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Regulatory Volume Increase in Rat Lacrimal Gland Acinar Cells

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Abstract. The volume of acinar cells isolated from rat lacrimal glands was measured during hypertonic shock. Cells shrank in hypertonic solutions, but a regulatory volume increase (RVI) was only observed under certain conditions. In HEPES-buffered solutions at 37°C, an RVI was observed. This was inhibited by 20 μM bumetanide, an inhibitor of Na^+-K^+-$2Cl^-$ cotransport. RVI did not occur in HEPES-buffered solutions at 20°C suggesting that Na^+-K^+-$2Cl^-$ cotransport is inactive at this temperature. In HCO_3^- buffered solutions however, an RVI was observed at 20°C. In these conditions, the RVI was inhibited by 500 μM 4,4′-diisothiocyanatodihydrostilbene-2,2′-disulfonic acid (H_2-DIDS) and 10 μM 5-(*N*-methyl-*N*-isobutyl)-amiloride (MIBA) indicating the involvement of Cl^--HCO_3^- exchange and Na^+-H^+ exchange respectively. RVI was also supported by a mixture of neutral amino acids, and by the nonmetabolizable amino acids 5 mM α-(methylamino)isobutyric acid (MeAIB) and 5 mM α-aminoisobutyric acid (AIB). These data suggest that the accumulation of amino acids, possibly by the system A Na^+-coupled amino acid cotransporter, contributes to RVI in these cells. In conclusion, rat lacrimal gland acinar cells are capable of undergoing RVI following shrinkage by hypertonic shock.

Key words: Exocrine gland — Na^+-K^+-$2Cl^-$ cotransport — Na^+-H^+ exchange — Cl^--HCO_3^- exchange — Na^+-amino acid cotransport

Introduction

Lacrimal gland acinar cells exhibit a regulatory volume decrease (RVD) when placed in hypotonic solutions (Park et al., 1994). RVD is thought to be the result of K^+ and Cl^- loss from the cells due to the activation of Ca^{2+}-activated K^+ (Park et al., 1994) and Ca^{2+}-activated Cl^- channels (Kotera & Brown, 1993). These channels appear to have a dual function in that they also have an important role in the secretory process in these cells (Petersen, 1992). Other ion transport pathways in the lacrimal gland acinar cells may also function both in secretion and volume regulation. The accumulation of Cl^- across the basolateral membrane is an important step in the secretion of Cl^-. Na^+-K^+-$2Cl^-$ cotransport, and a combination of Na^+-H^+ and Cl^--HCO_3^- exchange are thought to be responsible for Cl^- accumulation (Saito et al., 1987). In many cells, these transporters also have an important role in cell volume regulation, i.e., they are activated by cell shrinkage and participate in regulatory volume increase (RVI; Chamberlin & Strange, 1989; Hoffman & Simonsen, 1989). However, it is not known if these transporters are activated by shrinkage in lacrimal gland cells, or indeed whether these cells are capable of RVI. In a previous study (Park et al., 1994) we showed that after undergoing an RVD in hypotonic solutions, lacrimal gland cells do not regulate their volume when returned to isotonic solutions (i.e., they did not exhibit post-RVD RVI).

In the present study, we have examined the effects of hypertonic solutions on lacrimal gland acinar cell volume. The results show that these cells exhibit an RVI. Na^+-K^+-$2Cl^-$ cotransport, Na^+-H^+ exchange and Cl^--HCO_3^- exchange all appear to contribute to this process. Furthermore, we have observed that amino acid transport, probably by System A, also contributes to RVI. Preliminary accounts of this work have been published in abstract form (Douglas & Brown, 1995 *a,b*).

Materials and Methods

Cell Preparation

Single lacrimal gland acinar cells were prepared as described by Kotera & Brown (1993). Small pieces of gland were incubated with trypsin

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Table 1. Composition of experimental solutions

	Isotonic	Hypertonic	Isotonic/ HCO_3^-	Hypertonic/ HCO_3^-	Hypotonic	Low Cl^- hypotonic
NaCl	140	140	118	118	90	
Na-glucuronate						90
KCl	5	5	5	5	5	5
$CaCl_2$	1	1	1	1	1	1
$MgCl_2$	1	1	1	1	1	1
HEPES	5	5			5	5
Glucose	5	5	5	5	5	5
$NaHCO_3$			22	22		
Mannitol		100		100		
Osmolality (mOsm/Kg)	280	379	280	378	198	196

Values are mM unless otherwise stated. HEPES-buffered solutions were gassed with 100% O_2 and HCO_3^--buffered solutions were gassed with 3% CO_2/97% O_2 at room temperature and 5% CO_2/97% O_2 at 37°C. In some experiments, amino acids were added to these solutions including: a mixture of neutral L-amino acids (MEM amino acids; Sigma), 5 mM aminoisobutyric acid (AIB; Sigma), 5 mM methyl-aminoisobutyric acid (Me AIB; Sigma), 5 mM L-cysteine (Sigma) and 5 mM 2-amino-2-norbornane-carboxylic acid (BCH; Sigma). The presence of these compounds caused only a slight increase in solution osmolality 379–383 $mOsm.Kg^{-1}$. The mixture of amino acids contained (mM): arginine, 0.6; cystine, 0.8; histidine, 0.2; isoleucine, 0.4; leucine, 0.4; lysine, 0.4; methionine, 0.1; phenylalanine, 0.2; threonine, 0.4; tryptophan, 0.1; tyrosine, 0.2; valine, 0.4.

and collagenase (Sigma). The tissue was then dissociated by repeated pipetting. The resultant single acinar cells were resuspended in 1 ml of Medium 199 (Sigma), and stored in an incubator for up to 2 hr at 37°C gassed with 5% CO_2.

Cell Volume Measurements

Glass coverslips (2 × 4 mm washed in acetone and ethanol) were placed on the bottom of a 35-mm culture dish, and covered with Medium 199 HEPES (Sigma). Six drops of the cell suspension (~50 μl) were added to the culture dish directly above each coverslip. The dishes were then placed in the incubator for 1.5 hr to allow the cells to adhere loosely to the coverslips. A single coverslip was then placed in a transparent experimental chamber (volume = 50 μl), on the stage of an inverted microscope (World Precision Instruments). The cells were superfused with solutions at a rate of 3 mls/min; the composition of the solutions used is shown in Table 1. Cells were exposed to the various isotonic solutions (*see* Table 1) for 5–10 minutes before each experiment, to allow the intracellular compartment to equilibrate with the extracellular solution. The osmolality of all the solutions was measured by the freezing point depression method using a Roebling micro-osmometer (Camlab, Cambridge, U.K.). Experiments were performed either at 37°C or at 20°C (range = 18–22°C).

Cell volume was measured by video-imaging as previously described (Park et al., 1994). Cells were observed through the 25× objective lens of the microscope, which was fitted with an EDC-1000 camera (Electrim, N.J.). Images were saved on hard disk at 30-sec intervals. The area of the image was subsequently measured using an AVS software package (Hewlett Packard). Cell volume was calculated assuming that cells are spherical. Volumes were normalized to the volume observed during an initial control period when cells were superfused with isotonic solution.

Statistics

Results are expressed as mean ± SEM. Each series of experiments was carried out on at least 3 cell preparations from different animals. The significance of volume changes was tested using Student's *t*-test for paired data to compare the minimum volume reached during hypertonic shock with the volume immediately before returning to the isotonic solution.

Results

HEPES-buffered Solutions (HCO_3^--free)

This first series of experiments was performed in HCO_3^--free solutions (Table 1), which are known to inhibit the contributions of Cl^--HCO_3^- and Na^+-H^+ exchange to volume regulation in other cells (Fisher & Spring, 1984; Kregenow et al., 1985; Hebert, 1986). Figure 1*A* shows results from nine experiments in which isolated lacrimal acinar cells were exposed to a hypertonic solution for 20 min at 37°C. On exposure to the hypertonic solution the cells shrank within 1 min to a mean relative volume of 0.84 ± 0.02. Cell volume then recovered over the next 10 min to 0.95 ± 0.02. They remained at this volume for a further 9 min in the hypertonic solution. Thus, lacrimal acinar cells are capable of RVI. When the cells were returned to the control solution, cell volume increased to 1.09 ± 0.02, before exhibiting a post-RVI RVD (Fig. 1*A*).

The contribution of the Na^+-K^+-$2Cl^-$ cotransporter to RVI was examined by performing experiments in the presence of bumetanide (an inhibitor of the cotransporter). Figure 1*B* shows results obtained from six experiments performed at 37°C in the presence of 20 μM bumetanide. On exposure to the hypertonic solution the cell shrank over 2 min to a relative volume of 0.83 ± 0.03. The cells then failed to show any volume recovery

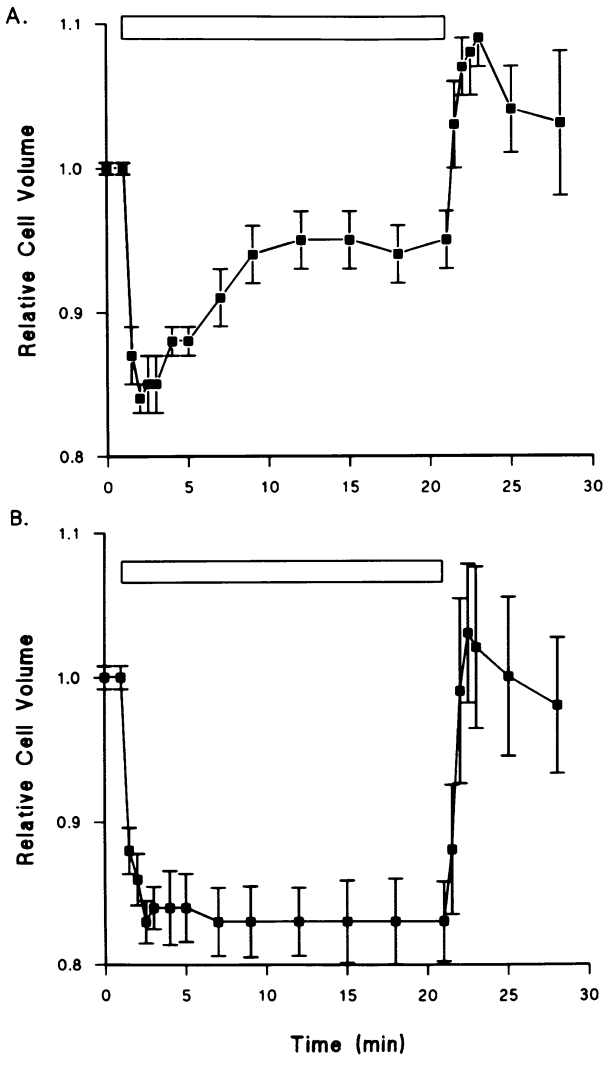

Fig. 1. Volume changes in lacrimal acinar cells exposed to hypertonic solutions in the absence (*A*) and presence (*B*) of 20 μM bumetanide. The solution bathing the cells was changed from isotonic to hypertonic for the period indicated by the bar (details of solutions in Table 1), and the experiments were performed at 37°C. Cell volume was monitored by measuring the area of a video image of the cell. Volumes were then calculated assuming the cell to be spherical, and expressed as a fraction of the control volume (measured over the first minute of the experiment in isotonic conditions). Data are mean ± SEM of 9 experiments in *A* and 6 experiments in *B*.

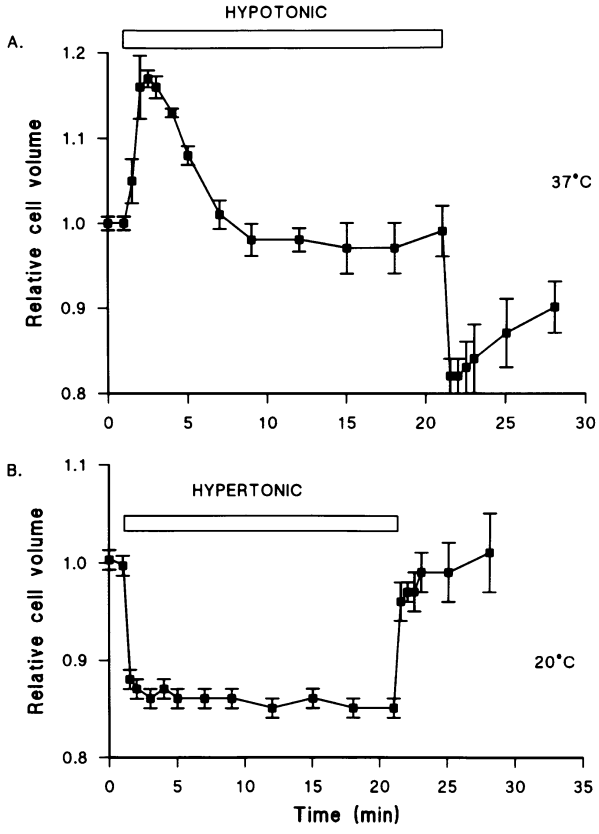

Fig. 2. Volume regulation in lacrimal acinar cells is temperature dependent. (*A*) A post-RVD RVI is observed at 37°C. Cells were exposed to hypotonic solutions for the period indicated by the bar ($n = 3$). (*B*) RVI is not observed at room temperature (20°C). Cells were exposed to HEPES-buffered hypertonic solutions ($n = 7$).

over the next 18 mins under hypertonic conditions. These results suggest that the Na^+-K^+-$2Cl^-$ cotransporter makes an important contribution to RVI in lacrimal gland acinar cells. In control experiments bumetanide did not affect the volume of cells in isotonic solutions ($n = 4$).

The ability of cells to undergo a post-RVD RVI was also examined. Figure 2*A* shows data from 3 cells exposed to HEPES-buffered hypotonic solutions (Table 1) at 37°C. On exposure to the hypotonic solution relative cell volume increased to 1.17 ± 0.01 and then quickly recovered to a volume of 0.99 ± 0.03. This RVD was caused by the loss of K^+ and Cl^- from the cytoplasm through ion channels activated by cell swelling (Kotera & Brown, 1993; Park et al., 1994). Thus, when the cells were returned to the isotonic solution, the relative volume shrank to 0.82 ± 0.02. Figure 2*A* shows that volume then recovered towards control volume (0.90 ± 0.03) over 7 min. Therefore, under these conditions (i.e., 37°C) lacrimal cells do exhibit a post-RVD RVI.

In a previous study, we failed to observe a post-RVD RVI in experiments performed at room temperature (*see* Fig. 1; Park et al., 1994). To investigate the effect of temperature on RVI in more detail cells were exposed to the hypertonic solution at 20°C. Results from seven such experiments are summarized in Fig. 2*B*. The cells shrank to a relative volume of 0.85 ± 0.01 on exposure to the hypertonic solution, but an RVI was not observed under these conditions. Similar results were also obtained when 4 cells were exposed to solutions made hypertonic by the addition of 50 mM NaCl (*data not shown*). The results from these experiments suggest that

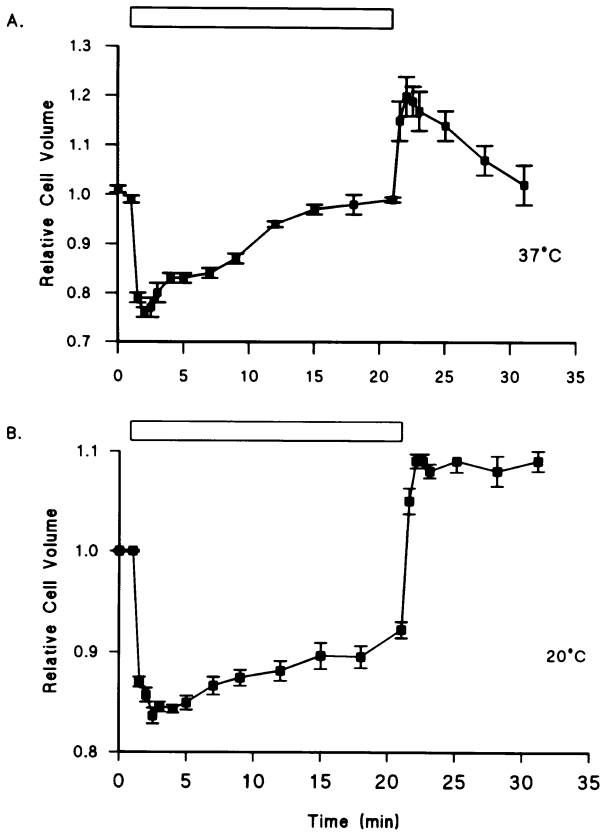

Fig. 3. RVI is supported by HCO_3^--buffered solutions. Cells were superfused with isotonic and hypertonic (bar) solutions buffered with CO_2/HCO_3^- (Table 1). Experiments were performed at 37°C (*A*), and 20°C (*B*). Results are mean ± SEM of 4 experiments in *A* and 5 experiments in *B*.

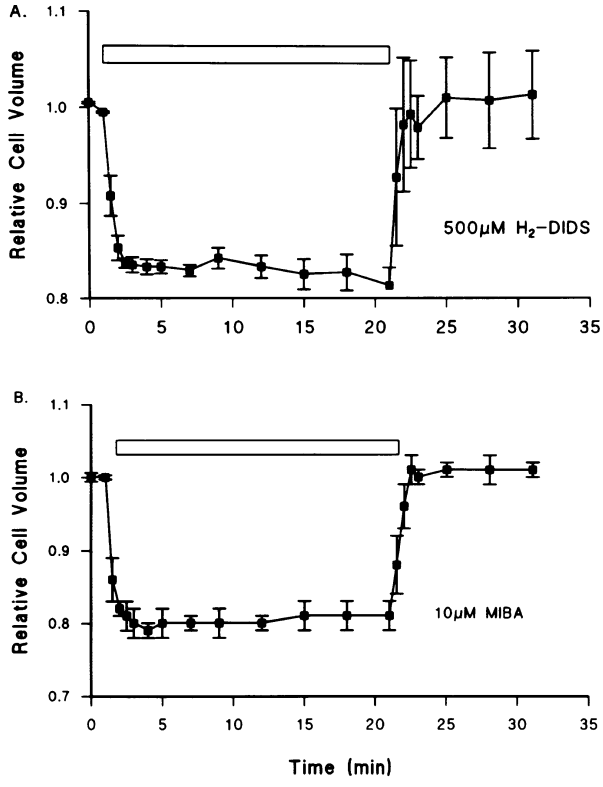

Fig. 4. RVI is inhibited by (*A*) 500 μM H_2-DIDS and (*B*) 10 μM MIBA. Experiments were carried out at 20°C in HCO_3^--buffered solutions and the bar indicates the period of superfusion with hypertonic solution. Data shown are mean ± SEM of 4 experiments in *A* and *B*.

the Na^+-K^+-$2Cl^-$ cotransporter is unable to mediate the uptake of Cl^- and K^+ at 20°C.

HCO_3-buffered Solutions

Experiments were performed in HCO_3-buffered solutions to investigate the involvement of Cl^--HCO_3^- exchange and Na^+-H^+ exchange in RVI. Exposing 4 cells to the HCO_3^--buffered, hypertonic solution at 37°C caused a rapid decrease in mean cell volume to 0.76 ± 0.01 (Fig. 3*A*). The decrease in cell volume was followed by a RVI, taking the cell volume to 0.99 ± 0.01 in 20 min (Fig. 3*A*). This recovery in cell volume was more complete than that seen in HEPES-buffered solutions at 37°C (Fig. 1*A* $P < 0.05$ by unpaired *t*-test), suggesting that HCO_3^- may enhance RVI. The experiments shown in Fig. 3*B* were performed in HCO_3^--buffered solutions at 20°C, to eliminate the contribution of the Na^+-K^+-$2Cl^-$ cotransporter to RVI. Under these conditions, five cells showed an RVI with the average volume increasing from 0.84 ± 0.01 to 0.92 ± 0.01 during a 20 min exposure to the hypertonic solution. These data again suggest that HCO_3^--dependent pathways, possibly Cl^--HCO_3^- exchange and Na^+-H^+ exchange, contribute to RVI in lacrimal gland acinar cells.

To investigate the possible involvement of ion exchangers in RVI, experiments were performed at 20°C in the presence of inhibitors of the ion exchangers. Figure 4*A* shows that 500 μM H_2-DIDS, an inhibitor of Cl^--HCO_3^- exchange, completely abolished RVI in the lacrimal cells (after a 2-min exposure to hypertonic solutions volume = 0.83 ± 0.01, and after 20 min = 0.81 ± 0.02; $n = 4$). RVI was also greatly reduced by 10 μM MIBA an inhibitor of Na^+-H^+ exchange (Fig. 4*B*). In these experiments, the relative volume increased only slightly from 0.79 ± 0.01 to 0.81 ± 0.02 ($n = 4$), over a 20 min exposure to the hypertonic solution. Neither H_2-DIDS nor MIBA effected cell volume in isotonic conditions. Together, these data indicate that Na^+-H^+ and Cl^--HCO_3^- exchangers do participate in RVI in lacrimal gland acinar cells.

Contribution of Amino Acids to RVI

Figure 5 shows data from experiments performed at 20°C using HEPES-buffered solutions to which a mixture of neutral amino acids had been added (Table 1).

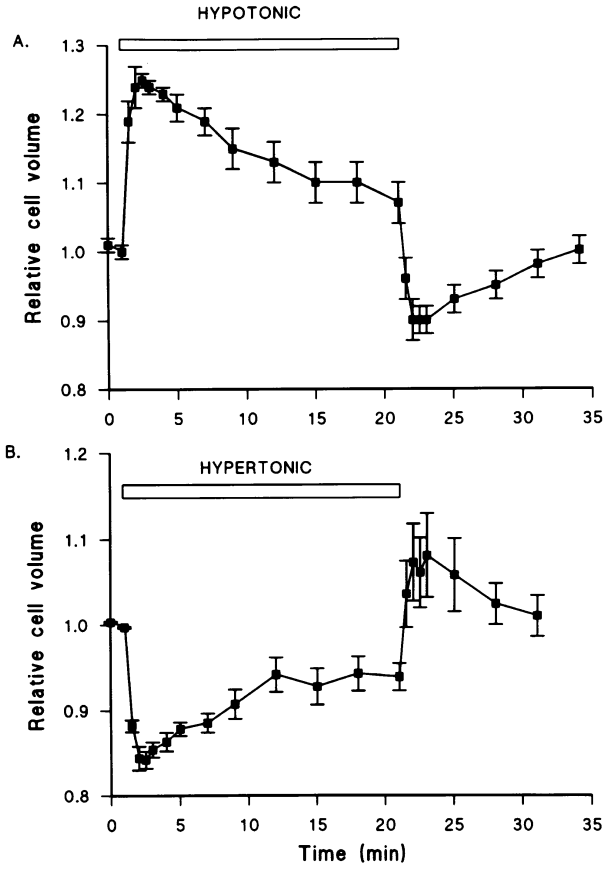

Fig. 5. Amino acids support RVI in lacrimal gland cells. (*A*) Cells were exposed to a hypotonic solution at 20°C as indicated by the bar. A post-RVD RVI was observed on return to isotonic solutions. All solutions contained a mixture of neutral amino acids (MEM; Sigma). A low Cl^- hypotonic solution (90 mM Na-glucuronate in place of 90 mM NaCl) was used to increase the rate of RVD and thus maximise the volume overshoot seen when returning to isotonic solution ($n = 6$). (*B*) An RVI is observed in HEPES-buffered solutions containing amino acids at 20°C ($n = 6$).

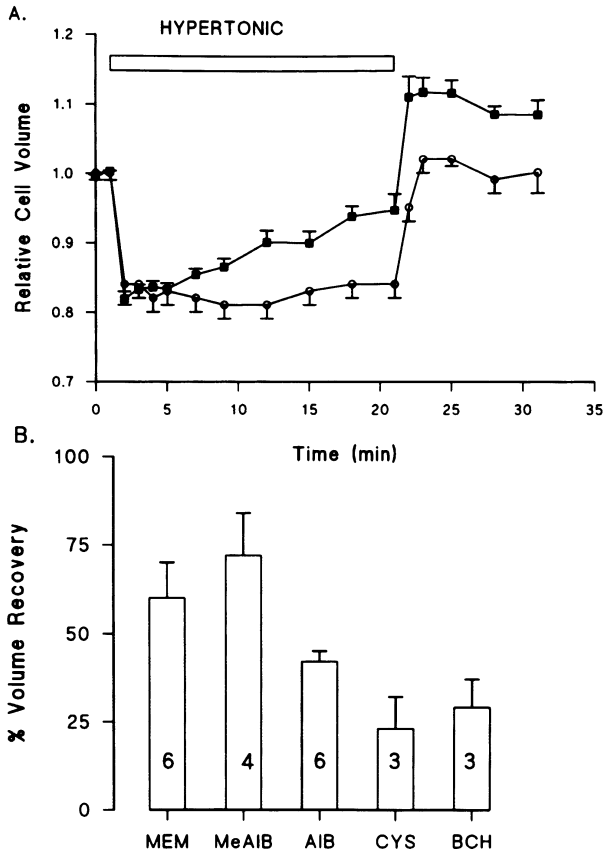

Fig. 6. RVI is supported to varying degrees by different amino acids. (*A*) RVI measured in the presence of either 5 mM methyl-aminoisobutyric acid (Me-AIB, ■; $n = 5$) or 5 mM L-cysteine (◯; $n = 4$). (*B*) Volume recovery in solutions containing: MEM amino acids, 5 mM Me-AIB, 5 mM AIB, 5 mM L-cysteine and 5 mM 2-amino-2-norbornane-carboxylic acid (BCH). The bars indicate mean (±SEM) volume recovery (expressed as a % of maximum volume change on exposure to hypertonic solutions). The number in the bar represents the number of experimental repeats.

A low Cl^- hypotonic solution was used in the experiments in Fig. 5*A*. This has been shown to enhance the rate of RVD in a previous study, however a post-RVD RVI is still not observed in these conditions (*see* Fig. 1*C;* Park et al., 1994). On exposure to the low Cl^--containing hypotonic solution in the presence of amino acids, cell volume quickly increased to 1.25 ± 0.01 (1.5 min), before recovering to 1.07 ± 0.03 over the remaining 18.5 min in the hypotonic solutions (Fig. 5*A*). The rate of RVD in the presence of amino aids was not greater than that in control experiments (*see* Fig. 1*C;* Park et al., 1994). On return to isotonic conditions, however, the cell exhibited a post-RVD RVI in the presence of the amino acids (volume increased from 0.90 ± 0.03 to 1.00 ± 0.02 over 12 min.; Fig. 5*A*). When exposed to hypertonic solutions in the presence of the amino acids at 20°C, cells also underwent a RVI (Fig. 5*B*). In six cells, exposure to the hypertonic solutions caused them to shrink in 1.5 min to a volume of 0.84 ± 0.01, before they recovered to a volume of 0.94 ± 0.02 over the next 18.5 minutes. On return to the isotonic solutions the cells exhibited a post-RVI RVD (Fig. 5*B*).

There are at least two ways by which the amino acids could support RVI in lacrimal cells. One possibility is that the amino acids are metabolized by the cells, providing energy for existing volume regulatory mechanisms (e.g., Na^+-K^+-$2Cl^-$ cotransport). To test this possibility experiments were performed using the nonmetabolizable amino acid amino-isobutyric acid (AIB). In six experiments 5 mM AIB supported RVI in response to hypertonic solutions. Over a 20-min period in these conditions volume increased from 0.83 ± 0.01 to 0.89 ± 0.01 (Fig. 6*B*). These data suggest that amino acid metabolism is not responsible for the RVI observed in the presence of amino acids.

An alternative explanation for the amino acid-supported RVI is that the amino acids themselves act as

intracellular osmolytes when accumulated by the cells. If amino acid accumulation does play a role, then Na^+-coupled active transport of the amino acids may be involved. To test this possibility, RVI was measured in the presence of substrates of two of the major classes of Na^+-amino acid cotransporter (A and ASC). Figure 6*A* shows the results of five experiments with 5 mM Methyl-AIB (■; Me-AIB; a specific substrate for system A), and four experiments with 5 mM L-cysteine (○; a substrate for system ''ASC''). In these experiments an RVI was observed in the presence of Me-AIB (0.82 ± 0.01 to 0.95 ± 0.02), however very little volume regulation was observed in the presence of 5 mM cysteine (0.82 ± 0.02 to 0.84 ± 0.02). Figure 6*B* summarizes the data on the amino acid supported RVI. The recovery in volume is expressed as a percentage of the initial decrease in cell volume for each individual experiment. The data show that 5 mM MeAIB and the MEM mixture of amino acids support the largest RVI. The RVI with 5 mM AIB is slightly smaller ($P > 0.05$ compared to 5 mM Me AIB). Cysteine (5 mM) and 5 mM 2-amino-2-norbornane-carboxylic acid (BCH; a specific substrate for the system L amino acid transporter), supported only modest increases in volume ($P > 0.05$ compared to MeAIB by unpaired *t*-test in both cases).

Discussion

In this paper, we have shown that acinar cells isolated from the rat lacrimal gland can volume regulate when bathed in hypertonic extracellular solutions, i.e., exhibit a RVI. The paper is complementary to a previous study in which we observed an RVD in lacrimal acinar cells exposed to hypotonic solutions (Park et al., 1994).

In the previous study of cell volume, we reported that lacrimal cells initially behave as perfect osmometers when bathed in hypotonic solutions, and swell to a theoretical maximum volume, before exhibiting RVD (Park et al., 1994). A similar conclusion can be made from the data in this paper. Figure 1*A* shows that cells shrank to a relative volume of 0.83 when bathed in the hypertonic solution. This change in volume is exactly what would be predicted if the cell behaved as a perfect osmometer for a change in osmolality from 280 to 379 mOsm (and assuming that 34% of the intracellular volume is osmotically inactive, *see* Park et al., 1994). The volume of the cells in Fig. 1*A* then recovered to 0.95 during the 20 min exposure to the hypertonic solution. On returning the cells to the isotonic solution the volume increased to 1.09. This ''overshoot'' of the cell volume occurs because the cells have accumulated osmotically active substances as part of the RVI. The size of the overshoot observed here is slightly less than predicted for a perfect osmometer (predicted volume 1.15), however this may be due to the fact that a post-RVI RVD is initiated before the cells have attained their maximum volume.

Three mechanisms by which osmotically active substances are accumulated in lacrimal acinar cells during RVI have been identified in the present study: (1) Na^+-K^+-$2Cl^-$ cotransporter, (2) the joint action of Na^+-H^+ exchange and Cl^--HCO_3^- exchange, and (3) the uptake of amino acids. In the remainder of this discussion the contribution of each mechanism to RVI will be examined, and the possible physiological importance of RVI in these cells discussed.

Na^+-K^+-2Cl Cotransport

RVI following hypertonic shock is often partly or wholly due to increased Na^+-K^+-$2Cl^-$ cotransport activity. This has been shown in various cell types (Hoffmann & Simonsen, 1989). An increase in cotransporter activity allows the intracellular accumulation of NaCl. This causes an influx of water into the cell, down the osmotic gradient, causing cell swelling. Several studies have reported the presence of the Na^+-K^+-$2Cl^-$ cotransporter in lacrimal acinar cells (Saito et al., 1987; Singh, 1988; Ozawa et al., 1988), and it is thought to maintain intracellular Cl^- at a concentration which is above equilibrium (Ozawa et al., 1988). The present study extends these observations by demonstrating that Na^+-K^+-$2Cl^-$ cotransport also has a role in RVI in lacrimal acinar cells. The evidence for this is that at 37°C volume regulation occurs in the absence of HCO_3^- and amino acids (Fig. 1*A*), and is abolished by bumetanide (Fig. 1*B*).

RVI is not observed in HEPES-buffered solutions at room temperature (Fig. 2*B*), suggesting the cotransporter is inactive under these conditions. This observation is also supported by data in a previous paper which showed that a post-RVD RVI is not seen in cells at room temperature (Park et al., 1994). The reason for the inactivity of the cotransporter is unclear. It is likely that the rate of the transporter 'turnover' is reduced by lowering the temperature, but this might be expected to simply reduce the rate of RVI rather than completely abolish it (*see* Fig. 2*B*). On the other hand activation of the cotransporter is a complex process, e.g., Matthews et al. (1993) showed that intestinal cell cotransporter activation involves changes in the cytoskeleton and particularly 'F-actin' filaments, and in avian erythrocytes activation of the cotransporter in hypertonic conditions involves phosphorylation of the protein (Pewitt et al., 1990). If a number of steps are involved in the activation of the cotransporter in lacrimal gland cells and each is temperature-dependent, then the combined effects of reducing the temperature could be sufficient to completely inhibit activation. This, however, seems unlikely since other transporters are activated at room temperature (e.g., Na^+-H^+ and Cl^--HCO_3^- exchangers). A more likely explanation for the lack of RVI at room temperature is that lowering the temperature reduces the driving force for the cotransporter. This is probably quite small in lacri-

mal glands since Cl^- is only accumulated slightly above equilibrium (Ozawa et al., 1988). Reducing the temperature may reduce the activity of the Na^+-K^+ pump either directly, or indirectly via effects of metabolism, to such an extent that the Na^+ gradient is unable to sustain K^+ and Cl^- transport into the cell, thus inhibiting RVI. Indirect evidence for this can be found in Fig. 2*A*, which shows that the rate of RVD is greatly increased at 37°C compared to room temperature (*see* Fig. 5*A*). The rate of RVD is known to be enhanced when there is a large gradient for Cl^- efflux (Park et al., 1994), which could be caused in Fig. 2*A* by an increase in Cl^- accumulation at 37°C.

Na^+-H^+ Exchange/Cl^--HCO_3^- Exchange

Many cells are known to undergo RVI by NaCl uptake via an increase in Na^+-H^+ and Cl^--HCO_3^- exchange activity (Hoffmann & Simonsen, 1989). As with the cotransporter, the result of increased exchanger activity is the net uptake of NaCl which is followed by water. The present study provides evidence that coupled Na^+-H^+ and Cl^--HCO_3^- exchangers are partially responsible for RVI in lacrimal cells. In HCO_3^--buffered medium at room temperature the Na^+-K^+-$2Cl^-$ cotransporter will be inactive. Volume regulation following hypertonic shock is therefore likely to be via a HCO_3^- dependent pathway (Fig. 3*B*). This pathway appears to be linked to a Na^+-H^+ exchange mechanism (which accounts for Na^+ uptake), since the RVI response is blocked by MIBA (Fig. 4*B*) an inhibitor of Na^+-H^+ exchange. The most likely pathway for HCO_3^--dependent Cl^- uptake appears to be Cl^--HCO_3^- exchange, as RVI is also completely inhibited by H_2-DIDS, which is known to block the exchanger (Lambert et al., 1988).

Ion exchangers have previously been described in lacrimal acinar cells. The Na^+-H^+ exchanger accounts for as much as 50% of the Na^+ influx seen during cholinergic stimulation of mouse lacrimal cells (Saito et al., 1987) and is also involved in pH regulation in rat lacrimal cells (Saito et al., 1990). The Cl^--HCO_3^- exchanger plays a role in the uphill transport of Cl^- in unstimulated mouse lacrimal cells (Ozawa et al., 1988). The mechanisms by which these exchangers can be activated by cell shrinkage have not been investigated in this study. Recent work, however, has shown that the activation of Cl^--HCO_3^- exchange (expressed in *Xenopus* oocytes) by hypertonic solutions is secondary to the activation of Na^+-H^+ exchange (Humphreys et al., 1995). Furthermore, it has also been shown that Na^+-H^+ exchange is activated by cell shrinkage in acinar cells from the salivary gland (Seo et al., 1995).

Amino Acids in Volume Regulation

In addition to mechanisms for RVI which increase intracellular ion concentrations, some cells are also capable of taking up organic osmolytes such as amino acids and sugars in response to hypertonicity (Chamberlin & Strange, 1989). This is advantageous to cell function, as large changes in intracellular ion concentrations are avoided. In the present study, amino acids supported RVI in lacrimal gland acinar cells. This was not due to the metabolism of the amino acids since AIB and MeAIB (both nometabolizable amino acids) supported the RVI (Fig. 6). Thus, the most likely explanation is that amino acids are accumulated in the cells, probably by secondary-active transport. Little is known about amino acid transport in lacrimal gland cells, although there is some evidence that Na^+-coupled pathways are present (Mircheff et al., 1983; Saito et al., 1987).

To investigate the role of amino acids in more detail, we examined volume regulation in the presence of MeAIB, L-cysteine and BCH, substrates for the A, ASC and L amino acid transport systems respectively (Yudilevich & Boyd, 1987). Figure 6 shows that MeAIB was most efficient in supporting RVI, suggesting that the System A Na^+-amino acid cotransporter is involved in RVI. System A has previously been reported to be involved in volume regulation in other cell types, e.g., vascular smooth muscle cells (Chen et al., 1994) and human fibroblasts (Dall'Asta et al., 1994) which respond to hypertonicity by an increase in System A amino acid uptake.

In these studies, the effects of hypertonicity on amino acid transport took several hours to develop, and were dependent on *de novo* synthesis of transport proteins (Chen et al., 1994; Dall'Asta et al., 1994). It seems unlikely that protein synthesis is important in lacrimal gland cells, since amino acid transport is stimulated within a few minutes of the onset of cell shrinkage (*see* Fig. 5*B*). A more likely explanation is that hypertonicity causes an increase in the rate of transport by proteins already in the cell membrane. The rate of system A transport is increased by both hyperpolarization of the membrane potential and by alkalinization of intracellular pH (McGiven & Pastor-Anglada, 1994). Hypertonic conditions are known to hyperpolarize the membrane potentials of many cells (e.g., thick ascending limb of Henle, Molony & Andreoli, 1988; astrocytes and oligodendrocytes, Kimelberg & Kettenmann, 1990; and MDCK cells, Ritter et al., 1991), and to cause an intracellular alkalinization of salivary gland acinar cells (Seo et al., 1995).

Relative Contributions of RVI Mechanisms

This study has shown that at least three separate systems can contribute to RVI in lacrimal gland acinar cells: Na^+-K^+-$2Cl^-$ cotransport, Na^+-H^+/Cl^--HCO_3^- exchange and Na^+-amino acid cotransport. It is not clear, however, whether all 3 systems are normally active, or whether one or two are simply recruited when another is inhib-

ited. RVI was perhaps most effective at 37°C in HCO_3^--buffered solutions (i.e., volume recovery is most complete in Fig. 3*A*), suggesting that both Na^+-K^+-$2Cl^-$ cotransporter and the exchangers function together. However, there appears to be little difference in the rate of volume recovery in the presence or absence of HCO_3^- at 37°C (*see* Fig. 3*A* and 1*A*), possibly because both sets of transporters involved are dependent on the Na^+ gradient, i.e., the activity of one will reduce the driving force for the other. For this reason, it would be difficult to assess the contribution of each individual pathway to volume regulation by simply using inhibitors of the different transport systems. Saito et al. (1987), however, estimated the relative contribution of the different systems to Na^+ influx into mouse lacrimal gland cells using Na^+-selective microelectrodes. They found that: Na^+-H^+ exchange contributed up to 50%, Na^+-K^+-$2Cl^-$ cotransport about 30% and Na^+ coupled nutrient transport about 30%. These experiments were carried out on ACh stimulated cells, but it is possible that the transporters may make similar contributions when activated by hypertonicity in unstimulated rat lacrimal glands.

Physiological Role for RVI in Lacrimal Gland Acinar Cells

This study has shown that lacrimal gland acinar cells can regulate their volume when exposed to hypertonic solutions. Volume regulation appears to involve several transport proteins which also have an important role in the secretory activity of these cells, e.g., Na^+-H^+ exchange, Cl^--HCO_3^- exchange and Na^+-K^+-$2Cl^-$ cotransport. While it is unlikely that lacrimal cells are exposed to anisotonic conditions *in vivo*, it is important to understand how ion transport in these cells can be modulated. Furthermore, recent studies of other secretory epithelia have shown that secretion is often associated with cell shrinkage, e.g., salivary gland acinar cells (Foskett & Melvin, 1989; Steward & Larcombe-McDouall, 1989; Nakahari et al., 1990), and intestinal crypt cells (Walters et al., 1992). It is therefore of interest to know what effects changes in cell volume will have on ion transport, and *vice-versa*. Future studies will be necessary to determine the effects of stimulating secretion on cell volume. However, the present study clearly shows that cell shrinkage simulates: Na^+-H^+ exchange, Cl^--HCO_3^- exchange and Na^+-K^+-$2Cl^-$ cotransport. Thus, a decrease in cell volume is unlikely to impair, and may even enhance secretion in lacrimal acinar cells.

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