Osmotic Swelling of Hepatocytes Increases Membrane Conductance but Not Membrane Capacitance

Jürg Graf,* Marjan Rupnik,‡ Gregor Zupančič,‡ and Robert Zorec‡

*Department of General and Experimental Pathology, University Hospital, Vienna, Austria, and ‡Laboratory of Neuroendocrinology, Institute of Pathophysiology, School of Medicine, Ljubljana, Slovenia

ABSTRACT We have used the whole-cell patch-clamp technique to study changes in membrane conductance and membrane capacitance after osmotic swelling in rat hepatocytes. Hypoosmotic solutions induced an instantaneous increase in the volume of patch-clamped cells that was followed by a slow decline reminiscent of regulatory volume decrease as seen in intact cells. These morphological changes were associated with a transient increase in membrane conductance. The rise in conductance was not correlated with changes in capacitance, neither in time after the initiation of cell swelling nor in magnitude. Therefore we conclude that an osmotically induced increase in conductance is probably a result of the activation of existent channels in the plasmalemma and not a result of the fusion of vesicle membrane containing ionic channels.

INTRODUCTION

Hepatocytes change their volume after alterations in cell metabolism or membrane transport or when exposed to nonisoosmotic solutions (Graf et al., 1988; Häussinger and Lang, 1991; Boyer et al., 1992; Graf et al., 1993). Cell swelling initiates a mechanism of compensatory cell shrinking (regulatory volume decrease; RVD) by which cells lose the appropriate amount of KCl and water to return toward their regular size. Efflux of K^+ and Cl^- is accomplished by a transient increase of the respective membrane conductances whereby the efflux of Cl^- is additionally supported by membrane hyperpolarization (Haddad et al., 1991; Wehner et al., 1992).

In intact liver, hypoosmotic cell swelling results in typical metabolic alterations (Häussinger and Lang, 1991) and, noteworthy, in an increase in bile flow, bile acid transport capacity, and transcytosis of fluid phase markers into bile (Bruck et al., 1992; Hallbrucker et al., 1992; Häussinger et al., 1992). The increase of these apical transport processes is blunted by microtubule inhibitors (Bruck et al., 1992, Häussinger et al., 1993). In addition, RVD is associated with an increased release of fluid phase markers across the basolateral cell membrane (Graf et al., 1993). These latter observations led to the assumption that increased exocytosis may not only mediate the release of fluid phase markers but could also serve for the incorporation of bile acid transporters into the apical cell membrane. Furthermore, it appeared possible that an insertion of new ion channels by exocytosis could be the mechanism by which membrane K^+ and Cl^- conductances increase during RVD, a mechanism already considered in other systems (Wills et al., 1991; Okada et al., 1992, Zorec and Tester, 1993; Ross et al., 1994).

To test whether the increase in membrane conductance during RVD is mediated by membrane channel insertion through exocytosis, we used the whole-cell patch-clamp technique to measure changes in membrane capacitance (C_m), a parameter related to cell surface area (Neher and Marty, 1982). If the osmotic swelling-induced conductance change was due to the insertion of channels with exocytosis, a correlation between conductance and capacitance was anticipated. Single rat hepatocytes were thus subjected to osmotic swelling by diluting the bath solution. We found that osmotically induced changes in conductance and capacitance are not correlated.

MATERIALS AND METHODS

Cell preparation

Male Louvain rats, 200 to 240 g, were obtained from the Institut für Versuchstierkunde (Himberg, Austria). Hepatocytes were isolated by collagenase perfusion of the isolated organ as previously described (Haddad et al., 1991). Isolated cells were collected in Leibovitz (L-15, Sigma Chemical Co., St. Louis, MO) tissue culture medium, filtered through a 40 μm nylon mesh, sedimented at unity gravity, and resuspended in L-15 medium to obtain $\sim 2 \times 10^6$ cells/ml. Fetal calf serum was added followed by gradual addition of dimethylsulfoxide to obtain concentrations of 20 and 10%, respectively. The cell suspension was frozen in 1-ml aliquots with the IceCube 1610 Computer Freezer (SY-LAB, Purkersdorf, Austria) and was stored under liquid nitrogen until use. Before use, a 1-ml frozen cell suspension was thawed in 50 ml of L-15 culture medium preheated to 37°C. Cells were allowed to sediment for 5 min. After resuspension, cell viability was tested by trypan blue exclusion, which was >90%. Cells were plated onto glass coverslips in petri dishes at 37°C for 1 h. The medium was then changed, and cells that adhered to coverslips were transferred to the microscope stage for electrical recording during the following 6 h. Using optical planimetry (compare Haddad et al., 1991), we could show that the rate of RVD after hypotonic stress was similar in these frozen and thawed cells as compared with freshly isolated cells, but the rate was reduced to ~25% when measured at room temperature (not shown).

Electrophysiological measurements and video imaging

With the whole-cell patch-clamp technique (Hamill et al., 1981), membrane capacitance (C_m) was recorded by using the standard noncompensated method with a two-phase lock-in amplifier (800 or 1600 Hz, 1 mV peak to

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Address reprint requests to Dr. Robert Zorec, Laboratory of Neuroendocrinology, Institute of Pathophysiology, School of Medicine, PO Box 11 61105 Ljubljana, Slovenia. Tel.: 011-386-61-310-841; Fax: 011-386-61-302-272; E-mail: robert.zorec@pafi.mf.uni-lj.si.

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peak) incorporated into a patch-clamp amplifier (SWAM IIA, Ljubljana, Slovenia; see Lindau and Neher, 1988; Zorec et al., 1991a). Borosilicate glass pipettes were pulled by a horizontal puller P-87 (Sutter Instruments, Novato, CA) with 1–4 MΩ, covered with Sylgard (Dow Corning, Midland, MI) and heat polished (Corey and Stevens, 1983). DC current (I_{dc}, low pass, 1–10 Hz, −3 dB), holding potential, and real and imaginary admittance signals (low pass, 1–10 Hz, −3 dB) were acquired (50–100 Hz) and used in calculations. The plots of the passive cell parameters, access conductance (G_a), parallel combination of leak and membrane conductance, and C_m were derived by a computer-aided reconstruction after an analog-to-digital conversion (CED 1401, Cambridge, UK) and an IBM-compatible personal computer (Zorec et al., 1991a). The software was written by Dr. J. Dempster (University of Stratchclyde, Glasgow, UK). Steady-state chord conductance (G_m) was estimated by application of ±10-mV pulses to the holding voltage (see artifacts on I_{dc} trace in Fig. 2). Changes in G_a did not affect the time course of C_m. Current transients induced by square voltage pulses as well as currents evoked by sinusoidal stimulation were readily nullified by the C_{slow} and G_s capacitance cancellation controls of the patch-clamp amplifier, suggesting that the whole-cell recordings of liver cells may be represented by a single time constant analog electrical circuit. Recordings were made at room temperature. Unless stated otherwise, the pipette solution contained 2 mM $MgCl_2$, 10 mM HEPES/KOH, 0.1 mM EGTA (ethyleneglycol-bis-(β-aminoethyl ether)-*N*, *N*, *N'*, *N'*-tetraacetic acid), 140 mM potassium gluconate, 2 mM (Na_2) ATP, pH 7.2 (KOH). Bathing solution was L-15, pH 7.4 (NaOH). With this pipette-filling solution, a liquid junction potential of −10 to −15 mV (potassium gluconate side negative) developed at the tip of the pipette while the pipette was immersed in external saline. The potential values given in the paper have not been corrected for this liquid junction potential. Hypoosmotically induced volume changes were obtained by a bolus addition of 500 μl of H_2O into the bath of 1000 or 2000 μl. This resulted in an abrupt offset in C_m (10 to 500 fF, see Fig. 2), as expected due to a change of stray capacitance of the recording configuration (Zorec et al., 1991b).

In some experiments hypoosmotically induced volume change of a voltage-clamped cell was monitored by a slow scan CCD video camera (Law and O'Brien, 1993) mounted to a Zeiss IM35 inverted microscope with phase-contrast optics (×40) and a Miracal image acquisition system (Life Science Resources, Cambridge, UK). Volume changes are represented as changes in diameters determined from gray level line profiles lined across centers of cell images and assuming cells were perfect spheres. Unless stated otherwise, statistics are in the format mean ± SE. Measurements of chord conductance were not performed at precisely the same time in all experiments. Therefore, interpolated values were used for calculation of mean values, obtained with a cubic spline function (SCoP, Simulation Resources, Berrien Springs, MI). All salts were obtained from Sigma Chemical Co.).

RESULTS

Fig. 1 displays a typical response of a voltage-clamped liver cell exposed to hypoosmotic swelling. Panel *A* shows the appearance of the cell before the application of a bolus of H_2O. Cell diameter increased to a peak value (*B*) and slowly declined as shown on panel *C* at the end of the experiment. Apparent diameter changes are more clearly shown on panel *D*. Gray level pixel line profiles were constructed by drawing a line through the axis of the cell image and perpendicular to the axis of pipette (arrow). Top (Da), middle (Db), and bottom (Dc) profiles were constructed from micrographs *A* to *C*, respectively. Note the increased distance between the two peaks representing the phase contrast ring around the cell, which is consistent with an increased diameter of the cell monitored. Panel *E* shows a time evolution of changes in the apparent cell diameter. These results show that these voltage-clamped cells compensate hypoosmotic swelling by a RVD response.

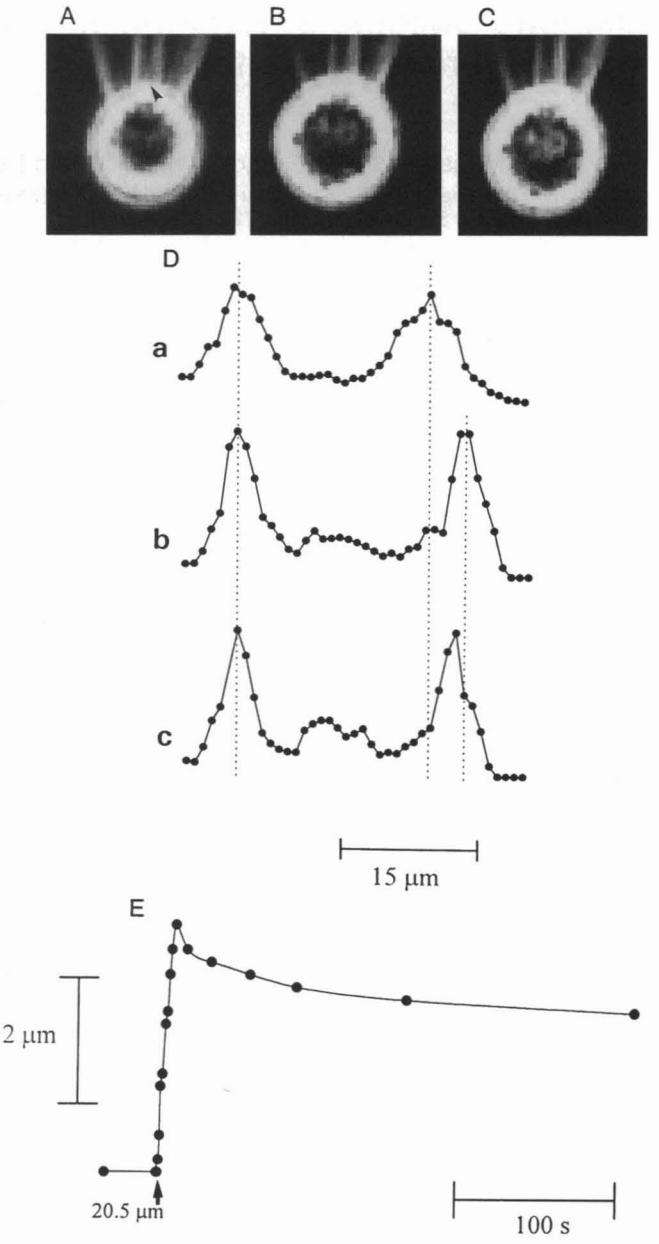

FIGURE 1 Typical morphological response of single patch-clamped hepatocytes exposed to hypoosmotic swelling monitored by a video camera under phase-contrast optics (×40). (*A–C*) A cell image before (*A*) and during (*B*) the peak response and at the end of recording (*C*). (*D*) Changes in apparent cell diameter are represented by gray level pixel profiles obtained through the axis of the cell and perpendicular to the axis of patch pipette; *a–c* are profiles obtained from images *A–C*, respectively. (*E*) A time evolution of changes in the apparent cell diameter after the start of hypotonicity (arrow). Note the slow decline after the peak was attained. Number adjacent to the trace indicates apparent resting cell diameter. Hypotonicity was obtained by dilution of 2 ml of L-15 bathing medium with 1 ml of water.

To investigate whether the osmotically induced changes in volume are correlated with changes in membrane conductance and surface area, cells were clamped to ~0 mV and a dual-phase lock-in amplifier was used to monitor membrane capacitance and conductance. A representative experiment is shown on Fig. 2. The application of extracellular hypoto-

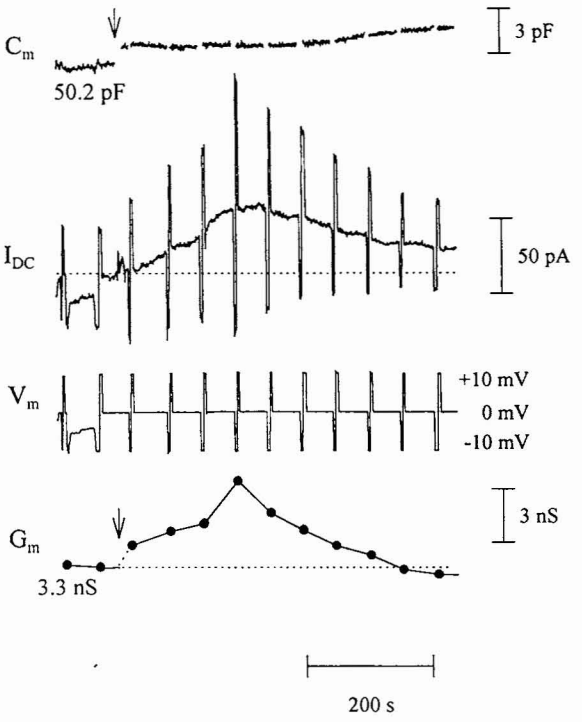

FIGURE 2 Time-dependent changes in membrane capacitance (C_m), DC current (I_{DC}), holding potential (V_m), and chord conductance (G_m), recorded in a hepatocyte exposed to hypoosmotic swelling (arrow adjacent to C_m trace). Resting capacitance is indicated 50.2 pF, and 3.3 nS (adjacent to chord conductance trace) indicates the resting conductance of the cell. Note the offset in C_m during addition of H_2O (arrow), which is due to an alteration of stray capacitance (Zorec et al., 1991b). Note that, before the application of hypotonicity (reduction of osmolarity by one-third; see Fig. 1), membrane potential was changed; thus, the rise in I_{DC} is not a result of a conductance change. Reversal potential was -5 ± 5 mV before application of hypotonicity and -7 ± 4 mV ($n = 6$) at the peak of conductance increase (not corrected for junction potential).

nicity resulted in a transient activation of an outward transmembrane current. The increase in conductance is demonstrated by increased currents driven by ±10-mV voltage pulses (middle trace) and by the bottom trace where the time course of estimated chord conductance (G_m) is shown. These transient changes in membrane conductance are consistent with previous observations (Haddad et al., 1991). The nature of the osmotically induced current was not investigated in detail, but it is consistent with both a potassium and a chloride current, the latter because the reversal potential was −20 to −30 mV, close to the predicted Nernst potential for our experimental conditions, taking into account a junction potential of approximately −10 to −15 mV. Furthermore, a positive shift of the reversal potential was observed after hypotonic swelling if the pipette contained high Cl^- solution (not shown).

Interestingly, the transient response in membrane conductance was not associated with a change in capacitance as would be predicted if changes in conductance were due to insertion of channels into the plasmalemma by exocytosis (Zorec and Tester, 1993). This lack of correlation is more evident during the late phase of the osmotically induced increase in conductance. However, during the rising phase of conductance change, a correlation between the two parameters could not be ruled out. For this we have plotted time-dependent changes in chord conductance and respective values of membrane capacitance recorded in six cells. Fig. 3 summarizes the result, which shows that the osmotically induced increase in membrane conductance is not associated with a change in C_m. These results additionally support the idea that the activation of membrane conductance is not related to an insertion of new channels under these experimental conditions.

DISCUSSION

We have used the patch-clamp technique to study changes in membrane capacitance and conductance of single rat hepatocytes subjected to osmotic swelling. Regulatory volume decrease of hepatocytes was shown previously to involve an increased membrane conductance leading to a subsequent loss of water (e.g., Boyer et al., 1992; Graf et al., 1993). The initiation of this process may be due to a number of cellular events affecting the activity of plasmalemma channels, such as stretch (Morris, 1990; Bear, 1990) or modulation of signaling pathways (Hoffmann and Simonsen, 1989; Pierce and Politis, 1990; Graf et al., 1993). However, an increased membrane conductance could also be a result of an increased insertion of channels by exocytosis (Wills et al., 1991; Okada et al., 1992; Bruck et al., 1992; Hallbrucker et al., 1992; Zorec and Tester, 1993; Ross et al., 1994). To test this hypothesis, we monitored simultaneously changes in membrane conductance and membrane capacitance during osmotic swelling. If an increase in conductance were due to an

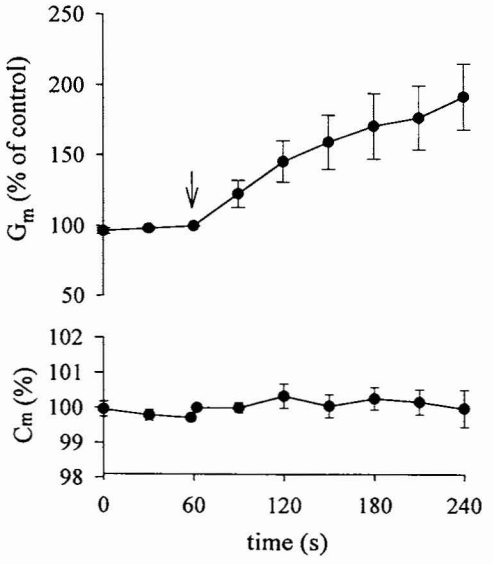

FIGURE 3 Relationship between changes in chord conductance (G_m%) and membrane capacitance (C_m%) relative to resting values in six cells after the start of hypotonicity (arrow). Resting values before osmotic swelling were $G_m = 4.06 \pm 0.76$ nS, and $C_m = 45.8 \pm 3.3$ pF. Access conductance (G_a) was stable during recordings and was 370 ± 47 nS. A small shift in C_m recorded immediately after the application of hypotonicity is a result of a stray capacitance change (see Fig. 2).

insertion of new channels by exocytosis, we would have expected to see a concomitant or a preceding increase in capacitance. The results show that osmotically induced changes in conductance in these cells are not correlated with those in capacitance. Thus, the increased conductance during osmotic swelling is a result of an activation of existent channels in the plasmalemma.

These experiments cannot rule out the possibility of increased membrane turnover in cells that do not respond with an increase in surface area to osmotic swelling. In this, membrane insertion and retrieval could proceed at higher but balanced rates. Specific changes of sorting mechanisms for individual membrane components (channels) during membrane recycling could result in changes in membrane conductance without leading to a change in total surface area.

The time course of hypotonicity-induced volume changes (Fig. 1) and conductance changes (Fig. 3) are quite different. This suggests that a stretch-activated channel may not be the only explanation of coupling a volume disturbance and compensatory membrane fluxes. In agreement with this, we have never observed stretch-activated conductances with pressure changes in the pipette in hepatocyte cell-attached or in whole-cell recordings. However, we have to consider that differences in relative time courses of osmotically induced volume (Fig. 1) and conductance changes (Fig. 3) may be a result of altered properties of patch-clamped and intact hepatocytes. Pipette pressure or dilution/dialysis of intracellular signals may affect responses to hypotonicity.

The delayed rise in membrane capacitance (Fig. 2) after transient hypotonicity-induced conductance change may represent a different phenomenon from incorporation of channels. After hypotonic liver perfusion, there was an increased bile flow and bile acid secretion (interpreted as insertion of bile acid transporters), and increased transcytosis with horseradish peroxidase as a marker had a delayed onset and reached a maximum after ~5 min (Graf et al., 1993).

Estimation of specific capacitance assuming liver cells as spheres resulted in a value of ~3 μF/cm^2 (cell radius, 9–12 μm; total membrane capacitance, ~50 pF; compare Figs. 1 and 2). This value substantially exceeds the value for biological membranes of ~1 μF/cm^2 (Hille, 1992), which suggests that most of the membrane of rat liver cells is folded. This membrane is unfolded during osmotic swelling (Pfaller et al., 1993) to withstand osmotic stress.

Using a similar technique, Ross et al. (1994) have observed a temporal dissociation between osmotically induced changes in chloride conductance and membrane capacitance, suggesting that in Jurkat T lymphocytes the whole-cell chloride conductance during osmotic swelling is provided by volume-sensitive Cl^- channels preexisting in the plasma membrane. Our results presented in this paper are consistent with such an explanation. However, during a rise in membrane capacitance at a late stage of hyperosmotic swelling of lymphocytes Ross et al. (1994) also report a concomitant increase in leak conductance. This may be a result of tears and ruptures that facilitate calcium entry, leading to a subsequent Ca^{2+}-induced rise in membrane capacitance. However, it cannot be ruled out that such a correlation may also be a result of insertion of nonspecific channels, which may additionally facilitate salt and water loss to withstand cell swelling. Correlated changes in conductance and capacitance to hypoosmotic swelling (Okada et al., 1992) and to a rise in hydrostatic pressure (Zorec and Tester, 1993) were recorded in epithelial and plant cells, respectively. These were interpreted to be a result of an insertion of channels into plasmalemma. Our results on hepatocytes clearly differ from the latter ones and it may be that different cell types possess different types of mechanisms to compensate for osmotic swelling. On the other hand, cells may posses both mechanisms, which may be activated at different physiological conditions or at different cell swelling states.

In summary, we have studied whether changes in membrane conductance and capacitance are correlated in intact liver cells subjected to osmotic swelling. Patch-clamped hepatocytes respond under these experimental conditions in a similar way as intact cells. We found a lack of correlation between these two parameters, suggesting that the activation of membrane conductance does not, under our conditions, involve insertion of new channels with exocytosis into plasmalemma.

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