Lack of Threshold for Anisotonic Cell Volume Regulation

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Abstract. Most cells possess mechanisms that are able to detect cellular volume shifts and to signal the initiation of appropriate volume regulatory responses. However, the identity and characteristics of the detecting mechanism remain obscure. In this study, we explored the influence of hypertonic and hypotonic challenges of varying magnitude on the characteristics of the ensuing regulatory volume increase (RVI) and regulatory volume decrease (RVD) of cultured bovine corneal endothelial cells (CBCECs). The main question we asked was whether a threshold of stimulation existed that would unleash a regulatory response. CBCECs (passage 1–3) were seeded on rectangular glass coverslips and grown for 1–2 days. We used a procedure based on detection of light scattering to monitor the transient volume changes of such plated cells when subjected to osmotic challenge. The osmometric responses were asymmetric: cells shrank faster than they swelled (by a factor of 3). Complete volume regulatory responses took 10–12 min. Bumetanide (50 μM) resulted in incomplete (50%) RVI. We found no threshold as the cells examined responded to hypertonic and hypotonic stimuli as low as 1%. There was some gradation as stimuli of <4% resulted in incomplete volume regulation. The degree of activation of the volume responses grew as an exponential buildup with the strength of the anisotonic challenge. We discuss how our observations are consistent with volume sensing mechanisms based on both ionic strength and the cytoskeleton.

Key words: Volume regulation — Regulatory volume increase/decrease — Anisotonic challenge — Threshold — Osmolarity — Corneal endothelium — Light scattering

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Introduction

Volume regulation is a fundamental property of most cells. Signals have been postulated that would trigger volume regulation in cells. The actual volume sensor is not yet known; candidates for it are swelling and shrinkage-induced changes in membrane tension (Wehner et al., 2003), cytoskeletal architecture (Ingber, 1997), cellular ionic strength (Motais, Guizouarn & Garcia Romeu, 1991; Nilius et al., 1998) and the concentration of cytoplasmic macromolecules (Minton, Colclasure & Parker, 1992; Parker, 1993). In this connection, it is not known whether cells respond with volume regulation to any change in volume or whether there is a threshold volume change required to trigger a response. The existence of a 6% threshold volume in *Giardia intestinalis* has been suggested (Park, Edwards & Schofield, 1998). On the other hand, KCl cotransporters (*see* Fig. 3 in Diecke & Beyer-Mears, 1997) and volume-sensitive osmolyte and anion channels (VSOACs) (*see* Fig. 1b in Jackson & Strange, 1993) respond to volume changes in a continuous fashion, suggesting there is no threshold.

The issue may stem from the difficulties inherent to the temporal detection of small cell volume changes. However, a technique we have developed based on light scattering (Fischbarg et al., 1989, 1993) has a sensitivity which is apparently higher than that of other methods. We have already used it to characterize volume regulatory responses in cultured bovine corneal endothelial cells (CBCECs) (Hara et al., 1999); in the current study, we quantified volume regulatory responses in more detail, utilizing challenges ranging 1–10% of isotonic solutions. We found that (1) partial volume regulation is observed with stimuli as small as 1%, (2) stimuli of 4% and higher are required for complete volume regulation to take place and (3) the osmometric response to hypotonic
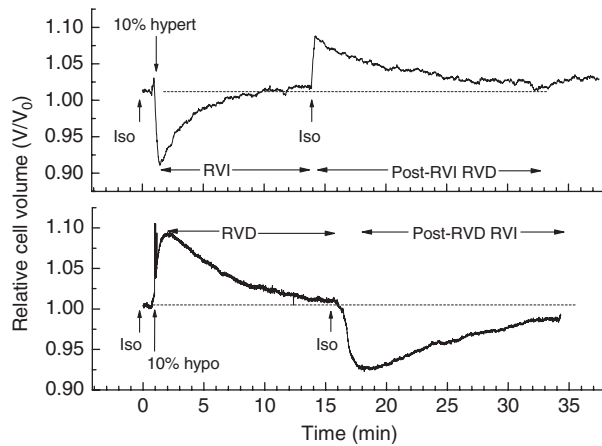
Fig. 1. Representative examples of (*top*) RVI and (*bottom*) RVD of CBCECs after 10% anisotonic challenge.

stimuli is slower than that to hypertonic stimuli. Our results suggest that there is no volume threshold as such.

Materials and Methods

CBCECs were cultured as previously reported (Narula et al., 1992), in Dulbecco's modified Eagle medium (GIBCO, Grand Island, NY) containing 6% fetal bovine serum, 100 U/ml of penicillin and 100 µg/ml of streptomycin (GIBCO), 2 ng/ml of basic fibroblast growth factor (Sigma, St. Louis, MO) at 37°C in a 5% CO_2/95% air mixture. After confluence, cells from passages 1–3 were used for volume regulation studies. Cells were plated onto 11×22 mm rectangular coverglasses (Thomas Scientific, Swedesboro, NJ) and grown for 1–2 days in the culture medium above. The coverslips would be used when the cell density was about 80–90% of confluence. The technique using scattered light intensity (Is) to monitor cell volume change has been described previously (Echevarria et al., 1993; Fischbarg et al., 1989, 1993). Briefly, rectangular coverslips affixed to plastic holders were inserted in a round glass vial (Fisher Scientific, Fair Lawn, NJ); this vial formed a perfusion chamber held at 37°C. Is was monitored with a photomultiplier; it corresponded to cell volume according to an expression previously described (Fischbarg et al., 1993). Is was determined in arbitrary units (mV); all experiments were performed with the photomultiplier at the same gain. For this study, the control perfusion solution was bicarbonate 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) Ringer's solution containing (in mM) NaCl 107.9, NaHCO$_3$ 37.0, KCl 4.7, NaH$_2$PO$_4$ 1.0, MgSO$_4$ (7 H$_2$O) 0.4, CaCl$_2$ (2 H$_2$O) 1.8, glucose 5.6, HEPES Na 10.0 and three factors found essential for regulatory volume decrease (RVD) (De Smet, Li & van Driessche, 1998), namely, 5 µg/l insulin, 5 µg/l transferrin and 5 ng/l sodium selenite. For osmotic challenge, the monolayer of CBCECs on the coverslip bathed in control isotonic medium was challenged suddenly by hypertonic or hypotonic solution (concentrations ±10%, 5%, 4%, 3%, 2% or 1% of control isotonic medium) obtained by varying the [NaCl]. Each challenge was followed by a return to isotonic solution, in which cells were allowed to recover for ~0.5 h. In some experiments, the perfusion solutions contained 50 µM bumetanide (using dimethyl sulfoxide as solvent, at a final concentration of 0.001 v/v). Solutions were prepared just before the experiments.

The maximum displacement in light intensity induced by a 10% change in osmolarity was arbitrarily equated to a 10% relative volume displacement, as shown in the y coordinates of the figures. Since the volume change results from two simultaneous processes, osmometric and regulatory, we used a double exponential fitting function to obtain the rates for both processes (Hara et al., 1999; Iserovich et al., 1998; Wu et al., 1997):

$$V_t = V_0 + \left\{ A_1 * [1 - exp(-t/\tau_{osm})] \right\} + \left\{ A_2 * [1 - exp(-t/\tau_{vr})] \right\} \quad (1)$$

Where V_t is volume as a function of time, V_0 volume at zero time, A_1 the signed amplitude of the osmometric response, A_2 the signed amplitude of the regulatory volume response, τ_{osm} the time constant for the osmotic response and τ_{vr} the time constant for RVD or regulatory volume increase (RVI). In this context, the volume recovery index (ρ) is the ratio:

$$\rho = [A_2]/[A_1] \quad (2)$$

It may be noted that the correct volume displacement in all cases can be calculated by equating the osmometric displacement (A1) to the osmometric displacement expected using:

$$V_i \, C_i = V_f \, C_f \quad (3)$$

A common expression linking initial and final volumes and osmolarities. From this, Vf is 1.11 and 0.909 of Vi for 10% hypotonic and hypertonic challenges, respectively.

Results

Figure 1 shows some typical responses: RVI (top panel) and RVD (bottom panel) after 10% hypertonic or hypotonic challenges and post-RVI RVD (top panel) and post-RVD RVI (bottom panel) after return to isotonic solution in each case. Responses to osmotic challenge were complete within 10–12 min. After RVI or RVD recovery, cells were perfused with isotonic solution, which resulted in postregulatory complementary volume changes (Fig. 1). These responses took longer (15–20 min).

REPRODUCIBILITY

In a typical experiment, after a given cycle of regulatory and postregulatory responses, cells were allowed to recover in isotonic solution, after which they could be challenged with another anisotonic solution. Cells proved resilient: six cycles of challenges followed by regulation and recovery could be elicited without significant changes in the magnitude and time course of the responses. In addition, the responses were reproducible from one experiment to another.

REGULATION AS A FUNCTION OF THE CHALLENGE

The reproducibility of the data was taken advantage of to explore the characteristics of the responses in more detail by using up to six sequential hypertonic
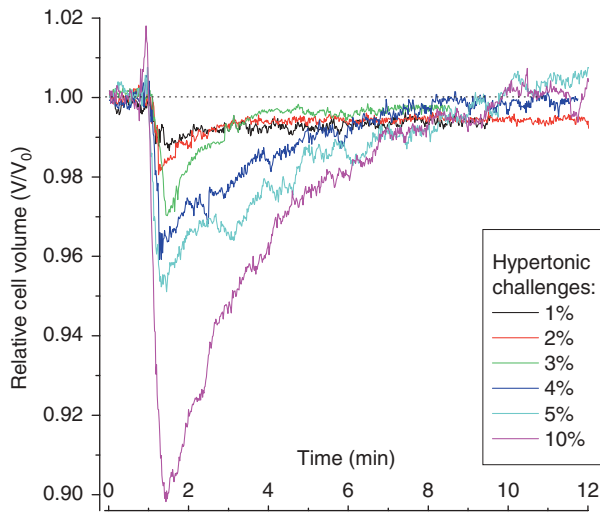
Fig. 2. Representative experiment showing volume responses obtained sequentially in a CBCEC monolayer to six different challenges with hypertonic solutions (1%, 2%, 3%, 4%, 5%, 10%). Challenges were applied at the 1-min mark. After each challenge, cells were returned to isotonic solution (298 mOsm).
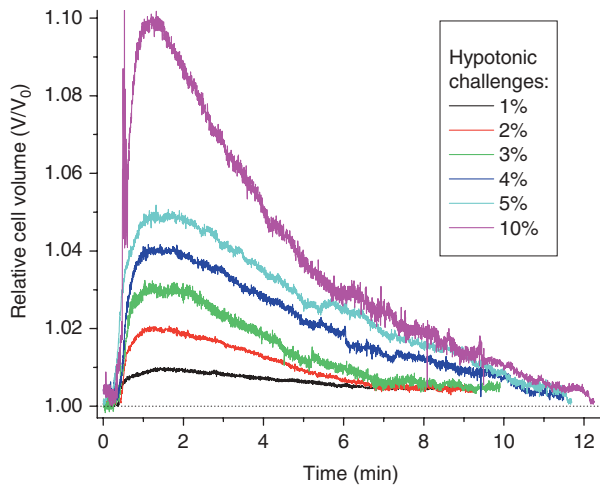

Fig. 3. As in Figure 2, except that the challenges were with hypotonic solutions (1%, 2%, 3%, 4%, 5%, 10%).

Fig. 4. (*A*) Examples of exponential association fits to experimental data obtained after 10% hypertonic (*top*) and hypotonic (*bottom*) challenges. Hypertonic challenge fit: $\chi^2 = 8 \times 10^{-6}$, $R^2 = 0.99$. Hypotonic challenge fit: $\chi^2 = 2 \times 10^{-5}$, $R^2 = 0.98$. (*B*) Time constants for osmometric and regulatory volume changes.

or hypotonic challenges of varying magnitude, ranging 1–10% of the isotonic concentration in the same preparation of plated cells. Usually, the progression went from lower to higher osmotic challenges; in some experiments, the order was inverted, with no appreciable difference in the results. The results of this series constitute the main findings and are given in Figures 2 and 3. As expected, the initial volume displacement after challenge is approximately osmometric. After that, in all cases, volume regulation ensued (Figs. 2 and 3). In other words, every challenge of 1% or more was followed by an
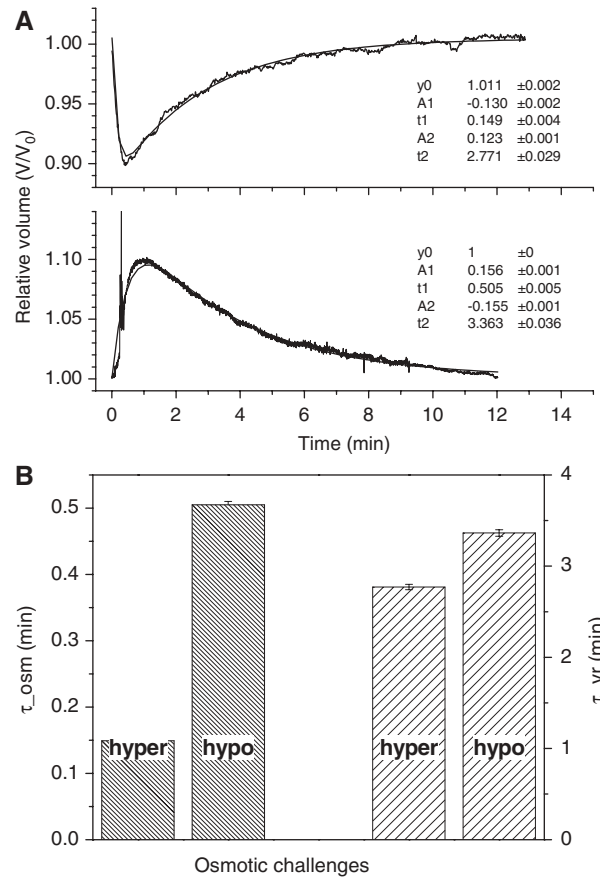
osmometric displacement and a volume regulatory response. However, interestingly, nominally complete volume regulation took place only after challenges of 4% or more (Figs. 2 and 3). Challenges of 1–3% resulted in incomplete volume recoveries, as discussed in more detail below.

QUANTITATION OF OSMOMETRIC AND REGULATORY RESPONSES

Figure 4 shows examples of fits to experimental data for RVI and RVD responses (top and bottom panels, respectively) using the fitting procedure described in Materials and Methods. The values of the fitting parameters y_o, A_1, A_2, τ_{osm} and τ_{vr} are given in the inserts; the time constants are shown separately in Figure 4B. Interestingly, the τ_{osm} for hypertonic challenge is significantly smaller than that for hypotonic challenge (by a factor of 3). In other words, cells shrink faster than they swell. Moreover, as can be seen in Figures 2 and 3, such a difference in the time course
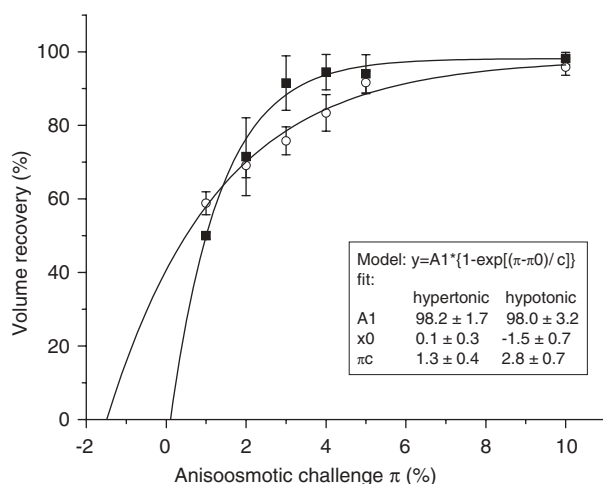
Fig. 5. Cell volume recovery as a function of the varying hypertonic and hypotonic challenges. *Lines*, exponential buildup fits to the data; *solid squares*, hypertonic; *open circles*, hypotonic. Deviations are standard error of the mean.
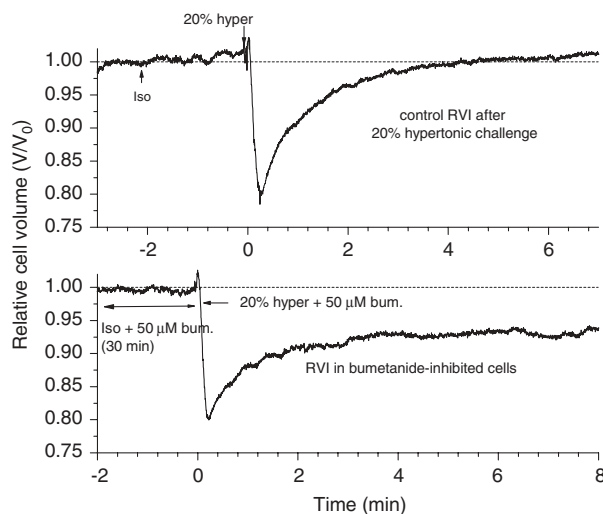

Fig. 6. The effects of 50 μM bumetanide (bum) on CBCEC RVI: two separate hypertonic challenges, without (*top*) or with (*bottom*) bum, were performed on the same CBCEC monolayer.

of osmometric responses is present regardless of the magnitude of the challenge. On the other hand, the time constants for volume regulation, τ_{vr}, do not appear significantly different.

As mentioned in Materials and Methods, the volume regulatory recovery index (ρ) can be obtained from the ratio of the magnitude of the regulatory response (A_2) and osmometric response (A_1). Figure 5 shows the recovery index for a series of different concentrations of hypertonic or hypotonic challenges (1%, 2%, 3%, 4%, 5% and 10%). To begin with, the value of the challenge influenced the extent of recovery. Moreover, strikingly, the values could be fit with simple exponential buildup functions both for
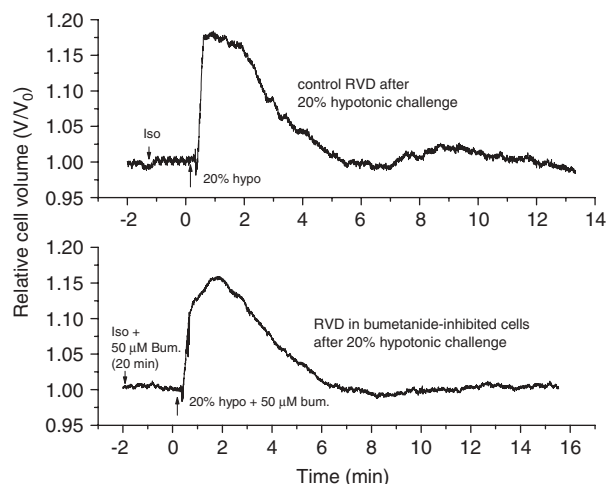

Fig. 7. The effects of 50 μM bumetanide (bum) on CBCEC RVD: two separate hypotonic challenges, without (*top*) or with (*bottom*) bum, were performed on the same CBCEC monolayer.

RVI and for RVD, and the fits closely approximate each other (Fig. 5). In both cases, the extrapolated curve passes through $y = 0$ at x values of 0 or less and not significantly different from each other. This suggests the absence of a threshold.

EXTENT OF RECOVERY AND TRANSPORT INHIBITION

We also examined the effects of bumetanide on CBCEC volume recovery after anisotonic challenge. In these experiments, CBCECs were first exposed to 20% anisotonic challenge as a control. We previously demonstrated that under such conditions the extent of RVI was $101 \pm 2\%$ and RVD was $98 \pm 3\%$ (Hara et al., 1999). After the control responses had been obtained, cells were preincubated with 50 μM bumetanide for 20 min and then exposed to a new anisotonic challenge with the inhibitor present. Figure 6 (top panel) shows full RVI recovery after exposure to 20% hypertonic solution but only partial (50%) recovery in the presence of the inhibitor (bottom panel). Figure 7 shows that there is no effect of 50 μM bumetanide on RVD.

Discussion

METHOD SENSITIVITY

There appear to be methodological advantages to the use of light scattering, as done in this study. Methods used in the past to quantify cell volume and its changes generally begin to detect volume responses only for osmotic challenges of 2% or larger. Thus, methods based on the use of radioisotopes detect responses for challenges of ~4% (Diecke & Beyer-Mears, 1997) or 5% (Parker, 1993). A method based

on the fluorescence intensity of calcein trapped in cells generates records of cell volume with an error band estimated at 3% (Hamann et al., 2005). A method based on the electrical resistance of a confined channel in contact with cells (O'Connor et al., 1993) can be estimated to detect cell volume changes in response to a 2% osmolarity shift. In contrast, the method utilized here (cf. Figs. 2 and 3) exhibits an error band of only 0.001–0.003 of the volume.

COMPARISON WITH PRIOR RESULTS

Volume changes detected by light scattering after anisotonic challenge of corneal endothelium have been previously determined (Hara et al., 1999; Srinivas et al., 2003). The RVI and RVD were found incomplete by Srinivas et al. (2003) and, instead, complete by Hara et al. (1999) and in the current results. A possible explanation for this discrepancy is that the media used by Srinivas et al. were nominally HCO_3^--free. That may have affected transporters or channels involved in volume regulation; for instance, also in corneal endothelium, absence of HCO_3^- resulted in a ~50% reduction of Na^+-K^+-2Cl- cotransporter activity (Diecke et al., 1998). Another difference in conditions was that the solutions in the Hara et al. and the current studies contained the factors essential for RVD described by De Smet et al. (1998), while those of Srinivas et al. apparently did not.

ASYMMETRY OF OSMOMETRIC RESPONSES

As mentioned in the Results section, these cells shrink faster than they swell (by a factor of 3). A similar finding is mentioned in passing in a prior paper on astrocytes (O'Connor et al., 1993). In the simplest of terms, the elastic elements of the cytoskeleton might resist volume expansion.

THRESHOLD FOR VOLUME REGULATION?

It is known that most cells exhibit volume regulatory responses when their water content is perturbed, as by exposure to an anisotonic environment (Parker, 1993). Cells also respond by volume regulation to accumulation or depletion of metabolites in the cytoplasm, as might occur during absorption and secretion (Foskett & Melvin, 1989; Haussinger & Lang, 1991). However, whether there has to be a displacement of cell volume of more than a given threshold value to trigger a regulatory response is not clear from the literature. In contrast, our results, detailed in Figures 2 and 3 and summarized in Figure 5, suggest that CBCECs respond to anisotonic stimuli without a threshold. Small anisotonic challenges elicit incomplete regulatory responses, which increase with osmolarity. Moreover, with stimuli >4% of the control osmolarity, volume regulation is

complete; that is, it has a volume regulatory index of 1 (equation 2). These results are consistent with data which show that volume regulatory transport mechanisms, such as VSOACs (Jackson & Strange, 1993), the K^+-Cl^- cotransporter (Diecke & Beyer-Mears, 1997) and the Na^+-K^+-2Cl$^-$ cotransporter (Diecke et al., 1998), are partially activated at ambient osmolarity and can be further activated by hypotonic solutions or inactivated by hypertonic solutions. Moreover, it has been shown that the VSOAC appears to be regulated by ionic strength and does not respond to swelling when ionic strength is maintained constant (Guizouarn & Motais, 1999; Motais et al., 1991; Nilius et al., 1998). It is conceivable that any change in ionic strength could affect a kinase, possibly a tyrosine kinase (Nilius et al., 1998) or Ste20-type kinases (Strange, Denton & Nehrke, 2006), which in turn would modulate the volume regulatory transport mechanism. In contrast, the KCl cotransporter is activated by cell swelling at constant ionic strength (Guizouarn & Motais, 1999). This would require a different mechanism for activation, such as molecular crowding or cytoskeletal distortion. Both sensing mechanisms, ionic strength and molecular crowding/cytoskeleton, are consistent with the lack of threshold described here.

As discussed above, most of the apparent threshold values present in the literature actually depend on the sensitivity of the recording method. In contradistinction, Park et al. (1998) working with *Giardia intestinalis* extrapolated a threshold of 6% for volume regulation by plotting the volume-related uptake of 2-aminoisobutyric acid against a range of different anisotonic challenges. However, as no challenges of <6% were used, the possibility of incomplete regulatory responses below that level was not examined.

EXTENT OF VOLUME REGULATION

From our data, bumetanide, a specific inhibitor of Na^+-K^+-2Cl$^-$ cotransport, only affects CBCEC RVI but not RVD. Bumetanide reduces the rate of RVI and results in an incomplete (50%) regulatory response. This can be explained if it is understood that K^+ gain is the ideal mechanism for RVI as the cell volume increases, but there is no activation of the Na^+-K^+-ATPase. Inhibition of the Na^+-K^+-2Cl$^-$ cotransporter of course negates this. In addition, aside from the Na^+-K^+-2Cl$^-$ cotransporter, there are two other mechanisms that can contribute to RVI in these cells: Na^+-H^+ exchangers (Bonanno & Giasson, 1992; Jentsch et al., 1985) and epithelial Na^+ channels (Kuang, Cragoe & Fischbarg, 1993; Rauz et al., 2003). Hence, a possible explanation for the incomplete regulatory response may be that after bumetanide inhibits Na^+-K^+-2Cl$^-$ cotransport, both remaining mechanisms tend to elevate the

intracellular Na^+ concentration and consequently tend to activate Na^+-K^+-ATPase. However, this ATPase transports Na^+ out of the cell and, thus, contributes to an RVD that counterbalances Na^+ uptake and leads to incomplete RVI. A similar incomplete volume regulatory response in the presence of p-chloromercuribenzoate has been observed in *G. intestinalis* (Park et al., 1998).

Since all stimuli tested here including the smallest one of 1% elicited regulatory responses, in this sense, there does not seem to be a "threshold" challenge for RVI or RVD. In this connection, when isolated proximal tubules (Lohr & Grantham, 1986) were subjected to slow, progressive osmolarity changes (1.5 mmol/min), cell volume remained constant for the range 167–361 mOsm. This suggests that no threshold was present in that case either and that volume regulatory mechanisms operated throughout the manipulation, driven by minute but continuous changes in volume and osmolarity. These observations are consistent with the possibility that any variation in volume activates cellular processes involved in volume regulatory responses. Interestingly, according to the data of Figure 5, the degree of activation grows as an exponential buildup function with the strength of the stimulus. The finding that all stimuli result in responses and the characterization of classes of responses (incomplete or complete) constitute novelties, as is the description of the degree of activation in terms of an exponential buildup.

References

Bonanno, J., Giasson, C. 1992. Intracellular pH regulation in fresh and cultured bovine corneal endothelium. I. Na^+/H^+ exchange in the absence and presence of HCO_3^-. *Invest. Ophthalmol. Vis. Sci.* **33:**3058–3067

De Smet, P., Li, J., Van Driessche, W. 1998. Hypotonicity activates a lanthanide-sensitive pathway for K^+ release in A6 epithelia. *Am. J. Physiol.* **275:**C189–C199

Diecke, F.P., Zhu, Z., Kang, F., Kuang, K., Fischbarg, J. 1998. Sodium, potassium, two chloride cotransport in corneal endothelium: Characterization and possible role in volume regulation and fluid transport. *Invest. Ophthalmol. Vis. Sci.* **39:**104–110

Diecke, F.P.J., Beyer-Mears, A. 1997. A mechanism for regulatory volume decrease in cultured lens epithelial cells. *Curr. Eye Res.* **16:**279–288

Echevarria, M., Kuang, K., Iserovich, P., Li, J., Preston, G.M., Agre, P., Fischbarg, J. 1993. Cultured bovine corneal endothelial cells express CHIP28 water channels. *Am. J. Physiol.* **265:**C1349–C1355

Fischbarg, J., Kuang, K., Hirsch, J., Lecuona, S., Rogozinski, L., Silverstein, S.C., Loike, J.D. 1989. Evidence that glucose transporters serve as water channels in J774 macrophages. *Proc. Natl. Acad. Sci. USA* **86:**8397–8401

Fischbarg, J., Li, J., Kuang, K., Echevarri, M., Iserovich, P. 1993. Determination of volume and water permeability of plated cells from measurements of light scattering. *Am. J. Physiol.* **265:**C1412–C1423

Foskett, J.K., Melvin, J.E. 1989. Activation of salivary secretion: Coupling of cell volume and $[Ca^{2+}]_i$ in single cells [published erratum appears in *Science* 245:343]. *Science* **244:**1582–1585

Guizouarn, H., Motais, R. 1999. Swelling activation of transport pathways in erythrocytes: Effects of Cl^-, ionic strength, and volume changes. *Am. J. Physiol.* **276:**C210–C220

Hamann S., Herrera-Perez J.J., Bundgaard M., Alvarez-Leefmans F.J., Zeuthen T. 2005. Water permeability of Na^+-K^+-$2Cl^-$ cotransporters. *J. Physiol.* 586: 123–135

Hara, E., Reinach, P.S., Wen, Q., Iserovich, P., Fischbarg, J. 1999. Fluoxetine inhibits K^+ transport pathways (K^+ efflux, Na^+-K^+-$2Cl^-$ cotransport, and Na^+ pump) underlying volume regulation in corneal endothelial cells. *J. Membr. Biol.* **171:**75–85

Haussinger, D., Lang, F. 1991. The mutual interaction between cell volume and cell function: a new principle of metabolic regulation. *Biochem. Cell Biol.* **69:**1–4

Ingber, D.E. 1997. Tensegrity: The architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59:**575–599

Iserovich, P., Reinach, P.S., Yang, H., Fischbarg, J. 1998. A novel approach to resolve cellular volume responses to an anisotonic challenge. *Adv. Exp. Biol. Med.* **438:**687–692

Jackson, P.S., Strange, K. 1993. Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. *Am. J. Physiol.* **265:**C1489–C1500

Jentsch, T.J., Stahlknecht, T.R., Hollwede, H., Fischer, D.G., Keller, S.K., Wiederholt, M. 1985. A bicarbonate-dependent process inhibitable by disulfonic stilbenes and a Na^+/H^+ exchange mediate $^{22}Na^+$ uptake into cultured bovine corneal endothelium. *J. Biol. Chem.* **260:**795–801

Kuang K., Cragoe E.J., Fischbarg J. 1993. Fluid transport and electroosmosis across corneal endothelium. In: H.H. Ussing, J. Fischbarg, E. Sten Knudsen, E. Hviid Larsen, N.J. Willumsen, editors. Proceedings of the Alfred Benzon Symposium 34, "Water Transport in Leaky Epithelia", pp 69–79. Munksgaard, Copenhagen

Lohr, J., Grantham, J. 1986. Isovolumetric regulation of isolated S2 proximal tubules in anisotonic media. *J. Clin. Invest.* **78:**1165–1172

Minton, A.P., Colclasure, G.C., Parker, J.C. 1992. Model for the role of macromolecular crowding in regulation of cellular volume. *Proc. Natl. Acad. Sci. USA* **89:**10504–10506

Motais, R., Guizouarn, H., Garcia Romeu, F. 1991. Red cell volume regulation: The pivotal role of ionic strength in controlling swelling-dependent transport systems. *Biochim. Biophys. Acta* **1075:**169–180

Narula, P.M., Xu, M., Kuang, K., Akiyama, R., Fischbarg, J. 1992. Fluid transport across cultured bovine corneal endothelial cell monolayers. *Am. J. Physiol.* **262:**C98–C103

Nilius, B., Prenen, J., Voets, T., Eggermont, J., Droogmans, G. 1998. Activation of volume-regulated chloride currents by reduction of intracellular ionic strength in bovine endothelial cells. *J. Physiol.* **506(pt2):**353–361

O'Connor, E.R., Kimelberg, H.K., Keese, C.R., Giaever, I. 1993. Electrical resistance method for measuring volume changes in monolayer cultures applied to primary astrocyte cultures. *Am. J. Physiol.* **264:**C471–C478

Park, J.H., Edwards, M.R., Schofield, P.J. 1998. Swelling detection for volume regulation in the primitive eukaryote *Giardia intestinalis*: A common feature of volume detection in present-day eukaryotes. *FASEB J.* **12:**571–579

Parker, J.C. 1993. In defense of cell volume? Am. *J. Physiol.* **265(pt1):**C1191–C1200

Rauz, S., Walker, E.A., Murray, P.I., Stewart, P.M. 2003. Expression and distribution of the serum and glucocorticoid regulated kinase and the epithelial sodium channel subunits in the human cornea. *Exp. Eye Res.* **77:**101–108

Srinivas, S.P., Bonanno, J.A., Lariviere, E., Jans, D., Van Driessche, W. 2003. Measurement of rapid changes in cell volume by forward light scattering. *Pfluegers Arch.* **447:**97–108

Strange, K., Denton, J., Nehrke, K. 2006. Ste20-type kinases: Evolutionarily conserved regulators of ion transport and cell volume. *Physiology (Bethesda)* **21:**61–68

Wehner, F., Olsen, H., Tinel, H., Kinne-Saffran, E., Kinne, R.K. 2003. Cell volume regulation: Osmolytes, osmolyte transport, and signal transduction. *Rev. Physiol. Biochem. Pharmacol.* **148:**1–80

Wu, X., Yang, H., Iserovich, P., Fischbarg, J., Reinach, P.S. 1997. Regulatory volume decrease by SV40-transformed rabbit corneal epithelial cells requires ryanodine-sensitive Ca^{2+} induced Ca^{2+} release. *J. Membr. Biol.* **158:**127–136