J Membrane Biol (2009) 230:83–91
DOI 10.1007/s00232-009-9189-x

Studies of the Mechanism of Activation of the Volume-Regulated Anion Channel in Rat Pancreatic β-Cells

Len Best · Peter D. Brown

Received: 13 May 2009 / Accepted: 13 July 2009 / Published online: 8 August 2009

Abstract There is evidence that depolarization of the pancreatic β cell by glucose involves cell swelling and activation of the volume-regulated anion channel (VRAC). However, it is unclear whether cell swelling per se or accompanying changes in intracellular osmolality and/or ionic strength are responsible for VRAC activation. VRAC activity was measured in rat β cells by conventional or perforated patch whole-cell recording. Cell volume was measured by video imaging. In conventional whole-cell recordings, VRAC activation was achieved by exposure of the cells to a hyposmotic bath solution, by application of positive pressure to the pipette, or by use of a hyperosmotic pipette solution. Increased concentrations of intracellular CsCl also caused channel activation, but with delayed kinetics. In perforated patch recordings, VRAC activation was induced by isosmotic addition of the permeable osmolytes urea, 3-*O*-methyl glucose, arginine, and NH_4Cl. These effects were all accompanied by β-cell swelling. It is concluded that increased cell volume, whether accompanied by raised intracellular osmolality or ionic strength, is a major determinant of VRAC activation in the β cell. However, increased intracellular ionic strength markedly reduced the rate of VRAC activation. These findings are consistent with the hypothesis that the accumulation of glucose metabolites in the β cell, and the resultant increase in cell volume, provides a signal coupling glucose metabolism with VRAC activation.

L. Best (✉)
School of Medicine, Manchester Royal Infirmary, University of Manchester, Oxford Road, Manchester M13 9WL, UK
e-mail: Len.Best@cmft.nhs.uk

P. D. Brown
School of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9WL, UK

Keywords Anion channel · Pancreatic β cell · Volume regulation · Ionic strength · Osmolality

Introduction

The stimulation of insulin release from the pancreatic β cell by a rise in glucose concentration requires metabolism of the sugar and involves depolarization of the cell membrane potential, resulting in activation of voltage-sensitive calcium channels and hence calcium entry into the cell (for reviews see Ashcroft and Rorsman 1989; Best and McLaughlin 2004; MacDonald et al. 2005). This process is manifest as a characteristic pattern of calcium-dependent electrical activity. The molecular mechanisms coupling glucose metabolism to depolarization have been the subject of intensive investigation during the last 20 years. The current 'consensus model' for glucose-induced depolarization is thought to be a rise in ATP/ADP ratio resulting from increased metabolic flux. This leads to an inhibition of K_{ATP} channel activity, producing a net inward current. However, there is increasing evidence that additional ionic mechanisms could couple glucose metabolism to β-cell depolarization (Best and McLaughlin 2004). In particular, activation of the volume-regulated anion channel (VRAC) in β cells by glucose appears likely to contribute a depolarizing current (via Cl^- efflux), particularly at increased glucose concentrations ($\sim$5–20 mM), where K_{ATP} channel inhibition is close to maximal (Best 1997, 2000, 2002; Jakab et al. 2006).

Previous studies from this laboratory have suggested that VRAC activation by glucose requires metabolism of the sugar (Best 2002) and is accompanied by an increase in β-cell volume (Miley et al. 1997). Thus, we have suggested that glucose metabolism, and the subsequent intracellular

accumulation of glucose metabolites and osmotic entry of water, causes β-cell swelling and hence VRAC activation (Best and McLaughlin 2004). Consistent with this hypothesis, β-cell swelling by exposure to hypotonic extracellular media also results in depolarization, electrical activity, and insulin release (Best et al. 1996a; Drews et al. 1998; Beauwens et al. 2006), thus mimicking to some extent the stimulatory effects of glucose. These observations suggest that glucose-induced β-cell swelling could be an important step in coupling glucose metabolism to VRAC activation and electrical activity. However, studies of the determinants of VRAC activation in nonislet cell types have concluded that VRAC activation in response to cell swelling is actually caused by a concomitant reduction in intracellular ionic strength rather than by an increased cell volume per se (Nilius et al. 1998, 2001; Voets et al. 1999; Sabirov et al. 2000). It has also been suggested that reduced ionic strength (Voets et al. 1999) or, possibly, intracellular [Cl^-] and other electrolytes (Jackson et al. 1996; Emma et al. 1997) could modulate channel activity in terms of its volume set-point. While such a mechanism could explain VRAC activation by exposure to hypotonic extracellular media, it would not readily explain how channel activation could be brought about by an intracellular accumulation of glucose metabolites, which, since these metabolites are charged, would be expected to cause cell swelling via a rise in both intracellular osmolality and ionic strength.

The aim of the present study was therefore to determine whether VRAC activation in pancreatic β cells is the result of changes in intracellular osmolality, ionic strength, or cell volume in an attempt to understand how channel activation might be brought about by increased glucose metabolism in the β cell. Our findings suggest that increased cell volume per se is a key step in VRAC activation, although the kinetics of activation are influenced by the ionic composition of the intracellular medium.

Materials and Methods

Pancreatic islets were isolated from a total of eight Sprague-Dawley rats (350–400 g, either gender, killed by stunning and cervical dislocation in accordance with local regulations) by collagenase digestion (Worthington type 4; Cambridge Biosciences, Cambridge, UK). Islets were dispersed into single cells by a brief exposure to Ca^{2+}-free medium containing (mM) NaCl (130), KCl (5), $MgSO_4$ (1), NaH_2PO_4 (1), glucose (4), EGTA (1), and HEPES-NaOH (25; pH 7.4). Cells were suspended in HEPES-buffered Minimal Essential Medium (Invitrogen, Paisley, UK) supplemented with 5% (v/v) fetal calf serum and 50 μg/ml gentamycin and cultured in 30-mm-diameter polystyrene dishes for 3–10 days in humidified air at 37°C. β cells were identified by their size (larger than non-β cells [Majid et al. 2001]) and characteristic granular appearance. HEK-293 cells were kindly provided by Dr. Jason Bruce, University of Manchester, and used immediately after plating onto dishes. Cells were superfused at approximately 2 ml/min with a bath solution consisting of (mM) NaCl (130), KCl (4), CsCl (1), $MgSO_4$ (1), NaH_2PO_4 (1), $CaCl_2$ (1.2), glucose (4) and HEPES-NaOH (25; pH 7.4; 305 mOsmol kg H_2O^{-1}). Experiments were carried out to test the effects of isosmotic addition of permeant osmolytes. In the case of the charged osmolytes arginine hydrochloride and NH_4Cl, these were substituted for an equivalent concentration of NaCl. For the uncharged osmolytes urea and 3-*O*-methyl glucose, the control medium contained 100 mM mannitol substituting for 50 mM NaCl, addition of osmolytes then being made by substituting for mannitol.

Whole-cell currents were recorded using either conventional or perforated patch whole-cell recording techniques using a List EPC-7 amplifier. In the former case, the basic pipette solution had a nominal ionic strength (Γ) of 155 (calculated from total ionic concentration; designated Γ_{155}) and consisted of (mM) CsCl (60), $MgCl_2$ (2), ATP (1), EGTA (1), and HEPES (10; pH 7.2). The measured osmolality of this solution (by freezing-point depression) was 135 mOsmol kg H_2O^{-1}. Mannitol or CsCl was added to this solution to increase the osmolality and/or ionic strength as detailed in the text and figure legends. For perforated patch recording, the pipette solution consisted of (mM) $MgCl_2$ (1), HEPES (10; pH 7.2), amphotericin B (240 μg/ml), and various concentrations of CsCl. Amphotericin is permeable to small monovalent cations and anions (Ermishkin et al. 1977), thus allowing the manipulation of Γ_i by altering the salt concentration of the pipette solution (Best 2005). Pipette resistance was approximately 2.5 MΩ when filled with the Γ_{155} solution. Access resistance was ~10 and 25 MΩ in conventional and perforated patch whole-cell configurations, respectively. No voltage compensation was made. In all cases, the liquid junction potential generated by asymmetrical pipette and bath solutions was nulled using the amplifier 'V_p offset' control prior to seal formation. Activity of the VRAC was measured routinely by subjecting the cells to 200-ms voltage pulses of ± 100 mV at 4-s intervals from a holding potential of 0 mV. This protocol provides a constant indication of VRAC activity. In addition, representative I/V relationships were examined under conventional and perforated patch conditions. This involved holding the cell at 0 mV and applying 500-ms pulses increasing in 20-mV steps from −100 to +100 mV. Zero current is indicated by the dashed lines on the recording traces. The majority of recordings are shown immediately following 'breakthrough' or, in the case of perforated patch recordings, following seal formation. In some cases, only

the section of recording following maximal current activation is shown. Cell size was within the range 8–12 pF and current amplitudes are expressed as picoamperes/picofarads. All current amplitudes depicted in the histograms represent equilibrium or 'steady-state' measurements. Relative cell volume was measured using a video-imaging technique as previously described (Miley et al. 1997; Best et al. 1996b). Data are expressed as mean ± SE, and statistical significance was ascribed using ANOVA with a Dunnett post hoc correction or Student's paired *t*-test as specified in the figure legends.

5-Nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), urea, 3-*O*-methyl glucose, arginine hydrochloride, and all other chemicals were obtained from Sigma (Poole, UK).

Results

The first series of experiments investigated VRAC activity under conventional whole-cell recording conditions. With the Γ_{155} pipette solution containing 100 mM mannitol (i.e., when a low-Γ pipette solution was used in the absence of cell swelling), no current activation was observed (Fig. 1a, b). This finding contrasts with previous reports that spontaneous channel activation in the absence of cell swelling could be observed with a low-Γ pipette solution in Chinese hamster ovary cells and pulmonary endothelial cells (Voets et al. 1999; Cannon et al. 1998). Such spontaneous activation due to reduced intracellular Γ (Γ_i) was not observed in β cells even when Γ_i was reduced to a nominal value of 75 (20 mM CsCl; not shown). On the other hand, VRAC activation could be induced by swelling the cells by exposure to a 33% hyposmotic bath solution (Fig. 1a). In addition, a similar current could be activated by imposing positive pressure on the pipette ($\sim$300 mm H_2O; Fig. 1b). Although we have not characterized the latter current in detail, its outwardly rectifying nature and reversal potential (approx −10 mV; compared with an estimated E_{Cl} of −20 mV) are consistent with our previous report of a significant cation permeability of the β-cell VRAC (Best et al. 1996b).

We have previously shown that the VRAC in rat pancreatic β cells, in common with several other cell types, can also be activated with essentially similar characteristics in conventional whole-cell mode by the use of a hyperosmotic pipette solution, typically by the addition of mannitol (Best et al. 1996b, 2001). This effect is shown in Fig. 1c, d, where 200 mM mannitol was added to the pipette solution in order to raise the intracellular osmolality (OSM_i) and hence induce cell swelling without an increase in Γ_i. Under such conditions, VRAC activation could be reversed by application of a hyperosmotic bath solution (Fig. 1c). Thus, activation of the current required cell swelling rather than

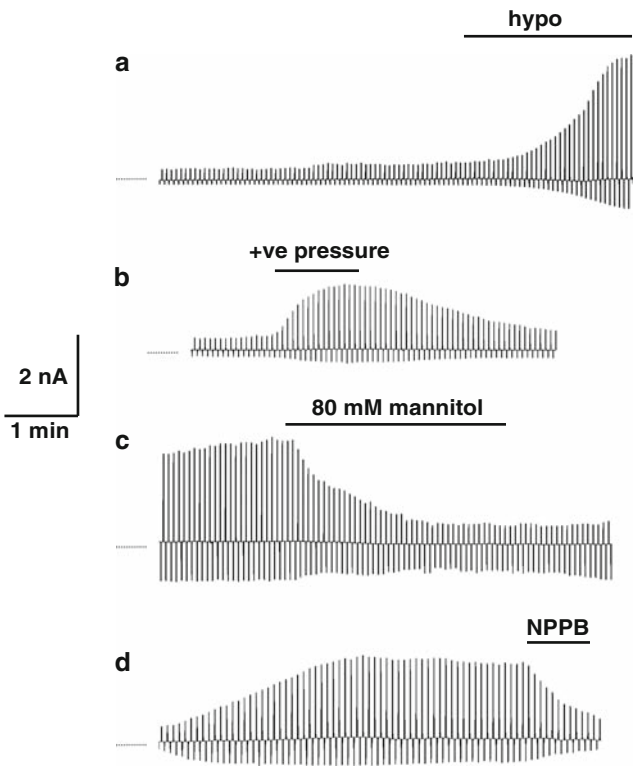

Fig. 1 Conventional whole-cell recordings of VRAC currents in rat pancreatic β cells. Mannitol (100 mM, **a** and **b**; 200 mM, **c** and **d**) was added to the Γ_{155} pipette solution in order to raise OSM_i but not Γ_i. Effects of a 33% hyposmotic bath solution (removal of 100 mM mannitol; **a**), positive pipette pressure (**b**), increasing osmolality of the bath solution (**c**), and the VRAC inhibitor NPPB (50 µM; **d**). Currents were elicited by voltage pulses of ±100 mV. Recordings a, b, and d were made immediately after breakthrough into the cell, whereas c was made $\sim$5 min after breakthrough. The dotted lines represent zero current

simply a rise in intracellular osmolality. As previously reported (Best et al. 1996b), the amplitudes of both outward and inward currents were also reduced by the VRAC blocker NPPB (by 74% and 57%, respectively; $n = 4$; Fig. 1d).

It is evident from Fig. 2 that, under conventional whole-cell recording conditions, a significant degree of activation of the outwardly rectifying current was achieved when the pipette solution contained 150 mM mannitol; that is, when the intracellular osmolality was approximately equivalent to that of the bath solution. This finding is consistent with the suggestion that, under conventional whole-cell recording conditions, an isosmotic pipette solution behaves as if it is hyperosmotic by $\sim$20% with respect to the bath solution (Worrell et al. 1989). This phenomenon presumably reflects the osmotic effects of intracellular proteins which remain undialyzed from the cell together with the hydrostatic pressure resulting from the column of liquid in the recording pipette. With a pipette solution containing 200 mM mannitol, current activation was maximal and rapid, with

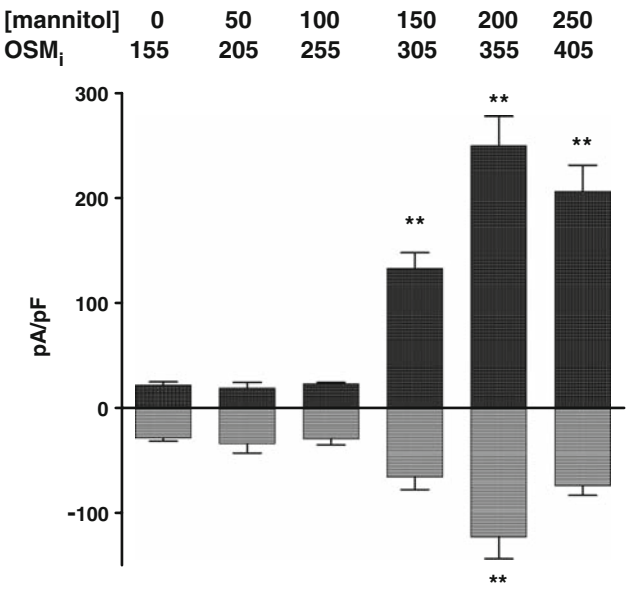

Fig. 2 Conventional whole-cell VRAC current amplitudes in rat pancreatic β cells: effect of increasing the intracellular osmolality (OSM_i) by the addition of mannitol. Maximum mean outward (+ve) and inward (−ve) currents from conventional whole-cell recordings. Currents were elicited by voltage pulses of ±100 mV. Values are mean ± SE for 5–10 cells. ** $p < 0.001$ vs. column 1 (ANOVA with Dunnett post hoc test)

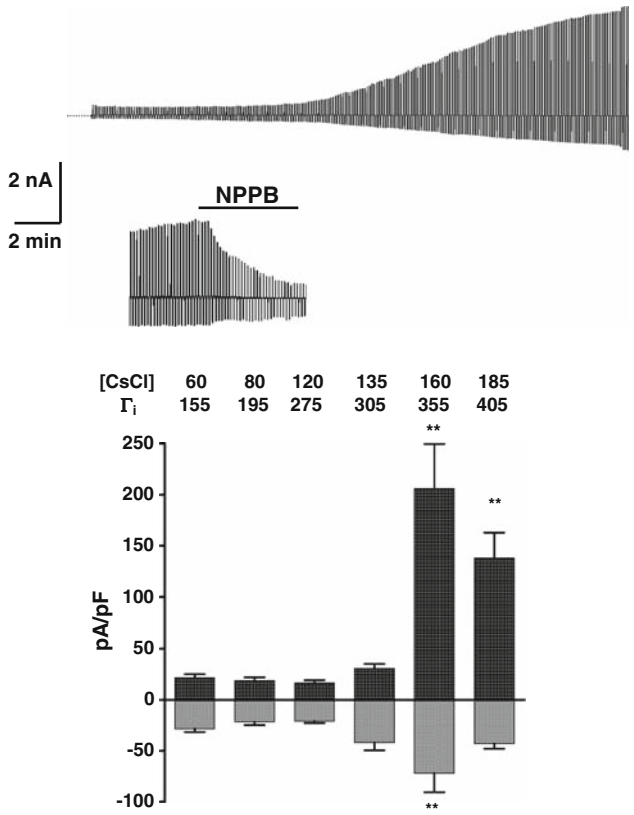

Fig. 3 Conventional whole-cell recordings of VRAC currents in rat pancreatic β cells: effect of increasing intracellular ionic strength (Γ_i) by addition of CsCl. *Upper*: Recordings from two cells; 100 mM CsCl was added to the Γ_{155} pipette solution (Γ_{155}) in order to raise both OSM_i and Γ_i to 355. Currents were elicited by voltage pulses of ±100 mV. *Lower*: maximum mean outward (+ve) and inward (−ve) currents with increasing concentrations (total) of CsCl in the pipette solution (±SE) for 5–10 cells. ** $p < 0.001$ vs. column 1 (ANOVA with Dunnett post hoc test)

half-maximal activity ($t_{0.5}$) observed 1.2 ± 0.1 min ($n = 10$) after formation of the whole-cell configuration (for examples, see Fig. 1a, d). Current reversal was −8.0 ± 0.5 mV ($n = 12$), again consistent with our previous report (Best et al. 1996b).

Taken together, the above findings suggest that increased cell volume, rather than reduced Γ_i, is the major determinant of VRAC activation in pancreatic β cells. This contrasts with previous reports using nonislet cell types in which VRAC activation or the volume set-point of the channel appeared to be inversely correlated with Γ_i (Nilius et al. 1998; 2001; Voets et al. 1999; Sabirov et al. 2000). The next series of experiments therefore investigated the effects of simultaneously raising OSM_i and Γ_i by the addition of CsCl (Fig. 3). No significant activation was observed when Γ_i and OSM_i were raised to 305 by the addition of 75 mM CsCl (Fig. 3, lower), consistent with an increase in volume set-point of channel activation by a rise in Γ_i. The further addition of CsCl to raise Γ_i and OSM_i to 355 was found to cause maximal VRAC activation to a degree similar to that seen with 200 mM mannitol. Channel activation was again sensitive to inhibition by NPPB and to exposure to a hypertonic bath solution (not shown), consistent with the characteristics of the VRAC currents. However, in this case the rate of channel activation was significantly reduced compared to raising the OSM_i alone, such that $t_{0.5}$ was now reached 16.1 ± 1.3 min ($n = 5$) after formation of the whole-cell configuration (Fig. 3, upper). The reversal potential of the current was −0.5 ± 1.3 mV ($n = 5$), comparable to E_{Cl} (approximately +5 mV) under such conditions. A further increase in Γ_i to 405 also caused VRAC activation, but with currents of reduced amplitude (Fig. 3, lower). A similar pattern of VRAC activation was observed when OSM_i and Γ_i were increased by the addition of 100 mM Cs lactate, Cs gluconate, or NMDG-Cl to the pipette solution, the respective whole-cell currents in response to voltage pulses of ±100 mV being 214 ± 37 and −61 ± 11 ($n = 5$), 188 ± 41 and −46 ± 9 ($n = 4$), and 160 ± 20 and −48 ± 9 ($n = 4$) pA/pF with $t_{0.5}$ values of 12.0 ± 3.4, 10.8 ± 1.8, and 11.3 ± 1.1 min, respectively (not shown). Thus, VRAC activation with a high Γ_i does not appear to depend on the ionic species present in the intracellular solution.

A limited number of experiments were carried out to investigate the influence of OSM_i and Γ_i on VRAC activation in nonislet cells. For these experiments, we used the

human embryonic kidney HEK-293 cell line, in which VRAC activation has been reported in response to a reduction in intracellular ionic strength (Nilius et al. 2001). As shown in Fig. 4, qualitatively similar results were obtained with these cells. Thus, no VRAC activation was apparent when the cell interior was perfused with a low-Γ_i pipette solution (Fig. 1a). On the other hand, raising OSM_i by the addition of 200 mM mannitol or raising Γ_i with 100 mM CsCl resulted in channel activation. However, it was again apparent that the kinetics of activation were considerably slowed with a high-Γ pipette solution.

We next investigated the influence of Γ_i on VRAC activity in intact β cells, using the perforated patch technique, which maintains the integrity of the cytosolic compartment. Amphotericin B was used as perforating agent since this antibiotic is known to form pores which are permeable to small monovalent ions. While it is not possible to predict exactly how closely Γ_i will correspond to the ionic strength of the pipette solution, Fig. 5 clearly shows that with 40 mM CsCl in the pipette solution, no significant VRAC activation was observed, the current amplitudes being 26 ± 4 and −21 ± 3 pA/pF ($n = 7$). Figure 5 also illustrates that raising the CsCl concentration to 140 mM or above leads to activation of the VRAC current. Subsequent exposure of the cell to a hypertonic bath solution gradually inhibited the current while NBBP also inhibited channel activity (not shown), again consistent with the characteristics of VRAC currents. As with the conventional whole-cell configuration, VRAC activation under perforated patch conditions was relatively slow with a high Γ_i, the $t_{0.5}$ values with pipette solutions containing 140, 240, and 340 mM CsCl being 13.7 ± 1.5 ($n = 6$), 13.8 ± 2.3 ($n = 10$), and

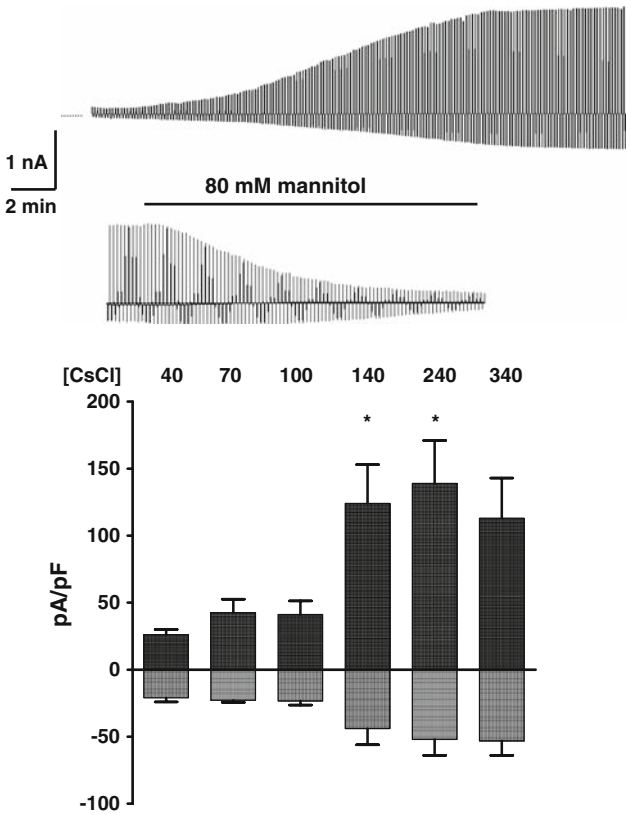

Fig. 5 Perforated patch recordings VRAC currents in pancreatic β cells: effect of increasing intracellular ionic strength by addition of CsCl. Upper: Recordings from two cells; the pipette solution contained 240 mM CsCl. Currents were elicited by voltage pulses of ±100 mV. Lower: maximum mean outward (+ve) and inward (−ve) currents with increasing concentrations of CsCl in the pipette solution (±SE) from 6 to 10 cells. * $p < 0.05$ vs. column 1 (ANOVA with Dunnett post hoc test)

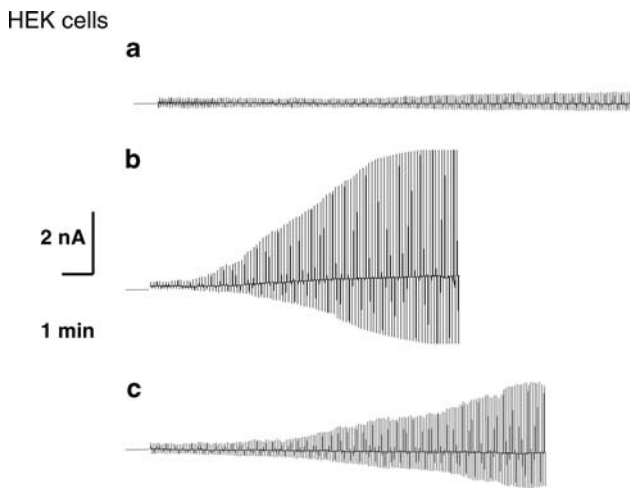

Fig. 4 Conventional whole-cell recordings of VRAC currents in HEK cells: hypotonic (Γ_{155}) pipette solution supplemented with **a** 100 mM mannitol, **b** 200 mM mannitol, or **c** 100 mM CsCl. Currents were elicited by voltage pulses of ±100 mV. Recordings are representative of those from three or four cells in each case

16.7 ± 1.7 ($n = 6$) min, respectively. Comparing Figs. 3 and 5, it is notable that VRAC activation could be achieved under perforated patch conditions using pipette solutions of considerably lower ionic strength than with conventional whole-cell recordings. The reasons for this are not certain but it is likely that the composition of the intracellular compartment is significantly different between the two experimental conditions, with a greater retention of the normal cytosolic constituents (ions, nucleotides, proteins, etc.) under perforated patch conditions. This suggests that VRAC activation may be more sensitive to changes in Γ_i in intact cells than in cells where the cytosol has been dialyzed by the pipette solution. VRAC current–voltage relationships under conventional and perforated patch conditions are depicted in Fig. 6; the characteristics of the current were essentially similar in both recording configurations. Again, in contrast to conventional whole-cell recordings, the reversal potential of the current was not apparently shifted to a more positive value by a high pipette CsCl solution. The reasons for this are unclear but could be related to the fact

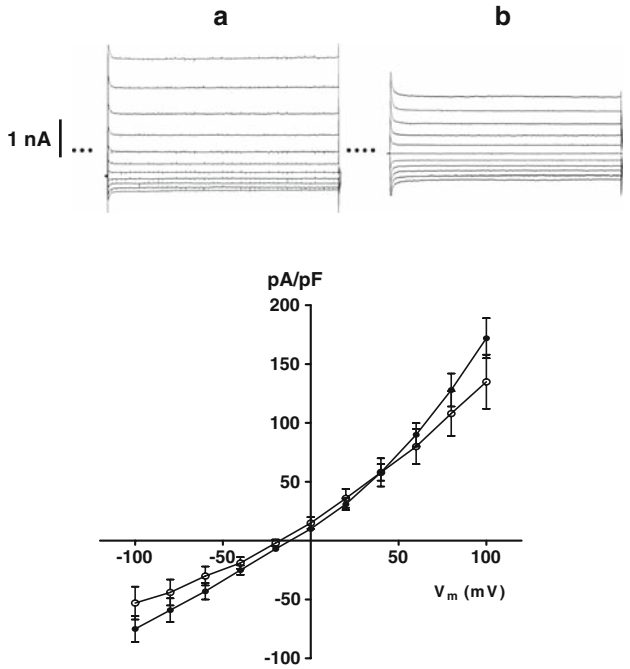

Fig. 6 VRAC current–voltage relationships in pancreatic β cells. Conventional whole-cell configuration with a pipette solution containing 200 mM mannitol (**a**; *filled circles*) and perforated patch recordings with a pipette solution containing 240 mM CsCl (**b**; *open circles*). The I/V plots are derived from five cells in each case

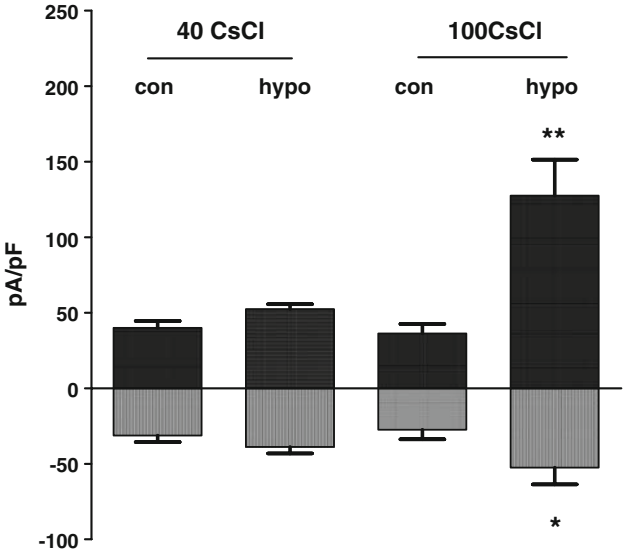

Fig. 7 VRAC amplitudes in pancreatic β cells in response to a hyposmotic bath solution: effect of Γ_i. Data were obtained from perforated patch recordings. The pipette solution contained either 40 or 100 mM CsCl. The bath solution was made ~33% hyposmotic by the removal of 100 mM mannitol. Currents were elicited by voltage pulses of ±100 mV. Mean outward (+ve) and inward (−ve) currents (±SE) from 5 to 10 cells. ** $p < 0.01$ and * $p < 0.05$ vs. control (paired *t*-test)

that the amphotericin B channel shows cation selectivity at physiological pH (Asandei and Luchian 2008), which could result in a higher intracellular concentration of Cs^+ than Cl^- under these conditions.

As shown in Fig. 1, the VRAC can be activated under conventional whole-cell recording conditions by exposure to a hypotonic extracellular solution. A similar effect of a 33% hypotonic bath solution could be demonstrated under perforated patch conditions (Fig. 7). However, VRAC activation by such a means was only observed with a pipette solution containing a raised 'threshold' concentration of CsCl (100 mM); no significant effect was apparent with a pipette solution containing 40 mM CsCl. This finding indicates that, in the perforated patch configuration, the composition of the pipette solution influences that of the cytosol and that a threshold level of Γ_i (or OSM_i) is required for channel activation.

An additional series of experiments was carried out to investigate further the effects of increased OSM_i or Γ_i on VRAC activity in intact β cells using the perforated patch technique. Initially, cell swelling was induced by exposing the cells to the permeant osmolytes urea and 3-*O*-methyl glucose under isosmotic conditions (i.e., substituting for an equivalent amount of the nonpermeant osmolyte mannitol). This maneuver would be expected to swell the cells through an increase in OSM_i with a concomitant reduction in Γ_i. As shown in Fig. 8, isosmotic addition of urea or 3-*O*-methyl glucose caused a significant increase in outward VRAC current, while the latter osmolyte also significantly increased the inward current amplitude. The corresponding $t_{0.5}$ values for channel activation were 8.5 ± 2.1 min ($n = 4$) and 6.3 ± 1.4 min ($n = 5$), respectively.

Figure 8 also shows the effect of isosmotic addition of arginine (substituting for Na^+), which enters the β cell presumably via the cationic amino acid transporter (Smith et al. 1997). Since arginine is a positively charged molecule, its uptake should lead to a net increase in Γ_i. Accumulation of this amino acid was associated with a significant activation of the VRAC, with a $t_{0.5}$ of 6.6 ± 1.8 min ($n = 5$). The isosmotic addition of NH_4Cl has been reported to cause cell swelling due to the passive entry of NH_3, which forms NH_4^+ accompanied by OH^- (or HCO_3^-)/Cl^- exchange, resulting in a net accumulation of NH_4Cl (Motais et al. 1991). In this case, as with isosmotic arginine addition, swelling would be associated with an increase in Γ_i. As shown in Fig. 8, the isosmotic addition of NH_4Cl (by substitution for NaCl) to pancreatic β cells increased both inward and outward current amplitudes, although with a relatively modest effect on the latter compared to arginine.

We also measured the effect of isosmotic addition of permeant osmolytes on β-cell volume (Fig. 9). The isosmotic addition of 100 mM urea or 3-*O*-methyl glucose

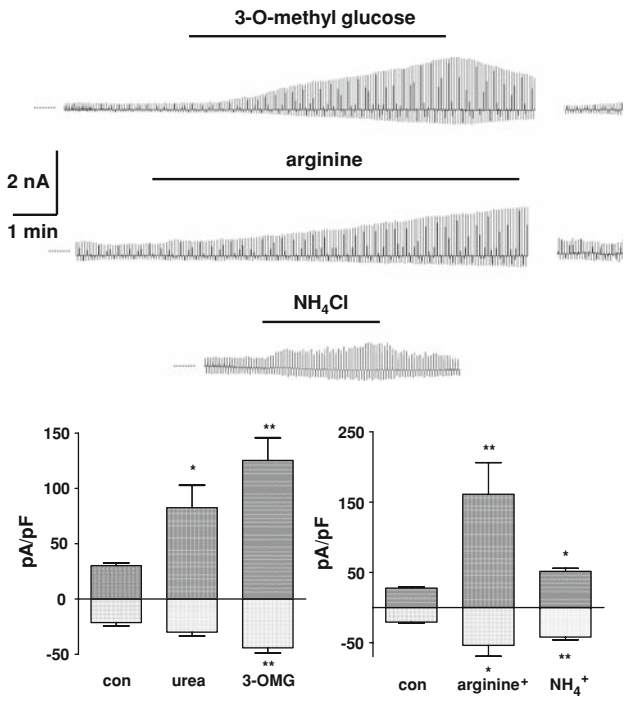

Fig. 8 VRAC amplitudes in pancreatic β cells: effects of permeable nonionic and ionic osmolytes. Data were obtained from perforated patch recordings. The pipette solution contained 100 mM CsCl. Urea or 3-*O*-methylglucose (both 100 mM) was added to the bath solution by substitution for mannitol. Arginine hydrochloride or NH_4Cl (both 50 mM) was added to the bath solution by substitution for NaCl. Currents were elicited by voltage pulses of ± 100 mV. Mean outward (+ve) and inward (−ve) currents (±SE) from five to seven cells. ** $p < 0.02$ and * $p < 0.05$ vs. control (paired *t*-test). The gaps in the upper and middle traces are 3–5 min

(substituting for mannitol) increased the relative cell volume by ~11.5% and 11%, respectively, within 3 min of application. Most notably in the case of urea, cell swelling was followed by a gradual regulatory volume decrease (RVD) response. The isosmotic addition of 50 mM arginine hydrochloride or NH_4Cl (in both cases substituting for NaCl) also increased β-cell volume by 3% and 6%, respectively, again with subsequent RVD responses.

Discussion

Activation of the VRAC in pancreatic β cells, in common with virtually all other cell types, can be elicited by exposure to hyposmotic extracellular media (Best et al. 1996a, b). There is also evidence that the channel can be activated specifically in β cells by a rise in glucose concentration and the ensuing increased glycolytic flux (Best 1997, 2000, 2002; Jakab et al. 2006), an effect also accompanied by cell swelling (Miley et al. 1997). As noted above, studies in nonislet cells have suggested that a reduction in Γ_i rather than the accompanying increase in cell volume could be responsible for activation or modulation of the channel during hypotonic cell swelling. However, such a mechanism is difficult to reconcile with activation of the VRAC in β cells by glucose metabolism, which would be expected to be accompanied by the intracellular generation of glucose metabolites and an increase in Γ_i. It should be emphasized that, at least on the basis of its anion selectivity sequence, the β cell VRAC

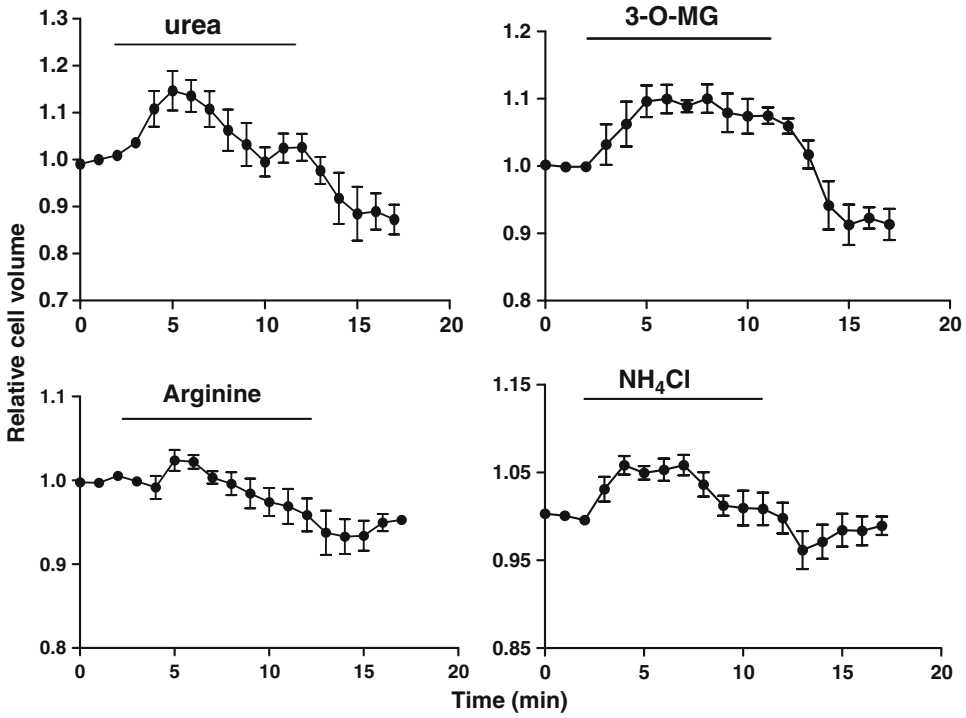

Fig. 9 Relative cell volume: effects of permeable nonionic and ionic osmolytes. Urea or 3-*O*-methylglucose (both 100 mM) was added to the bath solution by substitution for mannitol. Arginine hydrochloride or NH_4Cl (both 50 mM) was added to the bath solution by substitution for NaCl. Data (mean ± SE) were obtained from four cells in each case

appears to be distinct from that in other nonislet cell types (Kinard and Satin 1995; Best et al. 1996b).

The present study provides several lines of evidence that, at least in the case of the pancreatic β cell, VRAC activation can be induced by an increase in cell volume, irrespective of any accompanying changes in Γ_i. First, the application of positive pressure to the pipette solution was sufficient to activate the channel, consistent with a previous report using CHO cells (Cannon et al. 1998). These authors argued that this maneuver should not alter the composition of the intracellular medium, so that VRAC activation was probably due to cell swelling. However, Voets et al. (1999) suggested that, under conventional whole-cell recording conditions, Γ_i could be affected by unequal rates of water flux across the cell membrane and via the pipette aperture. Presumably, a similar argument could apply, perhaps to a lesser extent, to the interpretation of perforated patch experiments. Differences in water and solute fluxes among the cell interior, the bath solution, and the pipette solution will presumably affect cell volume in addition to Γ_i. Such factors might possibly explain why, in contrast to a previous report (Nilius et al. 2001), we were unable to observe VRAC activation with a pipette solution of low Γ and osmolality in either β cells or HEK-293 cells.

The second line of evidence supporting a primary role for increased β-cell volume in VRAC activation is that raising the Γ_i with increasing concentrations of CsCl was found to cause VRAC activation under both conventional and perforated patch conditions. Third, the uptake of urea, 3-*O*-methyl glucose (both uncharged), or arginine (charged) evoked a comparable degree of VRAC activation. To a lesser extent, NH_4Cl uptake was also found to activate the channel. Incidentally, the observed VRAC activation following urea and 3-*O*-methyl glucose uptake could explain the stimulation of insulin release previously reported to be evoked by such maneuvers (Lund et al. 1992). Furthermore, activation of the channel by arginine accumulation could contribute towards the depolarizing action of this amino acid (Smith et al. 1997).

Despite the fact that maximal VRAC currents were comparable whether accompanied by increased or decreased Γ_i, there was clear evidence that both the set-point and the kinetics of VRAC activation could be influenced by changes in Γ_i. Specifically, the rate of VRAC activation appeared to be reduced at high Γ_i values in both conventional and perforated patch configurations. This effect of ionic strength on VRAC activation rate is qualitatively similar to that in previous reports (Jackson et al. 1996; Emma et al. 1997; Cannon et al. 1998), although these studies did not demonstrate that maximal current magnitudes attained were similar whether with a high or a low Γ_i. The physiological significance of VRAC modulation by Γ_i is at present unclear. Since it was not possible in the present study to make accurate comparisons of cell volume changes under patch clamp conditions, it is uncertain whether this effect of high Γ_i on VRAC activation kinetics was due to a reduced rate of cell swelling. However, visual inspection of the cells during the experiments clearly revealed cell swelling with both low- and high-Γ pipette solutions, while earlier studies also suggested this to be an unlikely explanation (Jackson et al. 1996; Emma et al. 1997). It is also highly unlikely that differential rates of equilibration between the pipette and the intracellular solutions could account for the effect of high Γ_i on VRAC activation kinetics. Thus, as in other cell types, the β-cell VRAC could be modulated, either directly or indirectly, by Γ_i independently of cell volume changes. In view of this possibility, it is curious that the kinetics of VRAC activation during uptake of urea, 3-*O*-methyl glucose, and arginine were essentially comparable ($t_{0.5}$ values, $\sim$6–8 min). While the rate of cell swelling and thus VRAC activation will depend on the relative rates of osmolyte uptake, cell volume measurements indicated that these rates were comparable for the above osmolytes.

The mechanism(s) by which VRAC activity is regulated by changes in cell volume remains to be established. Numerous intracellular components and signaling pathways have been proposed to participate in this coupling, including the cytoskeleton, the caveolae, and the swelling-induced release of arachidonate (for reviews see Eggermont et al. 2001; Hoffmann 2000). The release of H_2O_2 during cell swelling through activation of an NAD(P)H oxidase has also been implicated in VRAC activation (Varela et al. 2004). Such a mechanism could theoretically be relevant to the β cell, where glucose has been reported to modulate NAD(P)H oxidase activity (Morgan et al. 2007). However, recent preliminary studies in our laboratory indicated that VRAC activity in rat pancreatic β cells was unaffected by H_2O_2 within the range 0.1–2 mM under perforated patch conditions (unpublished observations). The results of the present study are consistent with the hypothesis that VRAC activation by glucose in the pancreatic β cell is the result of the accumulation of glucose metabolites, leading to raised OSM_i and/or Γ_i and, hence, cell swelling (Best and McLaughlin 2004). However, it remains to be established which glucose metabolite(s) is important in this context. Lactate production accounts for a significant fraction ($\sim$50%) of glucose metabolism (Sener and Malaisse 1976; Best et al. 1989), and it is noteworthy that pancreatic β cells do not show lactate transport activity (Best et al. 1992) or express the MCT lactate transporter (Zhao et al. 2001). Thus, it is possible that accumulation of lactate could be a determinant of cell swelling and VRAC activation during increased glycolytic flux.

In conclusion, our findings suggest that increased β-cell volume is an important determinant of VRAC activation.

Further studies are clearly required to define fully how glucose metabolism results in cell swelling and how VRAC activity is regulated by such volume changes.

References

Asandei A, Luchian T (2008) Ion selectivity, transport properties and dynamics of amphotericin B channels studied over a wide range of acidity changes. Colloids Surfaces B Biointerfaces 67:99–106

Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic β-cell. Prog Biophys Mol Biol 54:87–143

Beauwens R, Best L, Markadieu N, Crutzen R, Louchami K, Brown PD, Yates AP, Malaisse WJ, Sener A (2006) Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. Endocrine 30:353–364

Best L (1997) Glucose and a-ketoisocaproate induce transient inward currents in rat pancreatic beta cells. Diabetologia 40:1–6

Best L (2000) Glucose-sensitive conductances in rat pancreatic beta-cells: contribution to electrical activity. Biochim Biophys Acta 1468:311–319

Best L (2002) Study of a glucose-activated anion-selective channel in rat pancreatic β-cells. Pflugers Arch 445:97–104

Best L (2005) Glucose-induced electrical activity in rat pancreatic β-cells: dependence on intracellular chloride concentration. J Physiol 568:137–144

Best L, McLaughlin J (2004) Nutrients as regulators of endocrine and neuroendocrine secretion. In: Taylor P, Winderickx J (eds) Topics in current genetics, vol 7. Springer, New York, pp 79–111

Best L, Yates AP, Meats JE, Tomlinson S (1989) Effects of lactate on pancreatic islets: lactate efflux as a possible determinant of islet cell depolarization by glucose. Biochem J 259:507–511

Best L, Trebilcock R, Tomlinson S (1992) Lactate transport in insulin-secreting β-cells: contrast between rat islets and HIT-T15 insulinoma cells. Mol Cell Endocr 86:49–56

Best L, Miley HE, Yates AP (1996a) Activation of an anion conductance and B-cell depolarization during hypotonically-induced insulin release. Exp Physiol 81:927–933

Best L, Sheader EA, Brown PD (1996b) A volume-activated anion conductance in insulin-secreting cells. Pflugers Arch 431:363–370

Best L, Speake T, Brown PD (2001) Functional characterization of the volume-sensitive anion channel in rat pancreatic β-cells. Exp Physiol 86:145–150

Cannon CL, Basavappa S, Strange K (1998) Intracellular ionic strength regulates the volume sensitivity of a swelling-activated anion channel. Am J Physiol 275:C416–C422

Drews G, Zempel G, Krippeit-Drews P, Britsch S, Busch GL, Kaba NK, Lang F (1998) Ion channels involved in insulin release are activated by osmotic swelling of pancreatic B-cells. Biochim Biophys Acta 1370:8–16

Eggermont J, Trouet D, Carton I, Nilius B (2001) Cellular function and control of volume-regulated anion channels. Cell Biochem Biophys 35:263–274

Emma F, McManus M, Strange K (1997) Intracellular electrolytes regulate the volume set point of the organic osmolyte/anion channel VSOAC. Am J Physiol 272:C1766–C1775

Ermishkin LN, Kasumov KM, Potseluyev VM (1977) Properties of amphotericin B channels in a lipid bilayer. Biochim Biophys Acta 470:357–367

Hoffmann E (2000) Intracellular signalling involved in volume regulatory decrease. Cell Physiol Biochem 10:273–288

Jackson PS, Churchwell K, Ballatori N, Boyer JL, Strange K (1996) Swelling-activated anion conductance in skate hepatocytes: regulation by cell Cl and ATP. Am J Physiol 270:C57–C66

Jakab M, Grundbichler M, Benicky J, Ravasio A, Chwatal S, Schmidt S, Strbak V, Furst J, Paulmichl M, Ritter M (2006) Glucose induces anion conductance and cytosol-to-membrane transposition of ICln in INS-1E rat insulinoma cells. Cell Physiol Biochem 18:21–34

Kinard TA, Satin LS (1995) An ATP-sensitive Cl^- channel current that is activated by cell swelling, cAMP and glyburide in insulin-secreting cells. Diabetes 44:1461–1466

Lund PE, Berts A, Hellman B (1992) Stimulation of insulin release by isosmolar addition of permeant molecules. Mol Cell Biochem 109:77–81

MacDonald PE, Joseph JJ, Rorsman P (2005) Glucose-sensing mechanisms in pancreatic β-cells. Phil Trans R Soc B 360: 2211–2225

Majid A, Speake T, Best L, Brown PD (2001) Expression of the $Na^+/K^+/2Cl^-$ cotransporter in α and β cells isolated from the rat pancreas. Pflugers Arch 442:570–576

Miley HE, Sheader EA, Brown PD, Best L (1997) Glucose-induced swelling in rat pancreatic beta-cells. J Physiol 504:191–198

Morgan D, Oliveira-Emilio HR, Keane D, Hirata AE, Santos da Rocha M, Bordin S, Curi R, Newsholme P, Carpinelli AR (2007) Glucose, palmitate and pro-inflammatory cytokines modulate production and activity of a phagocyte-like NADPH oxidase in rat pancreatic islets and a clonal beta cell line. Diabetologia 50:359–369

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

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 506:353–361

Nilius B, Prenen J, Wissenbach U, Bodding M, Droogmans G (2001) Differential activation of the volume-sensitive cation channel TRP12 (OTRPC4) and volume-regulated anion currents in HEK-293 cells. Pflugers Arch 443:227–233

Sabirov RZ, Prenen J, Tomita T, Droogmans G, Nilius B (2000) Reduction of ionic strength activates single volume-regulated anion channels (VRAC) in endothelial cells. Pflugers Arch 439:315–320

Sener A, Malaisse WJ (1976) Measurement of lactic acid in nanomolar amounts. Reliability of such a method as an index of glycolysis in pancreatic islets. Biochem Med 15:34–41

Smith PA, Sakura H, Coles B, Gummerson N, Proks P, Ashcroft FM (1997) Electrogenic arginine transport mediates stimulus-secretion coupling in mouse pancreatic β-cells. J Physiol 499:625–635

Varela D, Simon F, Riveros A, Jorgensen F, Stutzin A (2004) NAD(P)H oxidase-derived H_2O_2 signals chloride channel activation in cell volume regulation and cell proliferation. J Biol Chem 279:3304

Voets T, Droogmans G, Raskin G, Eggermont J, Nilius B (1999) Reduced intracellular ionic strength as the initial trigger for activation of endothelial volume-regulated anion channels. Proc Natl Acad Sci USA 96:5298–5303

Worrell RT, Butt AG, Cliff WH, Frizzell RA (1989) A volume-sensitive chloride conductance in human colonic cell line T84. Am J Physiol 256:C1111–C1119

Zhao C, Wilson MC, Schuit F, Halestrap AP, Rutter G (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50:361–366