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The glucokinase activator GKA50 causes an increase in cell volume and activation of volume-regulated anion channels in rat pancreatic β-cells

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Abstract

Glucokinase plays a key role in the metabolism of glucose by pancreatic β -cells. In this study the effects of the glucokinase activator GKA50 on cell volume and electrical activity in rat β -cells were examined. 1 μ M GKA50 caused an increase in β -cell volume in the presence of 4 mM glucose. GKA50 also caused a depolarisation of β -cell membrane potential and increased electrical activity. These changes were associated with the activation of inward whole-cell currents, and were attenuated by the anion channel inhibitor 5-nitro-2-(3-phenylpropylamino) benzoic acid. In single channel experiments, the open probability of volume-regulated anion channels (VRAC) was increased from 0.03 ± 0.01 to 0.19 ± 0.04 (n=3) by the GKA50. The data suggest that a GKA50-evoked increase in glucose metabolism causes an increase in β -cell volume. This in turn activates VRAC leading to a depolarisation of the cell membrane potential.

Keywords – islets of Langerhans, pancreatic β -cell, cell volume, volume-regulated anion channel, glucokinase.

Abbreviations – Vm, membrane potential; K_{ATP} channel, ATP-sensitive potassium channel; VRAC, volume-regulated anion channel; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; G_{input}, whole-cell input conductance.

1. Introduction

Insulin secretion by pancreatic β -cells is stimulated by an increase in plasma glucose concentrations. Secretion occurs because glucose metabolism causes a depolarisation of the βcell membrane potential (Vm). This results in the opening of voltage-gated Ca²⁺ channels, leading to an increase in intracellular Ca²⁺ activity which triggers the exocytosis of vesicles containing insulin (Ashcroft, 2007; Ashcroft et al., 1989; Yang & Berggren, 2006). The consensus model is that glucose metabolism evokes a depolarisation of Vm by promoting an increase in the ratio of ATP to ADP concentrations in the cytosol which is sufficient to close KATP channels (Ashcroft, 2007; Ashcroft et al., 1989; Detimary et al., 1998). However the last decade has produced a growing body of evidence indicating that the activation of anion channels may also contribute to the glucose-induced depolarisation (Best et al., 2010). It is hypothesized that glucose metabolism activates volume-regulated anion channels (VRAC) in β-cells, and that the efflux of Cl⁻ (and possibly other anions) through these channels contributes to the glucoseinduced depolarisation of Vm (Best et al., 2010). The VRAC hypothesis also explains why exposure of β -cells to hypotonic solutions (which induces cell swelling and activation of VRAC) causes insulin secretion (Best et al., 1996; Blackard et al., 1975), and also why inhibitors of Cl transport attenuate glucose-induced electrical activity and insulin secretion (Best, 1997; Best, 2002a; Best et al., 2000; Best et al., 2004).

The mechanism by which glucose metabolism activates VRAC in pancreatic β -cells is not fully established. *In vitro* studies, however, have shown that elevating the extracellular glucose concentration causes an increase in rat β -cell volume which is sufficient to activate VRAC (Miley *et al.*, 1997). More recent experiments have found that rat pancreatic α -cells, but not

intestinal Caco2 cells, also exhibit glucose-induced swelling (Davies *et al.*, 2007). These data demonstrate that the transport of glucose into the cells *per se* is not responsible for swelling, because Caco2 cells express the same GLUT2 glucose transporter as the rat pancreatic β -cells (Davies *et al.*, 2007). Glucose-induced cell swelling is dependent on glucose metabolism, because it is not evoked by 3-o-methyl-D-glucose (a non-metabolisable glucose analogue; Davies *et al.*, 2007; Miley *et al.*, 1997). A key step in glucose metabolism by both rat α -cells and β -cells is glycolysis by the enzyme glucokinase. Glucokinase is a highly specialised member of the hexokinase family of enzymes, which is characterized by a low affinity for glucose. Thus the rate of glucose metabolism increases over the physiological range of plasma glucose concentrations (Matschinsky *et al.*, 1998). Glucokinase is therefore thought to be essential in the activation of glucose-responsive cells, and as such it may have an important role in the generation of glucose-induced swelling.

Glucokinase is also a potential target for the treatment of type 2 diabetes mellitus, and a number of compounds have been developed which stimulate glucokinase activity (Matschinsky, 2009). GKA50 is one such compound which increases the affinity of glucokinase for glucose via an allosteric interaction with the enzyme (Brocklehurst *et al.*, 2004). Recent experiments have shown that GKA50 stimulates an increase in intracellular Ca²⁺ activity and insulin secretion in the absence of an increase in the extracellular glucose concentration (Johnson *et al.*, 2007). The aim of the present study was to investigate the effects of GKA50 on the volume of pancreatic β cells and on the electrophysiological properties of these cells. The results show that GKA50 caused both cell swelling and activation of a chloride channel, probably VRAC, at constant extracellular glucose concentrations.

2 Materials and Methods

2.1 Isolation of pancreatic β-cells

Islet cells were prepared from pancreatic tissue isolated from male Sprague Dawley rats (250-300g; Charles River, UK). Animals were killed by stunning and cervical dislocation as prescribed by Schedule 1 methods of the UK Animals (Scientific Procedures) Act. Islets were prepared by collagenase digestion, and then dispersed into single isolated cells by a brief incubation in Ca²⁺-free medium (Miley *et al.*, 1997). Single cells were harvested by centrifugation and re-suspended in MEM medium (Invitrogen) supplemented with 5% foetal calf serum and 50 µg/ml gentamycin. The re-suspended cells were plated at the centre of 30 mm sterile plastic culture dishes (Nunc), and incubated in humidified air at 37°C. Experiments were performed between 24 and 96 h after cell isolation. In an additional series of experiments the effects of GKA50 on the volume of pancreatic acinar cells were allowed to loosely adhere to glass coverslips for 10 min before the start of each experiment. They were used within 3h of isolation.

2.2 Cell volume measurement

Cell volume was measured by an imaging method using a Nikon Diaphot 200 microscope fitted with a Canon Powershot A620 camera as previously described (Davies *et al.*, 2009; Miley *et al.*, 1997). Cells were viewed through the 40x objective lens and images saved as JPEG files at 1 minute intervals for subsequent analysis. Cells were superfused at a rate of 6.5 ml/min with a control solution containing (mM): 140 NaCl, 5 KCl, 1.2 CaCl₂, 1 MgCl₂, 16 mannitol, 4 glucose and 5 HEPES (adjusted to pH 7.4). The temperature of the superfusate was maintained at 35-37

^oC throughout each experiment. Solution glucose concentrations were varied in some experiments to 0, 12 or 20 mM by equimolar substitution of mannitol in order to maintain a constant osmolality. Thus the osmolalities of all solutions were approximately 289 mOsmol kg H_2O^{-1} (range 285 to 293 mOsmol kg H_2O^{-1}), measured using an Osmomat 030 osmometer (Gonotec, Germany).

β-cells were selected for experiments on the basis of cell volume (i.e. greater than 1.8 pl) and granular appearance as described by Majid *et al.* (2001). The mean volume of the cells used in this study was 3.5 ± 0.3 pl (n=25). The area of each recorded cell image was measured using ImageJ software (NIH, USA), and volume calculated assuming that cells maintained a spherical shape (Miley *et al.*, 1997). Data are quoted as relative cell volume, i.e. cell volume divided by control volume recorded in the first 2 minutes of each experiment.

2.3 Electrophysiological Experiments

Vm was recorded from single β -cells using the perforated patch technique with a List EPC-7 amplifier in current-clamp mode (Darmstadt, Germany). Cells were selected on the basis on their granular appearance and capacitance (range 8-14 pF) as discussed by Yang & Berggren (2006). The pipette solution contained (mM): 130 KCl, 5 NaCl, 1 MgCl₂, 25 HEPES-KOH (pH 7.2) and 240 µg/ml amphotericin B. The amplifier was switched for defined periods to voltage-clamp mode to record VRAC current (Best, 2005; Best, 1997). Cells were superfused at a rate of 2 ml.min⁻¹ with an extracellular medium which contained (mM): 130 NaCl, 5 KCl, 1 MgSO₄, 1 NaH₂PO₄, 1.2 CaCl₂, 25 Hepes–NaOH (pH 7.4) and 4 glucose. In some control experiments a 0

glucose extracellular solution was employed. The access resistance in the experiments described was always less than 25 M Ω .

VRAC activity was also assessed at the single channel level in cell-attached patches as previously described (Best, 1999; Best, 2002b). In these experiments the pipette solution contained (mM): 140 CsCl, 1 MgCl₂ and 10 Hepes–CsOH (pH 7.4). With no applied pipette potential, channel openings were observed as downward deflections indicative of negative currents. Recordings were filtered at 200 Hz and stored for subsequent analysis. Channel records were analysed using pClamp 9.2 software (Molecular Devices), and open propabilities (Po) for multi-channel patches estimated as described by Jackson (1985).

Whole-cell input conductance (G_{input}), an index of K_{ATP} channel activity (Best *et al.*, 2004; Smith *et al.*, 1990) was also measured in perforated patch experiments. Cells were subjected to 50 ms pulses of -60 and -80 mV alternatively at 2 s intervals from a holding Vm of -70 mV. In these experiments the extracellular solution contained no glucose. All electrophysiological experiments were performed at temperatures of 28-30 °C.

2.4 Chemicals

GKA50 was a gift from AstraZeneca Pharmaceuticals, it was prepared as a 10 mM stock solution in DMSO, and diluted in the experimental solutions to a final concentration of 1 μ M. All other chemicals were obtained from Sigma-Aldrich.

2.5 Data analysis

Data are presented as mean \pm SEM. Volume changes were quantified by using Prism 5 (GraphPad software) to measure the "area under the curve" for relative cell volumes different to 1.0. Differences between mean volume changes were assessed by ANOVA followed by Tukey's post hoc test, and between electrophysiological parameters by Student's t-test for paired data.

3 Results

3.1 GKA50 induces an increase in β -cell volume in the presence of 4 mM glucose

Figure 1A shows the changes in β -cell volume caused by increasing the superfusate glucose concentration from 4 mM to 20 mM. This manoeuvre resulted in a sustained increase in relative cell volume, and the volume only decreased to control values when the glucose concentration was returned to 4mM. Similar observations were made by Miley et al. (1997). An increase in cell volume was also observed in seven β cells exposed to a superfusate containing 1µM GKA50 in the continued presence of 4 mM glucose (Figure 1B). The increase in volume was again sustained during the exposure to the GKA50, with volume returning to control values only when the drug was removed from the superfusate. By contrast, application of 1µM GKA50 did not evoke an increase in cell volume when the superfusate contained 12 mM glucose throughout the experiment (Figure 1C). Figure 1D shows that 0.01% (v/v) DMSO (the solvent for the GKA50) was without effect on the cell volume in cells superfused with the 4 mM glucose solution. The data from all the volume experiments are summarised in Figure 1E, which shows that the effects of both 20 mM glucose and of 1µM GKA50 in 4 mM glucose are significantly different from the DMSO controls (P<0.05 by ANOVA). By contrast, GKA50 had no significant effect on cell volume in the presence of 12 mM glucose (P>0.1). GKA50 was also without effect on cell volume in the absence of extracellular glucose (GKA50 0, n=5; Figure 1E).

The effects of GKA50 on the volume of pancreatic acinar cells, which do not express the glucokinase enzyme (Schuit et al, 1999), were examined a separate series of control experiments. Superfusing the acinar cells with 1 μ M GKA50 in the presence of 4 mM glucose had no effect on the volume of 4 cells (i.e. the volume change during a 10 min exposure to GKA50 = -0.06 ± 0.09

RCV.min, which is not significantly different to that observed in 4 time-matched control cells = -0.15 ± 0.21 RCV.min, P>0.1 by unpaired t-test).

3.2 GKA50 evokes electrical activity and activates VRAC in pancreatic β-cells

The effects of GKA50 on electrical activity and VRAC in β-cells are illustrated in Figure 2. In the presence of 4 mM glucose, the addition of 1 µM GKA50 for 4 min caused a gradual depolarisation of Vm resulting in electrical activity (i.e. Ca^{2+} -dependent action potentials; Figure 2A). Vm slowly recovered to control values when the GKA50 was removed. Similar data were obtained from a total of 12 cells. Figure 2B shows another example; in this experiment the anion channel blocker 50 µM 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) was added to the superfusate for 100 s at the peak of GKA50-induced electrical activity. This manoeuvre resulted in a hyperpolarisation of Vm, in this cell and in two others. These data suggest that anion channels may contribute to both the resting Vm of the β -cell and to the GKA50-induced depolarisation. In the experiment shown in Figure 2C the amplifier was briefly switched from current-clamp to voltage-clamp mode, prior to and during stimulation with GKA50, in order to record the noisy, inward (negative) currents characteristic of VRAC in β-cells (see Best, 1997; Best, 2000). In these experiments inward current amplitude increased during the exposure to 1 μ M GKA50. In a total of four cells the mean inward current at Vm = -65 mV was -2.75 \pm 0.43 pA in control conditions, and -6.33 ± 1.08 pA in the presence of GKA50 (P< 0.05 by paired ttest).

Single channel methods have previously been used to characterise VRAC in pancreatic β -cells (Best, 1999; Best 2002). Figure 3A shows the effects of 1 μ M GKA50 on channel activity in a

cell-attached patch containing three VRACs (see Figure 3C). Addition of GKA50 for 150s caused an increase in single channel activity (downward current deflections). Figures 3Bi and 3Bii show 25 s periods of single channel activity i) before and ii) during the exposure to GKA50. The data indicate that GKA50 caused an increase in channel open probability (Po). In three similar experiments, mean Po was 0.03 ± 0.01 during the 2 min control period, this increased to 0.19 ± 0.04 in the presence of GKA50 (P<0.05) and decreased to 0.13 ± 0.03 during a 3 min wash-out period (P>0.1).

3.3 GKA50 has no effect on VRAC or on K_{ATP} channel activity in the absence of extracellular glucose

GKA50 failed to stimulate β -cell swelling in the absence of extracellular glucose (Figure 1E). Thus studying the effects of GKA50 on VRAC activity in the absence of glucose should help determine whether cell swelling is required for VRAC activation, or whether GKA50 has a more direct stimulatory mechanism. Vm exhibited a gradual hyperpolarisation over an 8.5 min period in the absence of extracellular glucose (Figure 4A, upper trace). In four cells this hyperpolarisation was from -63.3 ± 2.0 mV to -71.0 ± 1.3 mV (P<0.05). The addition of 1µM GKA50 (bar) failed to cause a depolarisation of Vm in the cell in Figure 4A or in three other cells.

The observed hyperpolarisation in Figure 4A (upper trace) was associated with the activation of outward (positive) currents in voltage clamp mode (Figure 4A, lower trace). These currents are probably carried by K_{ATP} channels which will activate in the absence of glucose. To test this hypothesis G_{input} , a measure of K_{ATP} channel activity (Best *et al.*, 2004; Smith *et al.*, 1990), was

monitored in voltage clamp mode. Vm was continually stepped to -60 or -80 mV for 50ms from a holding Vm of 70 mV, and the resultant currents recorded. The experimental recording in Figure 4B commenced as G_{input} approached a maximum of 14.0 ± 1.5 nS (n=3) after 4 minutes of superfusion with the 0 glucose solution. Addition of 1 μ M GKA50 to the superfusate for 80 s had no effect on the whole cell currents (13.7 ± 0.9 nS; P>0.1), again indicating that VRAC is not activated in the absence of glucose. By contrast, the addition 100 μ M tolbutamide (a K_{ATP} channel inhibitor) dramatically reduced the conductance to 1.2 ± 0.1 nS (P<0.01), showing that K_{ATP} channels are activated in 0 glucose solutions. The data in Figure 4B also show that GKA50 has no direct action on K_{ATP} channels.

4 Discussion

Glucokinase is a member of the hexokinase family of enzymes which catalyze the first step in glucose metabolism. Glucokinase has low affinity for glucose (Km \approx 8-10 mM; Coghlan *et al.*, 2008; Matschinsky *et al.*, 1998), so that the rate of glucose phosphorylation is roughly proportional to plasma glucose concentrations in the physiological range of 4 to 12 mM. This property makes the enzyme a key component of glucose-sensing cells (Matschinsky *et al.*, 1998). In pancreatic β -cells an increase in the rate of glucose metabolism is essential for modulating the intracellular ATP/ADP concentration ratio which regulates K_{ATP} channel activity, and hence insulin secretion (Ashcroft, 2007; Detimary *et al.*, 1998). Glucokinase activators are thought to potentiate these responses in β -cells by increasing the rate of glucose metabolism at a constant concentration of glucose (Matschinsky, 2009). The present study provides evidence that increased β -cell volume and VRAC activation could also contribute to the insulinotropic action of these drugs.

Superfusing cells with 1 μ M GKA50, at a constant extracellular glucose concentration of 4 mM, caused an increase in cell volume to a relative volume approximately 1.08, and this increase was sustained in the presence of the drug. Thus the change in volume was both qualitatively and quantitatively similar to the changes observed when the glucose concentration was increased from 4 to 20 mM in this study and also by Miley *et al.* (1997). The fact that these changes are sustained suggest that the mechanisms causing cell swelling, presumably related to increased glucose metabolism (see below), are constantly activated by the GKA50. This is in contrast to the transient changes in volume observed when β -cells are exposed to hypotonic solutions (Miley et al, 1997). Here cell volume establishes a new equilibrium following an initial swelling phase

and a subsequent regulatory volume decrease (see Hoffman et al, 2009). The sustained increase in volume also explains the sustained depolarisation of Vm with GKA50 and glucose, whereas only a transient depolarisation of Vm (and increase in insulin secretion) is observed in hypotonic solutions (see Blackard et al, 1975; Miley et al, 1997).

GKA50 did not induce an increase in β -cell volume in the absence of extracellular glucose, suggesting that glucose metabolism is an essential pre-requisite for cell swelling. GKA50 also failed to cause a significant increase in cell volume at an extracellular glucose concentration of 12 mM. This lack of effect may be because at 12 mM glucose glucokinase activity, and hence cell volume, are already close to maximum, i.e. Miley et al, (1997) showed that increasing extracellular glucose from 4 to 12 mM caused an increase in cell volume to more than 80% of the maximum volume change observed with 20mM glucose in the same study. Thus, at 12 mM glucose GKA50 has little influence on the rate of glucose metabolism (Coghlan et al., 2008), and any resultant changes in cell volume will be small and difficult to detect. On the other hand, at 4 mM glucose GKA50 causes a much greater enhancement of glucokinase activity (Coghlan et al., 2008), resulting in a significant acceleration of glucose metabolism and consequently an increase in cell volume. These differential effects of GKA50 at the two glucose concentrations suggest that the increase in β-cell volume cannot be attributed to non-specific actions of GKA50 on cellular processes unrelated to glucose metabolism. This conclusion is further supported by the lack of effect of GKA50 on the volume of pancreatic acinar cells which do not express the glucokinase enzyme (Schuit et al, 1999).

GKA50 also caused a depolarisation of Vm which was associated with an increase in inward whole cell current. These events are almost certainly linked to an increase in glucose metabolism and cell swelling, because they were sustained and because GKA50 was without effect on Vm in the absence of glucose (Figure 4A). Furthermore, the depolarisation and current activation are similar to those observed when β cells are stimulated by supra-threshold concentrations of extracellular glucose (Best, 2005; Best, 1997; Miley *et al.*, 1997), suggesting cell swelling may cause the activatation of VRAC channels leading to the depolarisation. Additional evidence for the involvement of VRAC in the present study comes from the fact that single channel activity associated with VRAC (see Best, 1999; Best, 2002b), was increased by GKA50. The GKA50-induced depolarisation was also inhibited by NPPB which a known blocker of VRAC (Best, 1997; Best 2002b). The properties of the inward current carried by VRAC and activated by metabolism have been described in detail elsewhere (Best, 2000; Best, 2002a; Best, 2005; Best & Brown, 2009).

Thus, our data indicate that activation of glucokinase causes a glucose-dependent increase in β cell volume which activates VRAC, thereby contributing to the depolarisation of Vm. The mechanism by which increased glucose metabolism causes cell swelling, however, is still unclear. The intracellular accumulation of products of glucose metabolism has been suggested to contribute to cell swelling (Miley *et al.*, 1997). The activation of Na⁺-H⁺ exchangers (NHE) and Cl⁻HCO₃⁻ exchangers (AE) may also be involved (Miley *et al.*, 1997). This latter hypothesis is supported by data showing that both NHE and AE contribute to β -cell volume regulation (Miley *et al.*, 1998), and by the fact both transporters are activated by glucose metabolism, in response to an initial reduction of intracellular pH (Shepherd *et al.*, 1995).

In conclusion, GKA50 causes an increase in cell volume, at constant glucose concentrations, which is sufficient to activate VRAC in pancreatic β -cells. The data highlight the key role of u ek glucokinase activity in coupling extracellular glucose concentrations to β-cell electrical activity

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Figure Legends

Figure 1. The effects of glucose and GKA50 on the volume of rat β -cells. **A**) Increasing the glucose concentration of the superfusate from 4 to 20 mM (bar) caused an increase in cell volume. Data are mean ± SEM (n= 6). **B**) 1 μ M GKA50 (bar) caused an increase in the volume of seven β -cells superfused with a solution containing 4 mM glucose (n=7). **C**) 1 μ M GKA50 failed to cause an increase in cell volume in cells superfused with 12 mM glucose (n=5). **D**) Vehicle control with 0.01% DMSO in the 4 mM glucose solution (n=5). **E**) Summary of volume changes caused by GKA50 and glucose. Superfusate composition shows whether the test solution contained 1 μ M GKA50 and also gives the respective glucose concentrations. They are as follows: DMSO and 4 mM glucose; 20 mM glucose; 1 μ M GKA50 and 0 mM glucose (n=5); 1 μ M GKA50 and 4 mM glucose; 1 μ M GKA50 and 12 mM glucose. * indicates significantly different to the DMSO vehicle control data (P < 0.05 by ANOVA).

Figure 2. GKA50 stimulates whole-cell electrical activity in rat β -cells. **A**) 1 μ M GKA50 (bar) evokes a depolarisation of Vm in current-clamp mode. **B**) The GKA50-induced depolarisation of Vm is reversed by the Cl⁻ channel inhibitor 50 μ M NPPB. The gap in the trace (arrow) indicates a wash-out period of ~ 3 min in the absence of either GKA50 or NPPB. **C**) Simultaneous recordings of Vm and whole-cell current (I) show that the GKA50-induced depolarisation of Vm is associated with the activation of a negative, inward whole-cell current. The amplifier was switched between current clamp and voltage clamp modes as shown (i and ii), and the holding potential in voltage-clamp mode was -65 mV. In all experiments whole-cell access was gained using the amphotericin perforated patch method. The pipette solution was

KCl-rich and the bath contained the standard extracellular solution which contained 4 mM glucose. Data are representative of: A) 12, B) 3 and C) 3 cells.

Figure 3. GKA50 increases single channel VRAC activity in β -cells. **A)** Data from a cellattached patch with no applied pipette potential. K⁺ channel activity was suppressed by using the Cs⁺ -rich pipette solution and the bath contained the standard extracellular solution with 4 mM glucose. **B)** Expanded sections of data (25s) from the trace in A), recorded in the absence (i) and presence (ii) of 1 μ M GKA50. The patch contained a total of three channels and in (ii) the current levels are indicated by the unbroken line (closed) and dotted lines (1, 2 or 3 channels open). The vertical calibration bar: (A) 20 pA and (B) 7 pA; horizontal time calibration bar: (A) 1 min and (B) 3 s. **C)** All point amplitude histograms from 2 s of channel activity in: i) control conditions (absence of GKA50) and ii) the presence of 1 μ M GKA50. In the control three peaks (closed and two open) can be observed, and 4 peaks can be identified (closed and three open) in the presence of GKA50.

Figure 4. The activities of neither VRAC nor K_{ATP} channels are directly modulated by GKA50. **A)** Perforated patch whole-cell experiments were performed using the standard KCl-rich pipette solution and an extracellular solution which contained no glucose. 1 µM GKA50 was added to the bath as indicated by the bar. The upper traced shows a current clamp recording of Vm, and the lower trace shows two periods of recording in voltage clamp mode (Vm = -65mV) in the absence (i) and presence (ii) of GKA50. **B)** GKA50 has no direct effect on input conductance (G_{input}) a measure of whole cell K_{ATP} channel activity. G_{input} was measured in voltage clamp mode by measuring the currents generated when 50 ms steps to -60 and -80 mV were applied

from a holding Vm or -70mV. The pipette solution was the standard KCl-rich solution, and the bath solution contained no glucose. The bars indicated periods of superfusion with solutions containing 1 µM GKA50 and then 100 µM tolbutamide. The data are representative of A) 4 and Accepter B) 3 experiments

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<u>Highlights</u>

- The paper examines the effects of a potential anti-diabetic drug glucokinase activator 50 (GKA50) on the function of insulin-secreting , pancreatic β-cells.
- GKA50 caused a sustained increase in β -cell volume, which was dependent on glucose metabolism.
- GKA50 stimulated a sustained depolarisation of the β-cell membrane potential by activating volume regulated anion channels (probably as a result of the increase in cell volume).
- The stimulatory effects of GKA50 on insulin secretion may at least partially be explained by cell swelling and activation of volume regulated anion channels leading to a depolarisation of membrane potential.

MANUS

Figure 1





Figure 3







1 min