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TRANSPORT PHYSIOLOGY

HCO_3^--dependent volume regulation in α-cells of the rat endocrine pancreas

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Abstract Ion transport activity in pancreatic α-cells was assessed by studying cell volume regulation in response to anisotonic solutions. Cell volume was measured by a video imaging method, and cells were superfused with either 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid-buffered or HCO_3^--buffered solutions. α-Cells did not exhibit a regulatory volume increase (RVI) in response to cell shrinkage caused by hypertonic solutions. A RVI was observed, however, in cells that had first undergone a regulatory volume decrease (RVD), but only in HCO_3^--buffered solutions. RVI was also observed in response to a HCO_3^--buffered hypertonic solution in which the glucose concentration was increased from 4 to 20 mM. The post-RVD RVI and the glucose-induced RVI were both inhibited by 10 μM *5*-(*N*-methyl-*N*-isobutyl) amiloride or 100 μM 2,2′-(1,2-ethenediyl) bis (5-isothio-cyanatobenzenesulfonic acid), but not by 10 μM benzamil nor 10 μM bumetanide. These data suggest that Na^+–H^+ exchangers and Cl^-–HCO_3^- exchangers contribute to volume regulation in α-cells.

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Introduction

Pancreatic α-cells are responsible for the synthesis and secretion of glucagon, the peptide hormone which plays a central role in maintaining plasma glucose concentrations [2, 10]. Glucagon is constitutively secreted by the α-cells, and secretion is inhibited by an increase in the plasma glucose concentration [10]. Glucose inhibition is thought to be mediated in part by the paracrine actions of insulin, GABA, and Zn^{2+} released from neighboring β-cells, and somatostatin which is released from δ-cells [10]. In addition, glucose has been demonstrated to exert a direct effect on glucagon secretion, but data are still equivocal as to whether glucose has a stimulatory or inhibitory action [10].

The mechanisms by which these paracrine agents, and possibly glucose, may inhibit secretion are not fully understood. This is mainly due to the lack of information on α-cell ion channel and ion transporter expression. Good progress has recently been made in the identification of ion channels which are associated with the electrical activity involved in glucagon secretion, e.g., K_{ATP} [3]; G-protein-activated Kir [11, 30]; $GABA_A$ [29]; voltage-gated Na^+ [8]; and L-type and N-type voltage-gated Ca^{2+} [1]. Much less is known, however, about the expression of ion transporters in α-cells. Knowledge of transporter expression is crucial since these proteins help generate the electrochemical gradients that drive ion movement through ion channels. Furthermore, they regulate intracellular pH and cell volume, parameters which can modulate ion channel activity [12].

Our laboratory has previously examined the expression of cation-chloride cotransporters in both pancreatic α-cells and β-cells. Immunocytochemical and functional data demonstrated that the Na^+–K^+–$2Cl^-$ cotransporter (NKCC1) is not expressed in α-cells [18]. This is in marked contrast to pancreatic β-cells where there is substantial evidence for the expression and function of NKCC1 [17, 19]. In addition, there is evidence for the expression and function of the K^+–Cl^- cotransporters (KCC1, KCC3, and KCC4) in the rat α-cells, but not in β-cells [6]. Much of our functional data have been obtained by studying cell volume regulation. In these experiments, cell volume is challenged by exposing the cells to anisotonic extracellular solutions, a maneuver that activates the variety of ion transporters and channels involved in cell volume regulation. Thus, in β-cells, cell shrinkage in hypertonic (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES)-buffered) solutions is followed by a regulatory volume increase (RVI) which involves the activation of NKCC1. The resultant influx of ions into the cell is accompanied by the movement of water [18, 19]. By contrast, a RVI was not observed in α-cells under such conditions [18], consistent with the lack of NKCC1 expression.

RVI, however, can also involve the activities of Cl^-–HCO_3^- exchangers and Na^+–H^+ exchangers (NHE) [13, 28]. Indeed these transporters contribute to the RVI in β-cells exposed to hypertonic solutions that are buffered with HCO_3^- [19]. To date α-cell volume regulation has only been studied in HEPES-buffered solutions [18]. Thus, the primary aim of this study was to investigate the effects of hypertonic, HCO_3^--buffered solutions on α-cell volume.

Some cell types, which do not show a RVI in response to cell shrinkage in hypertonic solutions, can exhibit a phenomenon known as a RVI-after-regulatory volume decrease (RVD) or post-RVD RVI [13]. This occurs in cells which have lost intracellular ions as part of a RVD in hypotonic solutions, and consequently shrink below their original volume on return to isotonic solutions. Post-RVD RVI can involve the activities of either NKCC1 or NHE in conjunction with Cl^-–HCO_3^- exchangers [13]. A second aim of this study therefore was to determine whether α-cells exhibit a post-RVD RVI.

The data obtained show that α-cells do not exhibit RVI in HEPES-buffered nor in HCO_3^--buffered solutions. α-cells did, however, exhibit a post-RVD RVI, but only in HCO_3^--buffered solutions. Furthermore, we found that increasing the glucose concentration in the experimental solutions from 4 to 20 mM enabled RVI in hypertonic solutions.

Materials and methods

Isolation of pancreatic α-cells and β-cells

Islet cells were prepared from pancreatic tissue isolated from male Sprague–Dawley rats (250–300 g; Charles River, UK). Animals were sacrificed by stunning and cervical dislocation as proscribed by Schedule 1 methods of the UK Animals in Scientific Procedures legislation. Islets were prepared by collagenase digestion and were dispersed into single isolated cells by a brief incubation in Ca^{2+}-free medium [20]. Single cells were harvested by centrifugation and resuspended in MEM medium (HEPES-buffered) supplemented with 10% fetal calf serum, 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM glutamine (Gibco). The resuspended cells were plated at the center of 35-mm sterile plastic culture dishes (Nunc) and incubated in humidified air at 37°C. Cells were allowed to loosely adhere to the plastic culture dish overnight, and volume measurement experiments were performed between 24 and 96 h after cell isolation.

Table 1 Solution composition

Solution	Concentration (mM)				Osmolality (mOsm. kg H_2O^{-1})
	NaCl	$NaHCO_3$	HEPES	Mannitol	
HCO_3^--buffered solutions					
Isotonic	110	25	0	20	282±3 (n=5)
Hypertonic (mannitol)	110	25	0	120	381±3 (n=4)
Hypertonic (NaCl)	170	25	0	20	373 (n=1)
Hypotonic	65	25	0	0	178±3 (n=4)
HEPES-buffered solutions					
Isotonic	140	0	5	20	285 (n=2)
Hypotonic	85	0	5	0	182 (n=2)
Hypertonic	140	0	5	120	384 (n=1)

All solutions contained: 5 mM KCl, 1 mM Mg Cl_2, and 1.2 mM $CaCl_2$. In the majority of experiments, 4 mM glucose was present, but in one series 20 mM glucose (in exchange for mannitol) was used. The pH of the HCO_3^--buffered solutions was 7.4 achieved by gassing with 5% CO_2/95% O_2. The pH of the HEPES-buffered solutions was adjusted to 7.4 using NaOH. Osmolalities were measured using a Roebling micro-osmometer (Camlab, UK)

Cell volume measurement

Cell volume was measured by video imaging using a Nikon Diaphot 200 microscope fitted with a Canon Powershot A620 camera. Cells were viewed through a ×40 objective and saved as JPEG files for subsequent analysis. The images were of 3.146 megapixels and equivalent to a measured area of 850 μm^2 (i.e., one pixel=0.00027 μm^2). The area of each cell image was measured using ImageJ software (NIH, USA), and cell volume was calculated assuming that cells maintained a spherical shape [20]. α-Cells were selected for study on the basis of size (0.33 to 0.80 pl) and appearance, as previously discussed [18]. A single series of experiments was performed on β-cells which had volumes of between 2.2 to 4.2 pl.

Volume changes were examined in cells exposed to hypertonic or hypotonic solutions buffered with either HCO_3^-/CO_2 or HEPES (Table 1). Culture dishes were fitted with a plastic insert which reduced the effective volume to 200 μl, and the cells were superfused with the experimental solutions at a rate of 5 ml. min^{-1}. The temperature of the superfusate was maintained at a 35–37°C by the use of a heated water jacket.

Experimental protocols

To study RVI, cells were exposed to solutions made hypertonic by the addition of either 100 mM mannitol or 60 mM NaCl (Table 1). Following an equilibration period (10 min) and control period (2 min) in isotonic solution, the cells were exposed to hypertonic solution for 15 min followed by a 5-min recovery period in isotonic solution. Images of the cells were recorded at 1-min intervals during the experiment. Post-RVD RVI was examined by exposing cells to hypotonic solution (Table 1) for 15 min with a 10-min isotonic recovery period. To investigate the involvement of specific transporters in RVI, the following inhibitors were added to the isotonic superfusate for the duration of the recovery period: 10 μM bumetanide, 10 μM *5*-(*N*-methyl-*N*-isobutyl) amiloride (MIBA), 10 μM benzamil, and 100 μM 2,2′-(1,2-ethenediyl) bis (5-isothiocyanato-benzenesulfonic acid) (DIDS). These compounds were all purchased from Sigma and prepared as stock solutions in dimethyl sulfoxide so that the final concentration of DMSO in any experimental solution was 0.1% (*v*/*v*).

Data analysis

Data (mean±SEM) are expressed as relative cell volume, i.e., volume divided by control volume measured in isotonic solution. For statistical analysis, the rate of volume increase was determined by regression analysis of the linear phase of volume recovery, i.e., for 7 or 5 min after the minimum volume for the post-RVD RVI and the RVI in 20 mM glucose, respectively. Rates of volume increase were compared by ANOVA followed by Dunnett's multiple comparison tests. The RVD in HEPES- and HCO_3^--buffered solutions were compared by Student's *t* test for unpaired data.

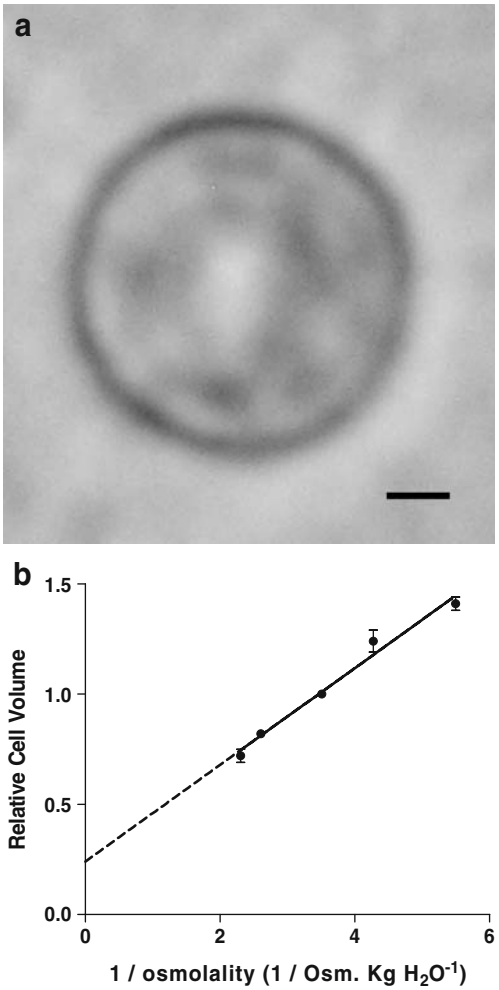

Fig. 1 Isolated pancreatic α-cells behave as osmometers in anisotonic solutions. **a** Image of an isolated α-cell with a volume of 0.65 pl in HCO_3^--buffered isotonic solution. The *bar* indicates 2 μm. **b** Boyle–van't Hoff plot of α-cell relative cell volume as a function of the reciprocal of superfusate osmolality (1/osmolality). Cell volumes are the maximum or minimum recorded when cells were superfused with hypotonic or hypertonic solutions, respectively. Solutions were all buffered with HEPES and include the isotonic (285 mOsm. kg H_2O^{-1}; *n*=1), hypotonic (182 mOsm. kg H_2O^{-1}; *n*=6), and hypertonic (384 mOsm. kg H_2O^{-1}; *n*=6) solutions described in Table 1. Two additional solutions were used: 433 mOsm. kg H_2O^{-1} (the hypertonic solution but with 150 mM mannitol; *n*=3) and 234 mOsm. kg H_2O^{-1} (the hypotonic solution but with 120 mM Na Cl; *n*=3). Data are mean±SEM and the line through the data fitted by linear regression analysis (R^2=0.982). Extrapolating this line to infinite osmotic strength (1/osmolality=0) gives an osmotically inactive space of 0.24

Results

α-Cells do not exhibit a RVI in HCO_3^--buffered solutions

Figure 1a shows the image of a pancreatic α-cell recorded 48 h after isolation. The cell is typical of the α-cells used in this study; it has a volume of 0.65 pl and is circular in appearance. The circular cross section suggests that the cells retain a spherical morphology, which is a major assumption in the volume measurement method. To further test this assumption, volume changes in response to a range of extracellular osmolalities were examined. Figure 1b shows a Boyle–van't Hoff plot of α-cell volume as a function of osmolality (see [28]). Over the range of osmolalities used in this study, the α-cells behave as osmometers so that volume was linearly related to superfusate osmolality. These data indicate that the method is working as one would predict and suggest that the α-cells must be retaining a spherical shape during the experiments. An osmotically inactive space of 0.24 was predicted by the linear regression line fitted to the data. This value is similar to 0.26 measured in pancreatic β-cells using similar methods [20].

It has previously been shown that rat pancreatic α-cells do not exhibit a RVI when exposed to hypertonic solutions buffered with HEPES [18]. Figure 2a, b shows the effects of hypertonic solutions buffered with HCO_3^- on α-cell volume. Solutions were made hypertonic either by the addition of (a) 100 mM mannitol or (b) 60 mM NaCl (Table 1). The α-cells quickly shrank to volumes of (a) 0.84±0.01 or (b) 0.83±0.02, when exposed to the hypertonic solutions. No changes in α-cell volume were observed during the remainder of the superfusion with either hypertonic solution. Cell volume, however, quickly returned to control values in the isotonic superfusate. Pancreatic β-cells, by contrast to the α-cells, exhibited a complete RVI in HCO_3^--buffered solutions (Fig. 2c), as has previously been reported [19].

Pancreatic α-cells exhibit a post-RVD RVI in HCO_3^--buffered solutions

Although most cells do exhibit a RVI in response to shrinkage in hypertonic solutions, some cells only show a RVI in response to the decrease in cell volume observed after a cell has first performed a RVD in hypotonic solutions (a post-RVD RVI; [14]). The possibility that pancreatic α-cells exhibit a post-RVD RVI was therefore examined by exposing cells to hypotonic solutions buffered with either HEPES or HCO_3^- (Fig. 3). The α-cells exhibited a RVD in both HCO_3^--buffered (Fig. 3a) and HEPES-buffered solutions (Fig. 3b). Table 2 shows that the maximum volumes attained were not different by unpaired *t* test, neither were the rates of RVD, nor the minimum volume observed at the end of the hypotonic period. Thus, when the hypotonic superfusate was replaced by the isotonic solutions, α-cell volume decreased to 0.85±0.02 in the HCO_3^- solutions (Fig. 3a) and 0.85±0.01 in HEPES-buffered solutions (Fig. 3b). In the HCO_3^--buffered solutions, this cell shrinkage was immediately followed by a significant increase in cell volume (a post-RVD RVI) over the next 8 min, so that the volume at the end of the experiment was 0.97±0.01 ($P<0.05$ by paired *t* test). By contrast, in HEPES-buffered solutions, the cell volume did not recover significantly (volume at the end of experiment= 0.88±0.01; $P>0.1$ by paired *t* test).

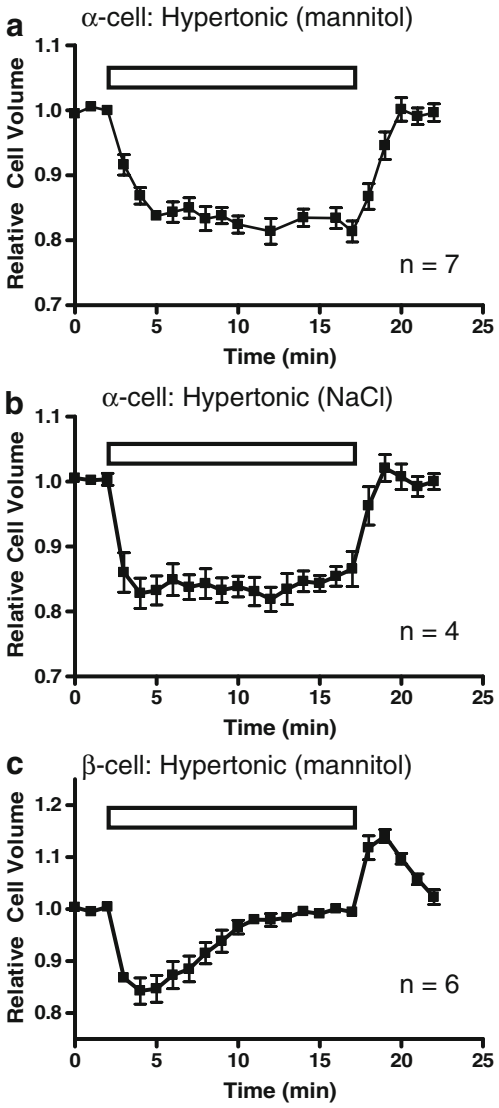

Fig. 2 RVI is not observed in pancreatic α-cells but is observed in β-cells. Isolated α-cells (**a**, **b**) and β-cells (**c**) were exposed to HCO_3^--buffered hypertonic solutions for the period indicated by the *bar*. The hypertonic solution (381 mOsm. kg H_2O^{-1}) in **a** and **c** contained an additional 100-mM mannitol compared to the isotonic solution (282 mOsm. kg H_2O^{-1}), whereas the solution in **b** contained an additional 60-mM NaCl (373 mOsm. kg H_2O^{-1}). Data are mean± SEM, for the number of cells indicated in each panel

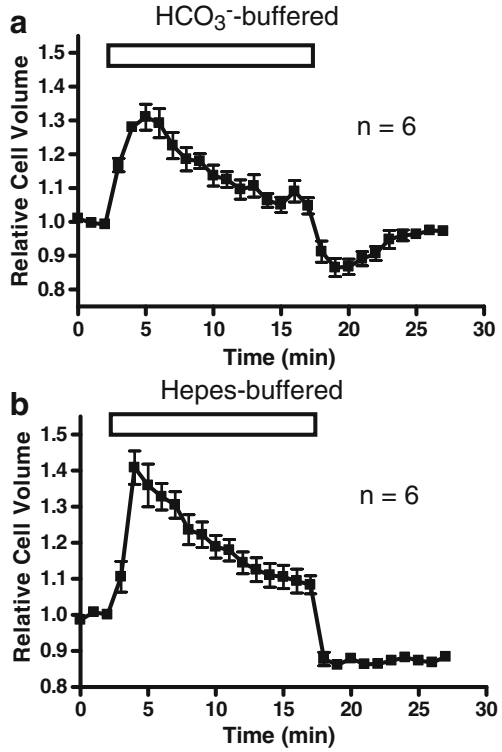

Fig. 3 A post-RVD RVI is observed in α-cells in HCO_3^--buffered solutions (**a**) but not in HEPES-buffered solutions (**b**). Cells were superfused with hypotonic solutions for the period indicated by the *bar*. At the end of this period, re-superfusion with the isotonic solutions caused cell volumes to decrease below those observed during the initial control period. An increase in cell volume (a post-RVD RVI) was then observed in HCO_3^--buffered solutions over this 10-min recovery period in isotonic solution

Effects of transport inhibitors on the post-RVD RVI in pancreatic α-cells

The mechanisms by which the post-RVD RVI occur were examined by using inhibitors known to act on transporters involved in RVI in other cells. All experiments were performed in HCO_3^--buffered solutions, and transport inhibitors were only present in the isotonic solution during the recovery period (Fig. 4; solid bars). Figure 4a shows that the post-RVD RVI was abolished by the anion transport inhibitor 100 μM DIDS [4]. The RVI was also greatly attenuated by 10 μM MIBA (Fig. 4b), a derivative of amiloride with a high specificity for NHE [15, 26, 27]. By contrast, 10 μM benzamil (an amiloride-derivative with a low affinity for NHE; [15]), was without effect on the post-RVD RVI (Fig. 4c). Bumetanide, at a concentration of 10 μM which specifically inhibits NKCC1 [23], was also without effect on the RVI (Fig. 4d).

Rates of volume increase, during the post-RVD RVI, in the presence of the transport inhibitors are plotted in Fig. 6a. The rates of increase were not significantly different between control experiments and those in the presence of 10 μM bumetanide or 10 μM benzamil. The rates of increase were, however, significantly reduced by 100 μM DIDS or 10 μM MIBA. In fact, in the presence of these inhibitors, the rates were not significantly different to those observed in HEPES-buffered solutions ($P>0.1$).

α-Cell volume regulation in solutions containing 20 mM glucose

We have previously reported that the volume of both α-cells and β-cells increases when the extracellular glucose concentration is elevated [5, 20]. Glucose-induced swelling requires glucose metabolism [5, 20], but the mechanism by which the volume increase occurs has not been determined. One possibility is that the activation of NHE and Cl^-–HCO_3^- exchangers, which in β-cells are stimulated by glucose due to changes in intracellular pH (pH_i; [17, 24]), may also cause the accumulation of Na^+ and Cl^- and hence result in cell swelling. In a final series of experiments, we therefore examined volume regulation in α-cells exposed to solutions containing 20 mM rather than 4 mM glucose. Cells were pre-incubated in isotonic solution containing 20 mM glucose for at least 10 min before recording the control isotonic period and the exposure to the hypertonic solution. Figure 5a shows that on exposure to HCO_3^--buffered hypertonic solutions (+100 mM mannitol), α-cells shrank, but then underwent RVI. Volume regulation was not, however, observed in cells bathed in HEPES-buffered solutions containing 20 mM glucose (Fig. 5b). The RVI in HCO_3^--buffered solutions was inhibited when 10 μM MIBA (Fig. 5c), but not 10 μM benzamil (Fig. 5d), was added to the hypertonic solution. The rates of volume regulation in these experiments are summarized in Fig. 6b.

Table 2 Regulatory volume decrease (RVD) in pancreatic α-cells is identical in HEPES-buffered or HCO_3^--buffered solutions

	Maximum volume (RCV)	Rate of volume decrease (RCV. min^{-1})	Minimum volume (RCV)
HEPES-buffered (n=6)	1.41±0.05	−0.035±0.006	1.09±0.03
HCO_3^--buffered (n=6)	1.34±0.03	−0.034±0.013	1.05±0.02

Maximum and minimum relative cell volumes (RCV) are for the period of cell exposure to the hypotonic solutions. The rate of volume decrease was calculated by linear regression analysis on the data for the 4-min period after the maximum volume was attained. Data are mean±SEM and there were no significant differences between the values in the two solutions ($P>0.1$ by Student's t test for unpaired data)

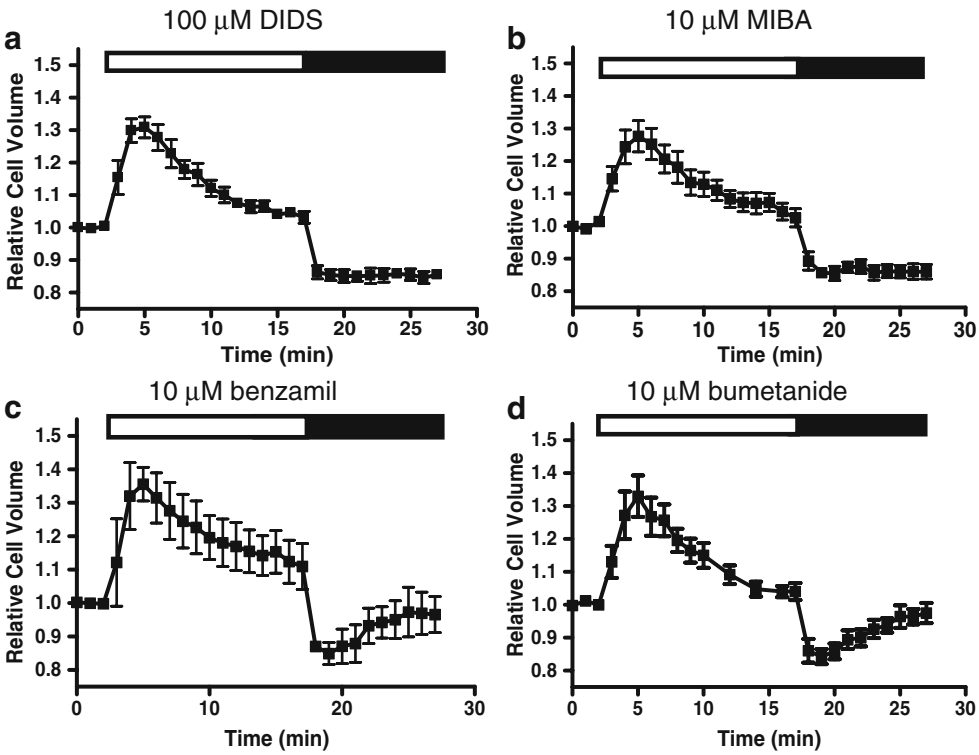

Fig. 4 The effects of transport inhibitors on the post-RVD RVI in α-cells. The cells were exposed to the hypotonic solution for the period indicated by the *open bars*. The *solid bars* indicate the period of superfusion with isotonic solutions containing: **a** 100 μM DIDS (n=6), **b** 10 μM MIBA (n=6), **c** 10 μM benzamil (n=6), and **d** 10 μM bumetanide (n=6). In each condition, the mean minimum volumes observed on switching from the hypotonic to isotonic solutions were not significantly different from each other by ANOVA (P>0.1), ranging from 0.83±0.02 to 0.85±0.01. All solutions were HCO_3^--buffered

Discussion

α-cells exhibit a post-RVD RVI in HCO_3^--buffered solutions

We have previously reported that pancreatic α-cells do not exhibit a RVI when exposed to hypertonic solutions buffered with HEPES [18]. This observation was made as part of a study which determined that NKCC1 is expressed in pancreatic β-cells, but not in α-cells [18]. This study did not, however, examine all the potential volume regulatory mechanisms in the α-cells. In the present study, we have therefore examined two such mechanisms, namely: (1) the possible involvement of HCO_3^--dependent ion transporters

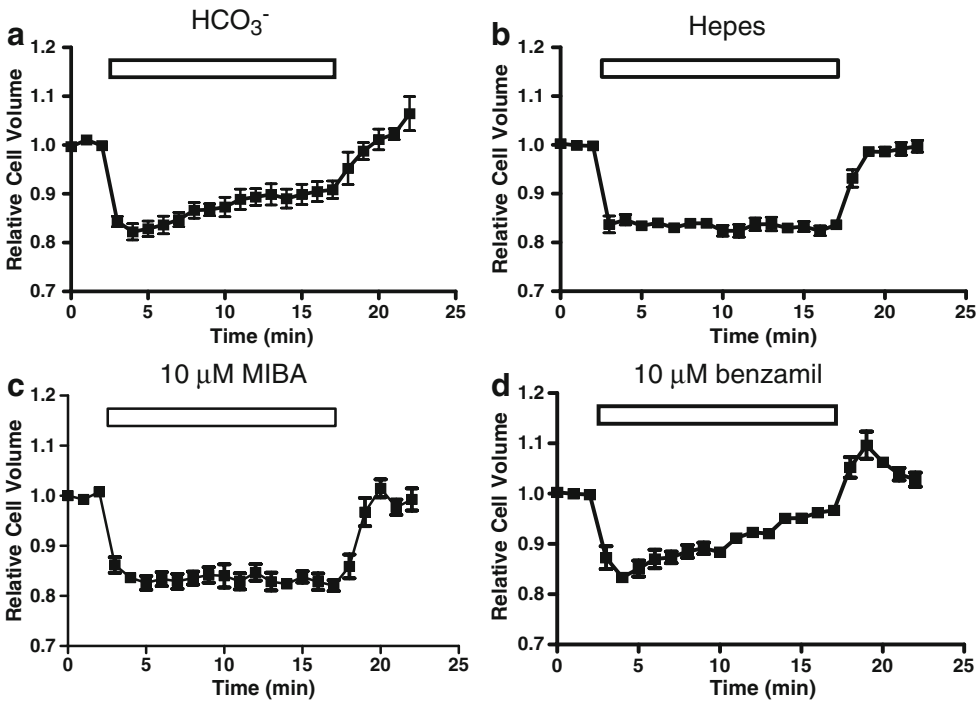

Fig. 5 Volume regulation in α-cells exposed to hypertonic solutions containing 20 mM glucose. **a** A RVI is observed in HCO_3^--buffered solutions (n=6). **b** A RVI was not observed in HEPES-buffered solutions (n=6). The RVI in HCO_3^--buffered solutions was inhibited by **c** 10 μM MIBA (n=6), but not by **d** 10 μM benzamil (n=6). The isotonic and hypertonic solutions contained 20 mM glucose

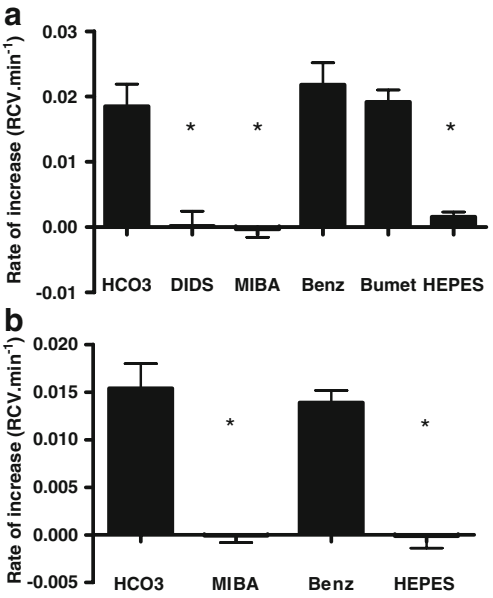

Fig. 6 Rates of volume increase in α-cells **a** during post-RVD RVI and **b** in the presence of 20 mM glucose. The rates of increase were calculated as the change in relative cell volume per minute (RCV min^{-1}) from the slopes of *straight lines* fitted to the linear phase of volume recovery by regression analysis. Data are mean±SEM (n=6), and *P<0.05 indicates significant difference to the control (HCO_3^-) by ANOVA

in volume regulation, and (2) the ability of cells to exhibit a post-RVD RVI.

α-Cells exposed to hypertonic solutions buffered with HCO_3^- did not exhibit volume regulation. This is in marked contrast to many other cells in which a combination of NHE and Cl^-–HCO_3^- exchangers contribute to Na^+ and Cl^- influx during volume regulation [13, 28]. This includes pancreatic β-cells in which NHE and Cl^-–HCO_3^- exchangers augment volume regulation mediated by NKCC1 [19]. Pancreatic of α-cells were found to exhibit a post-RVD RVI, but this was observed only in HCO_3^--buffered solutions. This ability of cells to post-RVD RVI, but not RVI in hypertonic solutions, is not unique. Indeed, some of the earliest studies of cell volume regulation reported that human lymphocytes [9] and Ehrlich ascites tumor cells [14] only exhibit a RVI in response to cell shrinkage following a RVD. Post-RVD RVI can be mediated by either NKCC1 [14] or by HCO_3^--dependent transporters (usually a combination of NHE and Cl^-–HCO_3^- exchangers; [9]). In α-cells, the lack of a post-RVD RVI in HEPES-buffered solutions indicates that NKCC1 is probably not involved. This conclusion is further supported by the observation that the post-RVD RVI in HCO_3^--buffered solutions was unaffected by the NKCC1 inhibitor 10 μM bumetanide. These data are consistent therefore with the finding that NKCC1 is not expressed in α-cells [18] and indicate that HCO_3^--dependent transporters must mediate the post-RVD RVI.

HCO_3^--dependent transporters in pancreatic α-cells

A variety of HCO_3^--dependent processes can potentially contribute to Na^+ and Cl^- influx during volume regulation. Na^+ influx for instance can be mediated by NHE, Na^+ channels (either amiloride-sensitive or insensitive; 28), and possibly by Na^+–HCO_3^- cotransporters, whereas Cl^- influx may be mediated by Cl^-–HCO_3^- exchangers or Cl^- channels. To investigate the contribution of NHE to the post-RVD RVI, we examined the differential effects of benzamil and MIBA. Both of these compounds are derived from amiloride, but have different efficacies against the ion transporters blocked by amiloride. Thus, benzamil is very effective against Na^+ channels and Na^+–Ca^{2+} exchange, but is relatively ineffective against NHE [15]. By contrast, MIBA is a potent inhibitor of NHE activity [15, 26, 27]. The post-RVD RVI was completely abolished by 10 μM MIBA, but unaffected by 10 μM benzamil. These data therefore suggest that NHE, and not amiloride-sensitive Na^+ channels, are the main mechanisms involved in RVI in the α cells. This conclusion is further supported by recent data showing that NHE1 is expressed in pancreatic α-cells [21, 25]. The possible contribution of Na^+–HCO_3^- to volume regulation cannot be discounted completely, but to date, there are no data to suggest that these transporters are inhibited by derivatives of amiloride [22].

It is more difficult to use anion transport blockers to differentiate between potential Cl^- entry pathways because these compounds lack specificity. Thus, DIDS, which inhibits the post-RVD RVI, acts on anion channels [7, 16] and Cl^-–HCO_3^- exchangers [4]. Two types of anion channel are expressed in pancreatic alpha cells: $GABA_A$ receptor-operated [29] and volume-activated anion channels (Best, L, unpublished data); furthermore, the electrochemical gradient for Cl^- favors Cl^- influx [2, 29]. However, these channels are unlikely to contribute to Cl^- influx during RVI, since the $GABA_A$ channels are opened only by GABA and the volume-activated channels are closed by cell shrinkage. Thus, the effect of DIDS on volume regulation is probably due to the inhibition of Cl^-–HCO_3^- exchangers. This conclusion, however, now requires the support of data from molecular localization studies of the expression of Cl^-–HCO_3^- exchangers in α-cells.

High concentrations of glucose activate volume regulation in α-cells

The reason as to why some cells exhibit a post-RVD RVI but not an RVI is not completely resolved [13, 28]. It is generally assumed, however, that the changes in intracellular ion activities which occur during RVD in some way facilitate the activation of the ion transporters which mediate RVI. For instance, NKCC1 and Cl^-–HCO_3^-

exchange may be activated by a reduction in the concentrations of K^+ and Cl^- [13], whereas a change in pH_i could be responsible for activating NHE and Cl^-–HCO_3^- exchange. Circumstantial evidence for the role of pH_i in activation of NHE and Cl^-–HCO_3^- exchangers is provided by our experiments in which volume regulation was studied in solutions containing 20 mM glucose.

Previous work in our laboratory has shown that α-cells and β-cells increase in cell volume by between 5% and 15% when the extracellular glucose concentration is changed from 4 to 20 mM [5, 20]. A possible explanation for this glucose-induced swelling is the activation of NHE and Cl^-–HCO_3^- exchangers. This hypothesis is supported by the observation that changes in intracellular pH occur in β-cells during glucose metabolism, which in turn stimulate NHE activity and then Cl^-–HCO_3^- exchange activity [17, 24]. The hypothesis was further tested in the present study by investigating volume regulation in α-cells bathed in hypertonic solutions containing 20 mM glucose. Under these conditions, the α-cells exhibited a RVI which was inhibited by MIBA but not by benzamil. Moreover, the RVI was observed only in HCO_3^--buffered solutions. Thus, NHE and Cl^-–HCO_3^- exchangers appear to be activated by the increase in glucose concentration and can contribute to volume regulation under these circumstances.

Conclusions

This study shows that α-cells exhibit a post-RVD RVI in HCO_3^--buffered solutions. The post-RVD RVI is inhibited by MIBA and DIDS. A MIBA-sensitive RVI was also observed in α-cells exposed to hypertonic solutions containing 20 mM glucose. The data indicate that NHE contribute to volume regulation in pancreatic α-cells and that Cl^-–HCO_3^- exchangers may also be involved. The data show that there are significant differences in ion transporter activity between pancreatic α-cells and β-cells.

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