the relationship between the vertical displacement and the change in current deflections was approximately linear (see Fig. 3b). The patch-electrode was again placed on the original cell surface and the
time dependence of the changes of current deflections were recorded during hypotonic cell swelling. It can safely be assumed that pressing the cell surface downward with the electrode and closing the tip of the electrode by a swollen cell are physically identical as regards electrical resistance changes. Changes of the current deflection could be converted to changes in the cell height.

Approach B: The electrode was moved downward and upward from the apparent cell surface before and during hypotonic superfusion (see Fig. 3b). The difference between the first and second slope was determined as the increase in cell height. In the present study, intersections between the horizontal line of current deflections (100%) and the regression lines of the slopes were chosen for the measurements.

All experiments were conducted at room temperature (24–26°C), and solutions were equilibrated with air. Values are expressed as mean±SE and were compared by paired Student’s t-test.

Results and discussion

Microscopic observation of single A6 cell. Figure 1a–i shows phase difference images of a single A6 cell cultured on a glass coverslip. Figure 1d–f and g, h are the images at a fixed focal plane (10 µm above the bottom) during and after the hypotonic superfusion, respectively. Two lines of evidence indicate that the cell height increased during hypotonic superfusion: First, nucleoli in Fig. 1b (6 µm above) (isotonic) was in focus in Fig. 1d–f (10 µm above) (hypotonic). Second, the area of the top of the cell was larger in Fig. 1d–f (hypotonic) than in Fig. 1c (isotonic). The A6 cell swelled and returned to its original volume during and after superfusion with a hypotonic solution, respectively. This suggests that the single A6 cell may be a convenient system for the study of the phenomena that underlie the cell volume regulation. However, measurement of the volume of non-isolated A6 cells seemed to be difficult because their shape was neither cylindrical nor spherical and because their circumference at each level was not clear (except the bottom border) (Fig. 1). The cell volume change may not be computed from either the area at each focal plane [3] or the change in cell diameter on the assumption that isolated single cells are spherical [18].

Electrical measurement of cell height. A patch-clamp electrode and its electrical recording system were used to detect the top of the cell in the present study. Since the radius of the shank (r_s) is much larger (>50 µm) than that of the tip (r_t) (0.6–0.7 µm), the major part of the resistance should be determined by r_t [19]. Therefore, a change in current deflections caused by rectangular pulses through the patch-clamp amplifier indicates a change in opening area of the tip.

While the tip of the electrode was stepped down in the bathing solution toward the cell (minimum step is 0.2 µm), the current deflections were unchanged. When it reached the cell surface, the current deflections promptly decreased (see Fig. 3a). This point should indicate the cell surface, and the difference between the top and bottom of the cell was determined as cell height. The mean cell height was 10.1±
Fig. 1. Phase difference images of a single A6 cell cultured on a glass coverslip. a–c: Images focused within the bottom, and 6 and 10 μm above the bottom, respectively. d–f: Images during 78% hypotonic superfusion (30, 60, and 120 s, respectively) focused 10 μm above the bottom. Arrowhead indicates enlargement of area at this focal plane. Beveled pipette for local superfusion is seen in the top of the figure. g–i: Images during restoration from the hypotonicity (30, 60, and 120 s, respectively). Focuses were 10 μm above (g, h) and within the bottom (i). Solid bar indicates 50 μm.

0.5 μm (n = 25), which was half of that obtained from the monolayer of A6 cells growing on a transparent filter (20 ± 1 μm) [11]. The lower cell height in single A6 cells is not due to the smaller cell size, but probably due to a much wider bottom of the cell (Fig. 1), because single cells were isolated from monolayer cell culture of the same cell line a few days before experiments (see METHODS). When A6 cells were in an isotonic NaCl Ringer's solution, a time-dependent change in cell height was not observed for at least 1 h.

Time course of hypo-osmotic effects on current deflections. Since the current deflection was changed by pressing the pipette to the cell surface, the cell height change may be monitored by the patch-electrode placed on the top of the cell. Figure 2a shows the time course of current deflection change for a typical experiment before, during, and after hypotonicity. The current deflection decreased and reached a plateau within 60 s after hypotonic superfusion. It recovered to
Fig. 2. Change in current deflections induced by constant rectangular pulses (10 mV, 2.5 Hz) during hypo-osmotic superfusion (a patch-electrode was placed on the top of the cell). a: Representative recording of the current deflections, before, during, and after hypotonicity. Note that the deflections are black in a slow time scale (refer to Fig. 3a). b: Decreases in current deflections by 78 and 55% hypotonicity: 10.4 (filled circle) and 26.8% (filled square), respectively.

the control level when the hypotonic superfusion was stopped. In most cases, these changes were reversible and repeatable, and there was little shift of the baseline. Therefore, if hypotonicity-induced cell swelling is in an appropriate range, a time course in the current deflection change may reflect the time course of the cell height change. In some experiments, when the pipette was displaced 2 μm above the cell, the current deflections decreased after a longer delay. However, the time until the maximum change was almost identical to that of the control, and the change in current deflections was smaller than that of the control. This indicates that when the electrode is appropriately placed on an optimal position of the surface, it does not inhibit the time course of cell swelling and may trace changes in cell height. In the present study the electrode was placed 0.5 μm above the position where the first change was recognized on a trace. In all cases, a regulatory volume decrease (RVD) [2] was not observed during hypotonic superfusion for 2–3 min. This result may be consistent with that A6 cells in a monolayer did not show the RVD for 30 min [11], although we do not deny a possibility that the electrode on the cell may inhibit the RVD in the present study. The mean decrease in current deflections was
Fig. 3. Current deflections change by pressing the tip of the electrode against cell membrane. a: The current deflections were decreased and increased by stepping the electrode downward and upward, respectively. b: Relations between displacements of the electrodes and % changes in the current deflections, in control (circles) and during 78% hypotonic superfusion (triangles). Open and filled symbols indicate descending and ascending of the electrodes, respectively.

10.4±1.2 (n = 13) and 26.8±4.5% (n = 8) by 78 and 55% hypotonic superfusion, respectively (Fig. 2b).

Quantification of cell height change. Figure 3a shows the current deflection change caused by downward and upward stepwise displacements of the patch-pipette. Figures on the top indicate the difference from the apparent cell surface (negative, downward; positive, upward). The current deflection decreased in downward steps and returned toward the original level in upward steps. The resolution for detecting the cell height change should be at least 0.5 μm. Figure 3b (circles) illustrates the relationship between displacement of the electrode and % change in current deflections in control. The current deflections changed approximately linearly between −1 and −3 μm from the apparent cell surface. There was no significant difference in slope between the descending and ascending portions of the current-displacement curve. Recovery was 99.1±0.3% of the control (n = 7). These findings suggest that if pressing the tip of the electrode against the cell surface is within an appropriate range, it may not be plugged by the cell membrane, and indicate that the decrease in current deflections probably reflects a decrease in the opening area of the tip. The relationship between displacement of the electrode and % change in current deflections could be used to calibrate the cell height change.
Table 1. Cell height change estimates during hypotonicity-induced cell swelling.

<table>
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<tr>
<th>Number</th>
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<td>Mean±SE</td>
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(Approach A in METHODS). The mean increase in cell height during 78% hypotonic superfusion was 2.1±0.2 \(\mu m\) (\(n=7\)).

Stepping down and up the electrode was also conducted on the same cell during superfusion with a 78% hypotonic solution (Fig. 3b, triangles). The current deflection changed approximately linearly between 0 and ±2 \(\mu m\) from the cell surface. The rightward shift of the slope probably reflects the hypotonicity-induced cell swelling. The difference between the two slopes could be determined as the increase in cell height (Approach B in METHODS). The mean value of the difference was 2.0±0.2 \(\mu m\). Table 1 summarizes the results obtained from the two methods.

Theoretically, if the single A6 cell behaves as an ideal osmometer, the cell volume is expected to increase up to 28% during 78% hypotonic superfusion. In the present study, the % change in cell height was 20.6±2.9, suggesting that the single A6 cell swelled by bulging upward. This result is consistent with those found in renal tubules and in monolayer cell culture during hypotonic superfusion: the cells swell by bulging into the lumen without significant changes in the outer tubular diameter or cell length [4, 5] and increase in cell height [11, 13]. This result also suggests that cells cultured on and attached to a rigid material (glass coverslip coated with collagen in the present study) may swell in the direction of the apical surface with or without space for lateral expansion.

We have described two methods for studying cell volume changes. Their common advantages are their ease of preparation and handling. Cell height changes could be studied using regular patch-clamp equipment. The major advantage of Approach A is the continuous measurement of cell height during a cell volume change. Conversely, the major disadvantage of Approach B and the microelectrode measurement [13] is the discontinuous measurement of changes in cell height. The use of an electromotive manipulator may shorten the time to determine the cell height and improve this disadvantage. Approach A is not useful...
for measurement of cell height during either severe hypotonicity-induced cell swelling or hypertonic cell shrinkage. On the other hand, Approach B and the microelectrode measurement is useful for both cell swelling and cell shrinkage.

In summary, we report a simple technique for continuous measurement of cell height change as an index of cell volume change using a patch-pipette by monitoring current deflections.

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REFERENCES


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