Glucose-induced swelling in rat pancreatic β-cells

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1. Changes in relative cell volume in response to hypotonic solutions and glucose were studied in single isolated rat pancreatic β-cells using a video-imaging technique. β-cell electrical activity was recorded under similar conditions using the perforated patch technique.

2. Exposure of β-cells to hypotonic solutions (10 and 33% hypotonicity) caused an immediate increase in cell volume to relative values of 1·09 and 1·33, respectively. This was followed by a gradual regulatory volume decrease.

3. Raising the concentration of glucose from 4 to 20 mM or 12 mM (with substitution of mannitol) increased β-cell volume by 12 and 10%, respectively. This effect of glucose persisted when Co^{2+} was added to inhibit insulin release. Glucose-induced volume increases were sustained for the duration of exposure to elevated hexose concentration. The addition of 16 mM 3-*O*-methylglucose, which is transported into the β-cell but not metabolized, produced only a transient 5% increase in β-cell volume.

4. Exposure of β-cells to a 15% hypotonic solution resulted in a transient depolarization and electrical activity. Raising the glucose concentration to 20 or 12 mM caused a sustained depolarization and generation of electrical activity. However, the addition of 16 mM 3-*O*-methylglucose had no effect on β-cell membrane potential. The glucose-induced increase in volume and induction of electrical activity, when measured in single β-cells simultaneously, showed comparable kinetics.

5. The secretion of insulin from intact pancreatic islets was stimulated by exposure to hypotonic solutions (10–33% hypotonicity). A 15% hypotonic solution stimulated insulin release to a peak value comparable to that elicited by raising the glucose concentration from 4 to 20 mM. Whereas hypotonic solutions caused a transient stimulation of insulin release, the effect of glucose was sustained.

6. It is suggested that glucose increases the volume in rat pancreatic β-cells by a mechanism dependent upon metabolism of the sugar. The extent of cell swelling evoked by raised glucose concentrations is sufficient to depolarize the cells and induce electrical and secretory activity and may involve activation of a volume-sensitive anion conductance.

The regulation of cell volume following exposure to anisotonic solutions is a response common to many types of vertebrate cell and has been the subject of numerous reviews (Hoffman & Simonsen, 1989; Sarkadi & Parker, 1991; Lang, Busch, Volkl & Haussinger, 1995). This process has been extensively studied in epithelial cells, where it enables the cell to withstand exposure to anisotonic extracellular solutions and changes in intracellular osmolarity due to transport of solutes. Volume regulation is probably also important in cells where high rates of metabolism could alter intracellular osmolarity (Haussinger & Lang, 1991). The most commonly studied volume regulatory process is regulatory volume decrease (RVD), which counteracts cell swelling following exposure to hypotonic extracellular solutions or due to intracellular hypertonicity. RVD results from loss of solutes, usually K^+ and Cl^-, from the cell either by activation of cotransport systems or of K^+ and anion conductance pathways.

We and others have recently identified a volume-sensitive anion conductance in insulin-secreting cells (Kinard & Satin, 1995; Best, Sheader & Brown, 1996*b*). Inhibition of this conductance by 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) prevents RVD following hypotonically induced swelling (Best *et al.* 1996*b*), suggesting that the conductance plays a role in volume regulation in β-cells. Furthermore, activation of this conductance by exposure to hypotonic solutions leads to depolarization and enhanced electrical

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(Britsch, Krippeit-Drews, Gregor, Lang & Drews, 1994; Best, Miley & Yates, 1996*a*) and secretory (Blackard, Kikuchi, Rabinovitch & Renold, 1975; Best *et al.* 1996*a*) activity. A similar phenomenon has been reported in chromaffin cells (Moser, Chow & Neher, 1995) and presumably results from a greater activation by cell swelling of the anion conductance relative to K^+ channel activation (Sarkadi & Parker, 1991).

In pancreatic β-cells, the most important physiological stimulus is a rise in glucose concentration. It has recently been demonstrated that glucose elicits transient inward currents in β-cells comparable to those observed upon exposure to hypotonic solutions (Best, 1997). It is thus possible that this current, which is inhibited by anion channel blockers, represents activation of the volume-sensitive anion conductance by glucose. We have therefore investigated in the present study whether glucose can cause changes in β-cell volume and whether such changes could contribute towards the induction of electrical and secretory activity.

METHODS

Islet and β-cell preparation

Sprague–Dawley rats (300–400 g) of either sex were killed by stunning and cervical dislocation. The pancreas was excised and islets prepared by collagenase digestion (Lacy & Kostianovsky, 1967). Islets were dispersed into single cells and small clusters by gentle mixing in a Ca^{2+}-free medium (composition (mM): NaCl, 135; KCl, 5; $MgSO_4$, 1·2; Hepes, 10; EGTA, 1; pH 7·4). The cells were then resuspended into Hepes-buffered RPMI medium (Gibco), plated into 30 mm diameter polystyrene dishes and cultured for 1–5 days in humidified air at 37 °C. Single β-cells, identified by their size and typical granular appearence, were used for both volume and electrophysiological studies.

Cell volume measurements

Cells were superfused at a rate of 4 ml min^{-1} with a medium consisting of (mM): NaCl, 125; KCl, 5; NaH_2PO_4, 1·0; $MgCl_2$, 1·0, $CaCl_2$, 1·2; Hepes–NaOH, 10 (pH 7·4); mannitol, 20; and glucose, 4; 293 mosmol l^{-1} (measured by depression of freezing point). An increase in glucose concentration or addition of 3-*O*-methylglucose was either substituted for an equivalent concentration of mannitol in order to avoid changes in osmolarity of the medium, or otherwise added to the above medium with no substitution. In experiments to study the effects of hypotonically induced cell swelling, the buffer contained 85 mM NaCl and 100 mM mannitol, and a hypotonic solution (10–33%) made by omission of mannitol (30–100 mM). Relative volume of β-cells was measured at 37 °C using a video-imaging technique. The cells were observed though a ×40 objective lens of the microscope which was connected to an ECD-1000 camera (Electrim Corporation, NJ, USA). Images of the cells were saved as 'tif' files on the hard disk and the area of each image subsequently measured using an AVS software package (Hewlett Packard). Cell volume was calculated assuming the cells to be spherical and expressed as relative volume with respect to the

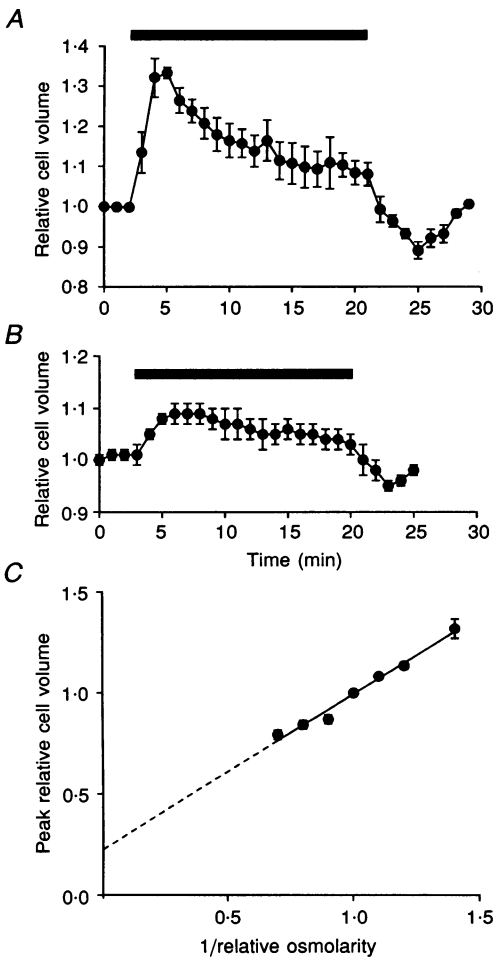

Figure 1. Volume changes in β-cells exposed to hypotonic solutions

Single isolated rat β-cells were exposed to 33% (*A*) or 10% (*B*) hypotonic solutions for the period designated by the filled bar. *C*, the relationship between peak relative cell volume, after switching to the hypotonic solution, and the reciprocal of relative osmolarity. The point at which the extrapolated line intersects the vertical axis represents the osmotically inactive volume (0·22). Each point represents the mean ± S.E.M. of 5 (*A*), 4 (*B*) or 4–5 (*C*) separate determinations.

volume measured during exposure to the control medium. In order to minimize flattening down of cells on the culture dish, cells for volume measurements were used within the first two days following preparation. The images were analysed in a blind and randomized manner in order to eliminate bias.

Electrophysiological experiments

The membrane potential in single, isolated β-cells was measured using the perforated patch configuration of the patch clamp technique (Rae, Cooper, Gates & Watsky, 1991). Recordings were made under current clamp conditions (zero current) using a List EPC-7 amplifier and stored on DAT tapes prior to playing onto a chart recorder. The pipette solution consisted of (mM): K_2SO_4, 60; KCl, 20; NaCl, 10; Hepes–NaOH, 10 (pH 7·2); and amphotericin B, 240 μg ml^{-1}. Access resistance was < 25 MΩ and whole-cell capacitance within the range 8–13 pF. Recordings were made at 29–31 °C since higher temperatures were found to cause seal disruption. In a number of experiments, simultaneous measurements were made of β-cell volume and electrical activity.

Insulin secretion was measured by radioimmunoassay using groups of twenty-five intact islets continuously perifused at a rate of 1 ml min^{-1} at 37 °C.

Chemicals

Collagenase was obtained from Boehringer Mannheim and all other chemicals from Sigma. ^{125}I-insulin was supplied by The Radiochemical Centre (Amersham, UK).

Results are given as means ± S.E.M.

RESULTS

β-cell volume regulation in response to hypotonic solutions

The volume of rat β-cells, estimated from the area of the cell image, was 2·52 ± 0·05 pl ($n = 20$). Figure 1 shows the effects of hypotonic solutions on relative volume in single, isolated rat β-cells. Exposure to a 33% hypotonic solution resulted in a rapid increase in relative volume to a peak value of 1·33 ± 0·01 ($n = 5$; Fig. 1*A*). This was followed by an RVD to 1·08 ± 0·03 over the next 16 min. Upon restoration of the isotonic solution, cell shrinkage occurred to 0·89 ± 0·02 followed by a regulatory volume increase to 1·01 ± 0·01. An essentially similar response was observed upon exposure to a 10% hypotonic solution, except that the maximum volume attained was 1·09 ± 0·01 ($n = 4$; Fig. 1*B*). The RVD response following exposure to a 10% hypotonic solution was inhibited by the anion channel blocker DIDS (results not shown), as reported previously with a 30% hypotonic solution (Best *et al.* 1996*b*). The relationship between osmolarity of the medium and peak cell volume is shown in Fig. 1*C*, and indicates that rat β-cells behave as osmometers with an osmotically inactive volume of 0·22. This value is identical to that previously obtained for hamster β-cells (Benson, Liu, Gao, Critser & Critser, 1993), but lower than the figure of 0·44 reported for a mixed

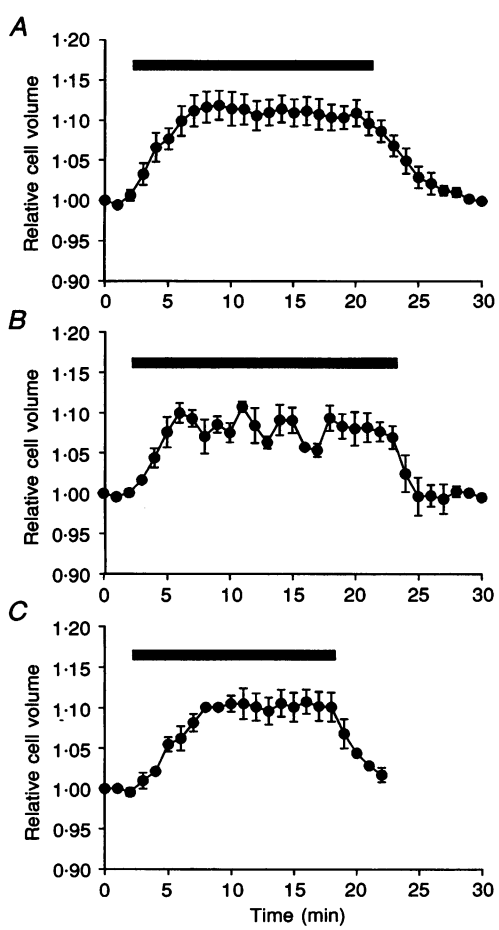

Figure 2. Volume changes in β-cells exposed to increased glucose concentrations

The concentration of glucose was raised from 4 to 20 mM (*A*) or 12 mM (*B*) with substitution of a corresponding amount of mannitol. *C*, the glucose concentration was raised from 4 to 20 mM with no substitution of mannitol. The increase in glucose concentration was maintained for the period designated by the filled bar. Each point represents the mean ± S.E.M. of 12 (*A*), 6 (*B*) or 4 (*C*) separate determinations.

population of hamster islet cells (Liu, Benson, Gao, Haag, McGann & Critser, 1995). The RVD response which follows hypotonically induced cell swelling probably involves activation of the volume-sensitive anion conductance recently described in insulin-secreting cells (Best *et al.* 1996*b*). Thus, a 9% increase in β-cell size appears to be sufficient to activate this conductance.

Glucose causes β-cell swelling

Since glucose is the major physiological stimulus for β-cells, we investigated the effects of increasing concentrations of the sugar on volume in single rat β-cells. This was done in two ways: (1) by substituting added glucose for an equal concentration of mannitol in order to avoid changes in osmolarity of the extracellular medium, and (2) by adding the sugar to the medium with no substitution, as is common in studies of β-cell physiology. Figure 2*A* shows the effects on volume of raising the glucose concentration from 4 to 20 mM with substitution of mannitol. This concentration of glucose, which is maximally effective with respect to insulin release (Hedeskov, 1980), was found to increase β-cell relative volume to a peak value of $1{\cdot}12 \pm 0{\cdot}02$ ($n = 12$). This value was attained within 6 min of raising the glucose concentration. Figure 2*B* shows the effects on cell volume of an intermediate concentration of glucose (12 mM). In this case, the peak volume attained was $1{\cdot}10 \pm 0{\cdot}01$ ($n = 6$). It was often found that irregular oscillations in cell volume occurred during exposure to intermediate concentrations of glucose (Fig. 2*B*). However, it is unclear at present whether these apparent oscillations are of any physiological significance. In contrast to the effects of hypotonic solutions, the increase in cell volume in response to glucose was sustained for the duration of exposure to high hexose concentrations. When the glucose concentration was restored to 4 mM, relative cell volume returned to control levels (Fig. 2*A* and *B*). When the glucose concentration was raised from 4 to 20 mM without substitution of mannitol, the peak relative volume increased to $1{\cdot}11 \pm 0{\cdot}03$ ($n = 4$; Fig. 2*C*).

Raising the concentration of glucose to 20 mM caused a similar degree of cell swelling in the presence of 2 mM Co^{2+}, a blocker of voltage-sensitive Ca^{2+} channels (Fig. 3*A*). This suggests that increased cell volume in response to glucose was not merely a consequence of an enhanced rate of exocytosis. The addition of 16 mM 3-*O*-methylglucose, a non-metabolizable analogue, which is transported into β-cells in the same manner as glucose (Hellman, Sehlin & Taljedal, 1973), resulted in a transient increase in relative volume of $1{\cdot}05 \pm 0{\cdot}01$ ($n = 6$; Fig. 3*B*). This value is significantly less than that attained in response to the equivalent concentration of glucose ($P < 0{\cdot}01$). The modest cell swelling observed upon addition of 3-*O*-methylglucose could reflect the fact that the glucose analogue, which is freely transported into β-cells, was substituted for an equivalent concentration of mannitol, which is relatively impermeant. This possibility is supported by the finding

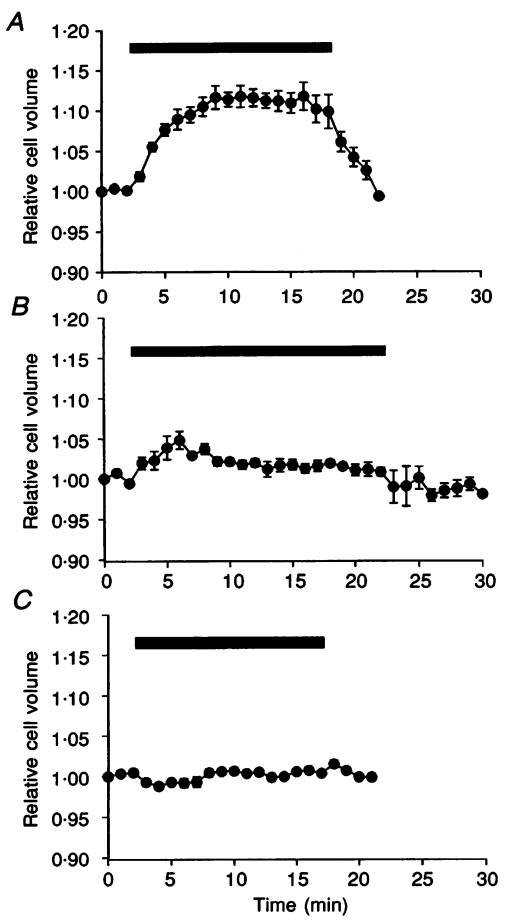

Figure 3. Glucose-induced β-cell swelling is independent of Ca^{2+} but requires glucose metabolism

Effect of 20 mM glucose on β-cell volume in the presence of 2 mM Co^{2+} (*A*). Effect of 16 mM 3-*O*-methylglucose on β-cell volume either with (*B*) or without (*C*) substitution of mannitol. The filled bars indicate the period during which the glucose concentration was raised (*A*) or 3-*O*-methylglucose was added (*B* and *C*). Each point represents the mean ± S.E.M. of 8 (*A*), 6 (*B*) or 4 (*C*) separate determinations.

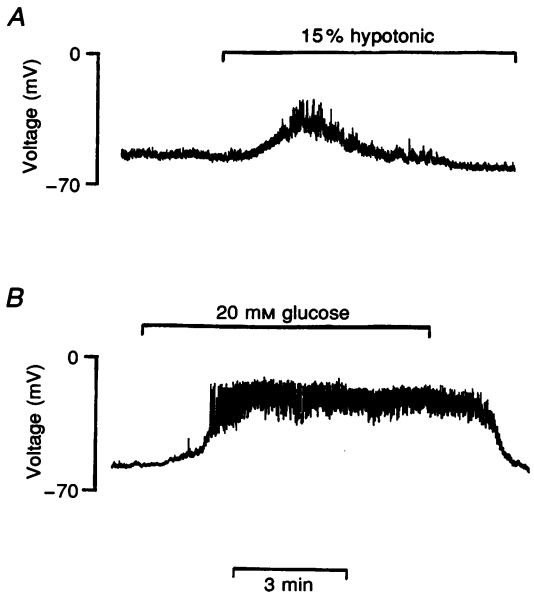

Figure 4. Electrical activity in response to a hypotonic solution and 20 mM glucose

Membrane potential was recorded from single isolated rat β-cells using the perforated patch technique under current clamp conditions. *A*, effect of a 15% hypotonic solution in the presence of 4 mM glucose. *B*, effect of raising the glucose concentration from 4 to 20 mM. These traces are typical of those from 3–15 similar experiments.

that addition of 3-*O*-methylglucose with no substitution of mannitol caused no significant increase in β-cell volume (Fig. 3*C*).

Hypotonic solutions and glucose induce electrical activity in β-cells

The effects of various treatments on membrane potential in single, isolated rat β-cells are shown in Figs 4 and 5. The resting membrane potential in the presence of 4 mM glucose was $-59{\cdot}7 \pm 0{\cdot}9$ mV ($n = 23$). We have recently shown that exposure of β-cells to a 30% hypotonic solution induces a marked, transient depolarization, presumably reflecting activation of the volume-sensitive anion conductance (Best *et al.* 1996*a*). A 15% hypotonic solution was also found to cause a transient depolarization and initiate electrical activity (Fig. 4*A*). This degree of hypotonicity would be predicted to swell the cells by approximately 12%, which is comparable to the value elicited by 20 mM glucose (see Figs 1*C* and 2*A*). A 10% hypotonic solution was found to be the smallest reduction in extracellular tonicity which consistently initiated electrical activity in rat β-cells. This finding is consistent with the cell volume measurements in suggesting that an increase in β-cell volume of 9% is sufficient to activate the volume-sensitive anion conductance, thus leading to RVD. In contrast, a 5% hypotonic solution had no detectable effect in three out of three cells (not shown).

Raising the concentration of glucose in the medium induced sustained electrical activity in single rat β-cells. A maximal concentration (20 mM) of the sugar depolarized the cell leading to a sustained plateau upon which action potentials were superimposed (Fig. 4*B*). The response to an intermediate concentration of glucose (12 mM) invariably consisted of a transient peak depolarization followed by a

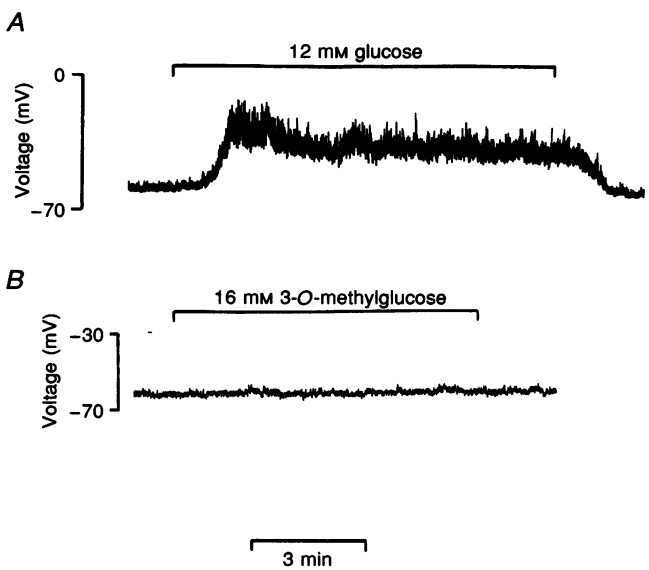

Figure 5. Electrical activity in response to 12 mM glucose and 16 mM 3-*O*-methylglucose

Membrane potential was recorded from single isolated rat β-cells using the perforated patch technique under current clamp conditions. *A*, effect of 12 mM glucose. *B*, effect of addition of 16 mM 3-*O*-methylglucose in the presence of 4 mM glucose. These traces are typical of those from 4–8 similar experiments.

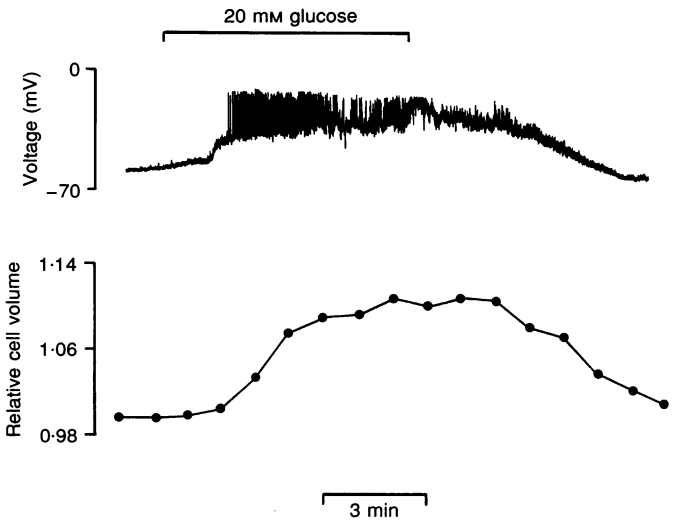

Figure 6. Simultaneous recording of volume and electrical activity in response to increased glucose concentration

Membrane potential was recorded from a single isolated rat β-cell using the perforated patch technique under current clamp conditions. Images of the cell were recorded at intervals of 1 min for the determination of relative cell volume. Six other experiments showed essentially similar results.

sustained plateau; no 'bursts' of electrical activity were observed (Fig. 5*A*). Similar observations were made in fifteen out of fifteen cells in response to 10 or 12 mM glucose. This finding is consistent with a previous report using single rat β-cells (Falke, Gillis, Pressel & Misler, 1989) and contrasts with the bursting pattern observed in single mouse β-cells exposed to 10 mM glucose (Smith, Ashcroft & Rorsman, 1990). In general it was found that exposure of single rat β-cells to increasing concentrations of glucose between 8 and 20 mM caused a progressively greater 'peak' depolarization (results not shown). In contrast, the addition of 16 mM 3-*O*-methylglucose had no effect on β-cell membrane potential (Fig. 5*B*). In the experiments shown in Figs 4 and 5, glucose and 3-*O*-methylglucose were added to the incubation medium with no substitution of mannitol. Virtually identical results were obtained when substitution for mannitol was made.

In order to assess the relative kinetics of β-cell volume and electrical responses to glucose, a number of experiments were performed where these responses were measured simultaneously. Figure 6 shows simultaneous recordings of volume changes and electrical activity in response to 20 mM glucose. In this cell, the onset of electrical activity in response to 20 mM glucose was accompanied by an increase in β-cell volume. Similarly, both membrane potential and volume returned to basal values when the glucose

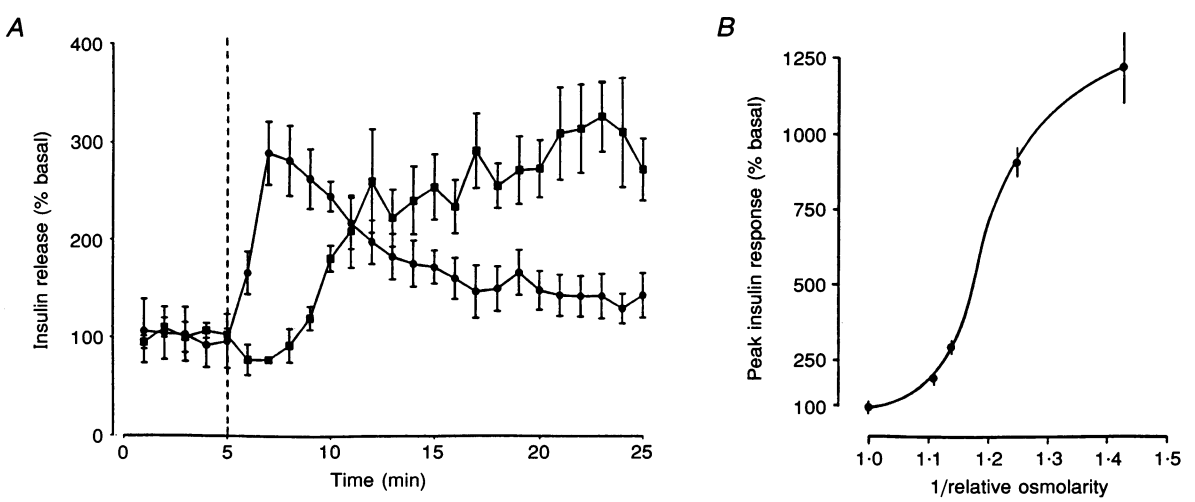

Figure 7. Effects of hypotonic solutions on insulin release from rat islets

Batches of 25 intact islets were continuously perifused and insulin measured by radioimmunoassay. *A*, kinetics of the insulin secretory responses to a 15% hypotonic solution in the presence of 4 mM glucose (●) and a rise in glucose concentration from 4 to 20 mM (■). *B*, relationship between peak insulin secretory response, measured after switching to a hypotonic solution, and the reciprocal of relative osmolarity. Each point represents the mean ± S.E.M. of 6 (*A*) or 4–6 (*B*) separate determinations.

concentration was restored to 4 mM. In seven experiments of this type, the peak level of depolarization upon raising the glucose concentration from 4 to 20 mM occurred at 2·8 ± 0·4 min, whilst the peak relative cell volume was attained at 5·2 ± 0·7 min. In the experiments where cell volume and electrical activity during glucose stimulation were measured independently, the corresponding values were 4·2 ± 0·4 min ($n = 15$) and 7·2 ± 0·5 min ($n = 12$), respectively. When comparing the kinetics of these responses, it should be borne in mind that cell swelling will activate both Cl^- and K^+ conductances, possibly at different rates (Sarkadi & Parker, 1991). Since activation of these conductances will have opposite effects on cell membrane potential, changes in the latter will not necessarily be expected to correlate exactly with changes in cell volume. It is also possible, in the simultaneous recordings, that the attachment of a patch pipette to the cell could affect the sensitivity and resolution of the video-imaging technique by partially restricting cell swelling. Therefore, whilst the kinetics of the volume and electrical responses to glucose were comparable, it is difficult to assess the precise timing of the former.

Stimulation of insulin release by glucose and hypotonic solutions

The effect of 20 mM glucose on insulin release from intact perifused islets is shown in Fig. 7*A*. As noted earlier, this concentration of glucose caused an increase in relative cell volume of 1·12 (see Fig. 2*A*). Therefore, in order to assess the relationship between cell volume and insulin release, islets were exposed to a 15% reduction in osmolarity, which would be predicted to cause cell swelling to a similar degree (see Fig. 1*C*). As shown in Fig. 7*A*, this manoeuvre stimulated insulin secretion to a peak level comparable to that observed with 20 mM glucose. However, as noted with electrical activity, the effect of the hexose on insulin release was sustained, whereas exposure to the hypotonic solution resulted in a transient stimulation of secretion. The magnitude of the peak insulin response to hypotonic solutions was directly related to the degree of hypotonicity within the range 10–30% (Fig. 7*B*).

DISCUSSION

The results of the present study confirm that, as previously shown with RINm5F insulinoma cells (Best *et al.* 1996*b*), single rat β-cells are able to regulate their volume following exposure to anisotonic extracellular media. A decrease in extracellular osmolarity of as little as 10% appeared to be sufficient to trigger RVD. This manoeuvre caused an apparent cell volume increase of 9%, implying that this degree of swelling should also be sufficient to activate the volume-sensitive anion conductance, which in part mediates the RVD response. This in turn would be predicted to depolarize the β-cell, leading to electrical activity and insulin release (Best *et al.* 1996*a*). Indeed, exposure to hypotonic solutions was shown to depolarize the cells and trigger insulin release.

In view of the finding that a 9% increase in cell volume was sufficient to induce electrical and secretory activity in rat β-cells, it was of interest to note that a rise in glucose concentration to levels effective in eliciting electrical activity and stimulating insulin release caused a sustained increase in β-cell volume of up to 12%. This finding is consistent with a previous study using the perfused rat pancreas in which raising the glucose concentration from 3·3 to 16·7 mM caused a rapid increase in β-cell size (Semino, Gagliardino, Bianchi, Rebolledo & Gagliardino, 1990). Thus, stimulatory concentrations of glucose could cause sufficient β-cell swelling to activate the volume-sensitive anion conductance. Activation of this conductance has been shown to generate an inward current (Best *et al.* 1996*a*) since the Cl^- equilibrium potential appears to be positive with respect to the resting membrane potential (Sehlin, 1978). This inward current could thus contribute towards the induction of electrical activity. The kinetics of the changes in β-cell volume and electrical activity in response to glucose suggest that these responses may be related. This relationship is further supported by the observation that a 15% hypotonic solution, predicted to swell the cells to a similar extent as 20 mM glucose, caused a peak value of insulin release comparable to that elicited by that concentration of the hexose.

The effects of hypotonic solutions on β-cell volume, and electrical and secretory activity were transient in nature. This is probably because, upon exposure of the cells to a hypotonic solution, RVD (and the associated activation of the anion conductance) would proceed to the point at which the intracellular medium was restored to isotonicity with respect to the extracellular solution. In contrast, the effects of glucose on β-cell volume, electrical activity and insulin release persisted for the duration of exposure to high concentrations of the sugar. This could in turn be explained by the continued metabolism of glucose maintaining the intracellular medium in a state of relative hypertonicity. The finding that 3-*O*-methylglucose (which is transported into the β-cell though not metabolized) caused a much smaller volume increase than an equivalent concentration of glucose also implies that metabolism of the hexose is the major determinant of its effect on β-cell volume. A major metabolite of glucose in β-cells is lactate (Malaisse, Sener, Levy & Herchuelz, 1976; Best, Yates, Meats & Tomlinson, 1989), whilst these cells appear to express extremely low levels of the $lactate^-$–H^+ cotransporter (Best, Trebilcock & Tomlinson, 1992). Thus, intracellular accumulation of this metabolite could lead to increased β-cell volume upon exposure to raised glucose concentrations. In addition it is possible that accumulation of Na^+ and Cl^- (in exchange for H^+ and HCO_3^-, respectively, also formed during glucose metabolism) could also contribute towards cell swelling.

In conclusion, we have demonstrated that glucose causes swelling in rat pancreatic β-cells to an extent that can enhance electrical and secretory activity. It is possible that activation by β-cell swelling of a volume-sensitive anion conductance could be involved in these processes.

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