Pflugers Arch - Eur J Physiol (2002) 444:506–512
DOI 10.1007/s00424-002-0842-9

ORIGINAL ARTICLE

Henrik Ortsäter · Per Liss · Karl E. O. Åkerman · Peter Bergsten

Contribution of glycolytic and mitochondrial pathways in glucose-induced changes in islet respiration and insulin secretion

Received: 16 January 2002 / Revised: 7 March 2002 / Accepted: 14 March 2002 / Published online: 8 June 2002

Abstract The different roles of glycolytic and mitochondrial pathways in glucose-induced metabolic activation and insulin secretion were studied in islets of Langerhans. Single islets were perifused with 3 mM glucose together with agents affecting the production or consumption of ATP. Subsequently, glucose was raised to 11 mM and the effects of the agents on metabolic and secretory responses were evaluated. Metabolism was monitored continuously with an oxygen-sensitive microelectrode inserted into the islet. Insulin secretion was determined by assaying insulin in perifusate with ELISA. Inhibitors of mitochondrial ATP production reduced the metabolic and secretory response to glucose. When glycolytic ATP production was reduced, initial but not sustained glucose-stimulated insulin release was observed. Inhibition of mitochondrial pyruvate transport reduced the glucose-induced decline in pO_2. Although mitochondrial metabolism was eventually similar to normal, insulin release was only 20% of normal. Increased energy expenditure also changed the kinetics of the glucose-induced decline in pO_2 and decreased the insulin release by 50%. In conclusion, glucose-induced enhancement of insulin release was only seen when the rise of the sugar concentration triggered a rapid and sustained increase of mitochondrial metabolism. This activation of mitochondrial metabolism required a good metabolic state prior to the glucose challenge.

Keywords ATP · Insulin · Metabolic inhibitors · Oxygen tension · Pancreatic islets

H. Ortsäter (✉) · P. Bergsten
Department of Medical Cell Biology, Box 571, Uppsala University, SE-751 23 Uppsala, Sweden
e-mail: Henrik.Ortsater@medcellbiol.uu.se
Tel.: +46-18-4714426
Fax: +46-18-4714059

P. Liss
Department of Radiology, Uppsala University, Sweden

K.E.O. Åkerman
Department of Physiology, Uppsala University, Sweden

Introduction

Prior to glucose-induced changes in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) or insulin secretion, metabolism of the pancreatic β-cell is activated by the sugar [1]. Glucose metabolism plays a key role in enhanced secretion of the hormone [2], consistent with the idea of the pancreatic β-cell being the body fuel sensor [3]. Metabolic impairment therefore results in a state of hyperglycemia caused by an inadequate secretory response [4]. Indeed, when postprandial hyperglycemia was chosen as a breeding criterion for establishing a non-insulin-dependent diabetes mellitus (NIDDM) animal model, the resulting strain, the GK-rat [5], showed metabolic and secretory impairment [6].

Glucose-derived ATP plays a key role in pancreatic β-cell glucose signalling. The nucleotide closes the ATP-sensitive K^+ channel [7] leading to membrane depolarization, the opening of voltage-dependent L-type Ca^{2+} channels [8] and an increase in $[Ca^{2+}]_c$ [9]. ATP is also needed for insulin granular movement and the exocytotic process [10]. However, prior to generation of ATP from the glucose molecule, ATP is required for phosphorylation of the sugar molecule to yield glucose-6-phosphate and fructose-2,6-bisphosphate [11]. These reactions depend on a sufficient β-cell ATP content prior to the glucose challenge. As a consequence any treatment that significantly lowers the ATP content of the β-cell would be anticipated to impair the secretory response upon glucose stimulation. Glycolysis accounts for approximately 10% of the ATP obtained from one glucose molecule, and mitochondrial metabolism for the remaining 90% [12]. Despite this relationship it has been shown that glycolytic ATP alone is able to sustain glucose-induced secretion [13]. In the present study we have challenged the idea of glycolytically sustained secretion by monitoring respiration and secretion of individual islets in the presence of agents that interfere with glycolytic and mitochondrial metabolism, or agents that affect the cellular energy state. We found that glucose-induced insulin release was reduced or even blocked in

the presence of these agents, which compromised the initial rapid increase in islet respiration. Furthermore, mitochondrial metabolism of glucose was obligatory for the initial and the sustained insulin secretory responses to the sugar. It can be concluded that the metabolic state of the β-cell prior to glucose stimulation is decisive for the secretory response.

Materials and methods

Chemicals

Reagents of analytical grade and deionized water were used. Collagenase, HEPES and bovine serum albumin (fraction V) were obtained from Boehringer Mannheim (Mannheim, Germany). D-Glucose, 3-*O*-methyl-D-gluco-pyranose, ouabain, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), oligomycin, α-cyano-4-hydroxycinnamic acid (CHC), arsenate, tetramethylbenzidine and insulin-peroxidase came from Sigma (St. Louis, Mo., USA). Gramicidin was obtained from Fluka (Buchs, Switzerland). The rat insulin standard was from Novo Nordisk (Bagsvaerd, Denmark). The mouse insulin antibodies were raised in our laboratory from guinea-pigs. IgG-certified 96-well microtitre plates were purchased from Nunc (Roskilde, Denmark).

Preparation and perifusion of islets

Animal experiments were approved by the local animal research ethics committee at Uppsala University. Pancreatic islets were collagenase isolated from male ob/ob mice [14] and cultured for 24–48 h in RPMI 1640 culture medium supplemented with 5.5 mM glucose and 10% fetal calf serum. Individual islets were attached to a poly-lysine-coated coverslip, which was used as the bottom in an open Sykes-Moore perifusion chamber thermoregulated to 37°C [9]. The islets were perifused at a rate of 150–160 µl/min with a medium supplemented with 1 mg/ml albumin and containing (in mM): NaCl 125, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 1.28, HEPES 25 and D-glucose 3, titrated to pH 7.4 with NaOH. Chelation of Ca^{2+} was performed by addition of 1.5 mM EGTA. After insertion of the oxygen-sensitive microelectrode into the islet, perifusion continued for approximately 50 min. Subsequently, 2 µg/ml oligomycin, 5 µM FCCP, 5 mM arsenate, 1 mM CHC with or without 1.5 mM EGTA, 100 µM ouabain or 1 µM gramicidin was added to the perifusion medium. After an additional 10 min five samples of perifusate for insulin determinations were collected every 5 min and recordings of pO_2 began. After 21 min the D-glucose concentration was raised to 11 mM and sampling and recording continued for another 30 min. During the first 6 min samples were taken every minute, then one sample after 3 min and subsequently four samples every 5 min. The time period of the sampling was 60 s for all samples. The perifusate samples were stored at –20°C until assayed for insulin. FCCP, oligomycin and gramicidin were dissolved in 95% ethanol giving a final ethanol concentration of 0.5–1‰ (v/v), CHC was dissolved in 100% DMSO giving a final DMSO concentration of 1‰ (v/v). Control experiments had the same concentrations of ethanol and DMSO.

Measurements of insulin release

Insulin in the perifusate was assayed by a competitive enzyme-linked immunosorbant assay (ELISA) [15] with the insulin antibody immobilized directly to the solid phase. The assay displayed linearity in a semilogarithmic diagram over the range of 10^{-2}–10^{5} amol of insulin and had an inter- and intra-assay variation of less than 10% in the specified range. The rate of insulin release was normalized to islet dry weight after freeze-drying and weighing the islets on a quartz fibre balance.

Measurements of oxygen tension

Oxygen tension in the isolated islets was measured by a modified Clark microelectrode [16, 17]. The tip of the microelectrode was positioned into the islets with the aid of a micromanipulator. A stereomicroscope was used to control the penetration depth of the electrode, which was approximately 30 µm. Electrodes were polarized at –0.8 V, which gave a linear response between the oxygen tension and the electrode current. Electrodes were calibrated in water saturated with $Na_2S_2O_5$ or air at 37°C before and after the experiments. The drift of the microelectrodes was less than 0.5% per hour. The electrical current was measured by a picoamperemeter (University of Aarhus, Aarhus, Denmark). The data acquisition rate of the A/D converter was 4 Hz providing good time resolution for further data analysis. Averages of data points corresponding to 60 s of recordings, during which perifusate was collected for insulin measurements, were calculated and used for figure presentation.

Data analysis

The starting and end points for the glucose-induced decrease in pO_2 were defined by dividing pO_2 recordings into consecutive periods of 10 s duration and calculating the average pO_2 for each period. The starting point of a decrease was defined as the time point at which the average pO_2 during a 10-s period was at least one standard deviation less than that of the preceding 10-s period and that this decrease continued during the next two 10-s periods. The end point was defined as the time point where this criterion was no longer fulfilled. The standard deviation was calculated from data obtained at 3 mM glucose. Between the starting and the end points the average slope was determined by linear regression analysis. The lag time of the perifusion system was determined in each experiment and was compensated for. Differences in oxygen tension were evaluated within each experimental group with an ANOVA for repeated measurements and between groups with factorial ANOVA with Fischer's posthoc test. *P*-values lower than 0.05 were considered significant. Values were expressed as means ± standard error of the mean (SEM).

Results

The influence of islet energy state for the secretory response to glucose was investigated by measuring glucose-induced changes in pO_2 and insulin release in individual islets. Energy state was affected by exposing the islet to different agents 30 min prior to elevation of the glucose concentration. Insulin release and pO_2 in the presence or absence of the agents were determined at 3 and 11 mM glucose. From the pO_2 measurements, the time from elevation of glucose to changes in pO_2 (lagtime), the duration of glucose-induced drop in pO_2 and the glucose-induced rate of decline in pO_2 were calculated. A prompt, initial glucose-induced rise in insulin release was only obtained under conditions when a rapid decline in pO_2 was observed. Prolonged, sustained glucose-induced insulin release required both a rapid initial decline in pO_2 and a sustained increase in respiration, i.e. sustained lowering in pO_2.

In order to assess the role of mitochondrial ATP synthesis in the kinetics of glucose-induced changes in

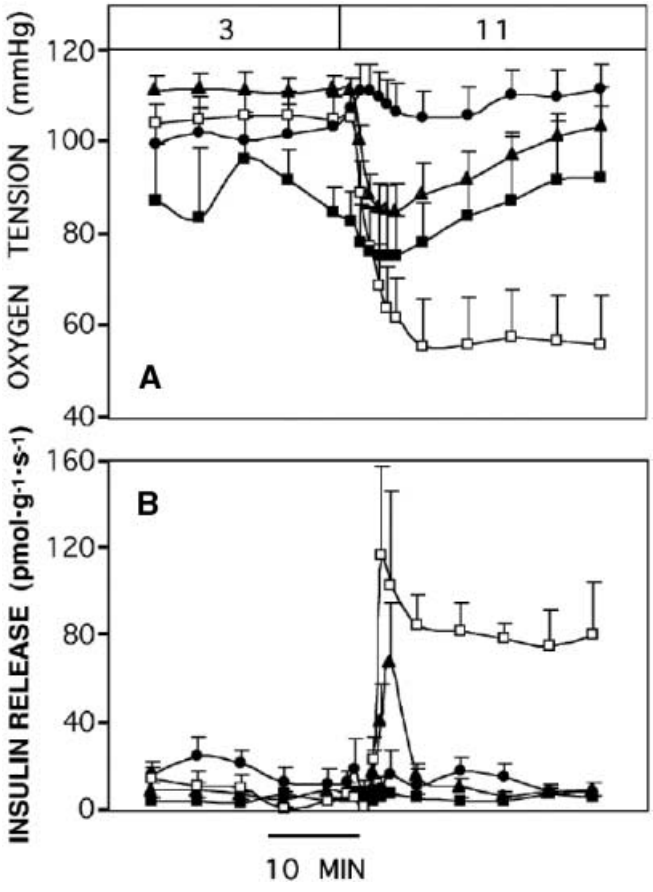

Fig. 1A, B Glucose-induced changes in oxygen tension and insulin release in the presence of oligomycin, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (*FCCP*) or arsenate. Oxygen tension (**A**) and insulin release (**B**) were monitored simultaneously from individual islets perifused with 2 μg/ml oligomycin (*filled circles*), 5 μM FCCP (*filled squares*), 5 mM arsenate (*filled triangles*) or no addition (*open squares*) when the glucose concentration was increased from 3 to 11 mM. Graphs show means ± SEM for 5–7 experiments

pO_2 and insulin release, islets were exposed to the inhibitor of mitochondrial ATP-synthase, oligomycin, or the uncoupler of oxidative phosphorylation, FCCP. Whereas mitochondrial metabolism is halted in the presence of the former compound, it can proceed in the presence of the latter. Oxygen tension and basal insulin release at 3 mM glucose were not affected by 2 μg/ml oligomycin (Fig. 1, Tables 1, 2). FCCP (5 μM) lowered pO_2 at 3 mM glucose by 13% but had no effect on insulin release (Fig. 1, Tables 1, 2). When the glucose concentration was raised to 11 mM in the presence of oligomycin there was no change in pO_2 or insulin secretory rate. In the presence of FCCP, 11 mM glucose decreased pO_2. The rate of decline in pO_2 was 40% compared with control. After reaching a nadir (75±9 mmHg) approximately 5 min after increasing the glucose concentration, pO_2 began to climb and did so for the rest of the experimental period. The rise in glucose concentration was not accompanied by any change in insulin release.

A minor contribution to the overall cellular ATP production comes from glycolysis. To evaluate the role of glycolytically derived ATP in glucose-induced insulin release, the reaction catalysed by phosphoglycerate kinase was uncoupled from ATP production with 5 mM arsenate. Oxygen tension and basal insulin release at 3 mM glucose were not affected by arsenate (Fig. 1, Tables 1, 2). When the glucose concentration was raised to 11 mM, pO_2 decreased as in controls. This was accompanied by an accelerated insulin secretory rate. However, after reaching a nadir in pO_2 (85±6 mmHg) approximately 5 min after increasing the glucose concentration, pO_2 started to climb. The increase in pO_2 continued for the rest of the experimental period and was paralleled by a rapid decrease in insulin release. Already 10 min after increasing the glucose concentration, insulin release was comparable to that observed at 3 mM glucose.

Apart from pyruvate, glycolysis also produces reducing equivalents in the form of NADH to be further metabolized by the mitochondria. To investigate the role of glycolytically produced NADH in glucose-induced insulin release, islets were exposed to CHC prior to the

Table 1 Oxygen tension and insulin release rate of single isolated islets of Langerhans in the presence of agents affecting ATP metabolism. Individual islets were perifused in the presence of 3 mM glucose. Thirty minutes prior to raising the glucose concentration to 11 mM, the compound was included in the perifusion medium. Means ± SEM show values obtained from five measurements obtained during the first 21 min prior to elevating the glucose concentration and during the last 21 min after raising the glucose concentration. The latter five measurements started 10 min after raising the glucose concentration. *n* indicates the number of experiments

Compound	Oxygen tension (mmHg)		Insulin release rate (pmol·g^{-1}·s^{-1})		*n*
	3 mM glucose	11 mM glucose	3 mM glucose	11 mM glucose	
None	105±5	57±10*	7±2	80±18*	7
2 μg/ml oligomycin	100±6	106±6**	16±6	13±5**	6
5 μM FCCP	91±7**	81±9**	5±2	6±1**	6
5 mM arsenate	111±3	96±5*,**	8±2	10±3**	5
100 μM ouabain	109±6	69±7*	16±6	81±13*	6
1 μM gramicidin	108±4	83±6*,**	8±4	39±14*,**	6

$^{*}P<0.05$ (ANOVA for repeated measurements) compared to corresponding value at 3 mM glucose. $^{**}P<0.05$ (ANOVA with Fischers posthoc test) compared to absence of compound

Table 2 Dynamics of glucose-induced decrease in oxygen tension in the presence of agents affecting ATP metabolism. Individual islets were perifused in the presence of 3 mM glucose. Thirty minutes prior to raising the glucose concentration to 11 mM, the compound was included in the perifusion medium. After raising the glucose concentration the decrease in pO_2 was measured and the kinetic parameters were calculated. Means ± SEM show values obtained from measurements for the indicated number of experiments

Compound	Decrease in oxygen tension			*n*
	Lagtime (min)	Duration (min)	Rate (mmHg·s^{-1})	
None	0.6±0.1	3.4±0.3	–0.23±0.03	7
2 µg/ml oligomycin	–	–	–	6
5 µM FCCP	0.7±0.1	3.3±0.6	–0.10±0.03*	6
5 mM arsenate	0.5±0.1	2.7±0.2*	–0.18±0.03	5
100 µM ouabain	0.9±0.1	3.0±0.2	-0.24±0.03	6
1 µM gramicidin	1.0±0.3	3.9±0.4	–0.10±0.01*	6

*$P<0.05$ (ANOVA with Fischers posthoc test) compared to absence of compound

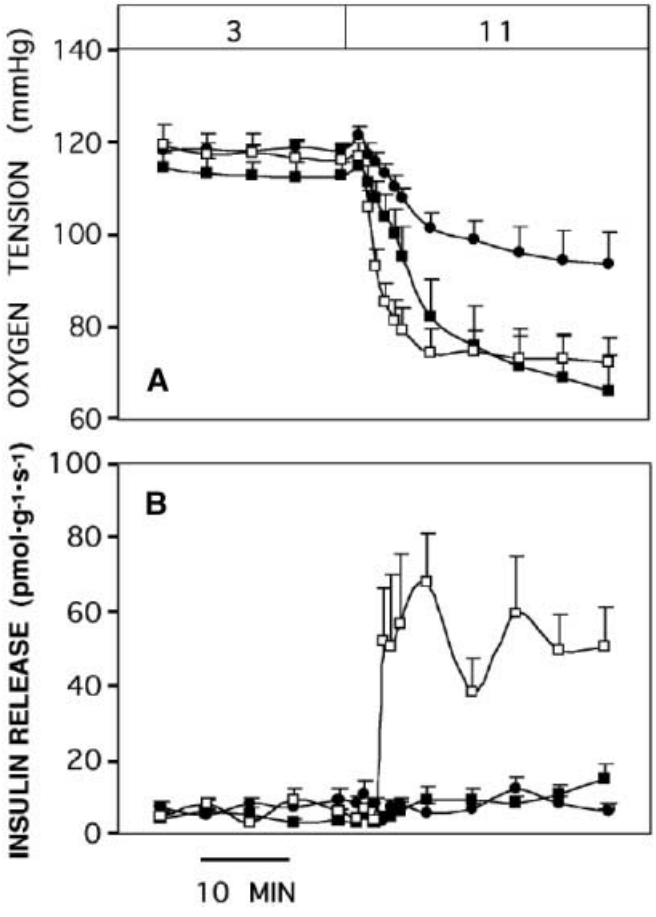

Fig. 2A, B Glucose-induced changes in oxygen tension and insulin release in the presence of α-cyano-4-hydroxycinnamic acid (*CHC*). Oxygen tension (**A**) and insulin release (**B**) were monitored simultaneously from individual islets perifused with 1 mM CHC (*filled squares*), 1 mM CHC and 1.5 mM EGTA (*filled circles*) or no addition (*open squares*) when the glucose concentration was increased from 3 to 11 mM. Graphs show means ± SEM for 5–6 experiments

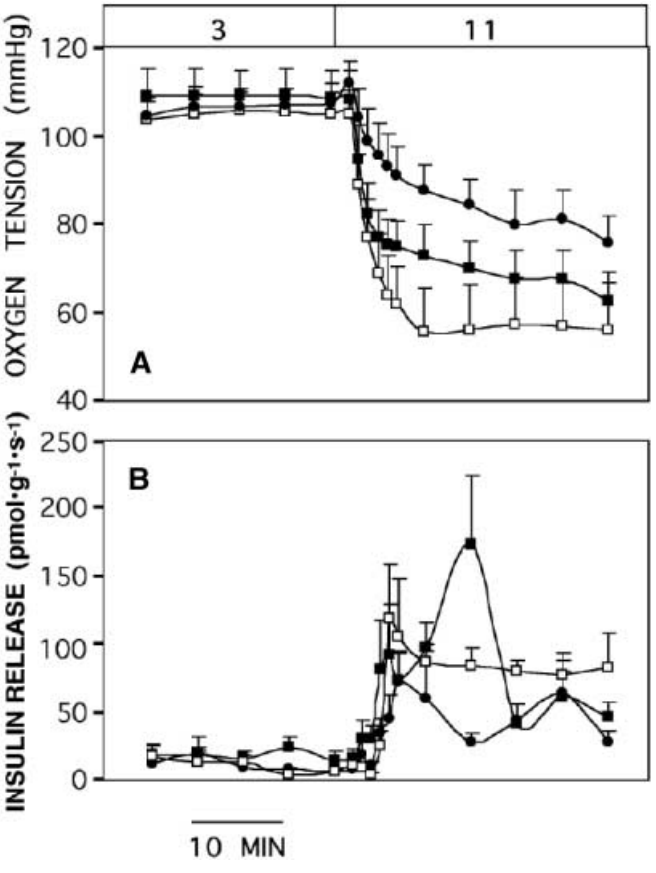

Fig. 3A, B Glucose-induced changes in oxygen tension and insulin release in the presence of ouabain or gramicidin. Oxygen tension (**A**) and insulin release (**B**) were monitored simultaneously from individual islets perifused with 100 µM ouabain (*filled squares*), 1 µM gramicidin (*filled circles*) or no addition (*open squares*) when the glucose concentration was increased from 3 to 11 mM. Graphs show means ± SEM for 6–7 experiments

rise of sugar concentration. Oxygen tension and basal insulin release at 3 mM glucose were not affected by 1 mM of the inhibitor of mitochondrial pyruvate transport (Fig. 2, Tables 3, 4). When the glucose concentration was raised to 11 mM, the lagtime of the glucose-induced decrease in pO_2 was more than doubled and the rate of decline was 30% of control. Although pO_2 in the presence of CHC eventually decreased to levels observed in control islets, the insulin secretory rate was only 20% of that observed in control islets. In islets exposed to 1.5 mM

Table 3 Oxygen tension and insulin release rate of single isolated islets of Langerhans in the presence of mitochondrial pyruvate transport inhibition. Individual islets were perifused in the presence of 3 mM glucose. Thirty minutes prior to raising the glucose concentration to 11 mM, α-cyano-4-hydroxycinnamic acid (*CHC*) in the absence or presence of EGTA was included in the perifusion medium. Means ± SEM show values obtained from five measurements obtained during the first 21 min prior to elevating the glucose concentration and during the last 21 min after raising the glucose concentration. The latter five measurements started 10 min after raising the glucose concentration. *n* indicates the number of experiments

Compound	Oxygen tension (mmHg)		Insulin release rate ($pmol \cdot g^{-1} \cdot s^{-1}$)		*n*
	3 mM glucose	11 mM glucose	3 mM glucose	11 mM glucose	
None	118±4	74±5*	6±1	55±5*	6
1 mM CHC	113±3	73±8*	5±2	11±2*,**	5
1 mM CHC, 1.5 mM EGTA	119±2	97±5*,**	8±1	9±2**	5

Table 4 Dynamics of glucose-induced decrease in oxygen tension in the presence of mitochondrial pyruvate transport inhibition. Individual islets were perifused in the presence of 3 mM glucose. Thirty minutes prior to raising the glucose concentration to 11 mM, CHC in the absence or presence of EGTA was included in the perifusion medium. After raising the glucose concentration the decrease in pO_2 was measured and the kinetic parameters were calculated. Means ± SEM show values obtained from measurements for the indicated number of experiments, *n*

Compound	Decrease in oxygen tension			*n*
	Lag time (min)	Duration (min)	Rate ($mmHg \cdot s^{-1}$)	
None	0.6±0.1	3.9±0.4	–0.19±0.01	6
1 mM CHC	1.4±0.5*	12.2±1.2*	–0.06±0.01*	5
1 mM CHC, 1.5 mM EGTA	1.2±0.4*	7.2±1.6*	–0.05±0.01*	5

*$P<0.05$ (ANOVA with Fischers posthoc test) compared to absence of compound

EGTA together with CHC prior to the rise of sugar concentration, the Ca^{2+} chelator counteracted the glucose-induced decrease in pO_2 and blocked the faint increase in secretion observed in the absence of the chelator (Fig. 2, Tables 3, 4).

By altering the consumption of ATP the energy state of the islet is also affected. The major consumer of cellular ATP is Na^+/K^+ ATPase. We therefore aimed to change the energy state by inhibiting Na^+/K^+ ATPase with ouabain or by stimulating ATPase by increasing the sodium permeability with gramicidin. Including 100 μM ouabain or 1 μM gramicidin had no effect on pO_2 or insulin release at 3 mM glucose (Fig. 3, Tables 1, 2). When the glucose concentration was increased to 11 mM in the presence of ouabain the effects on pO_2 and insulin release were similar to those in the control situation. In the presence of gramicidin, 11 mM glucose caused a rate of decline in pO_2, which was 40% of that of control islets. The decline in pO_2 resulted in a sustained level, which was 50% of that observed in control islets. Corresponding measurements of insulin release showed a glucose-induced increase, which was 50% of that observed in control islets.

Discussion

Metabolic activation is a key step in β-cell glucose signalling leading to insulin secretion [18]. In this study the increase in cellular respiration induced by 11 mM glucose was 41%, which is in good agreement with the previously reported 50% [19]. The prompt and sustained enhancement of respiration was paralleled by a rapid and maintained increase in secretion. The failure to correlate the blood glucose concentration to an appropriate metabolic signal disrupts the function of the β-cell as a controller of glucose homeostasis. In the search for factors important for this coupling of metabolism to the release of insulin, intermediaries of glycolytic and mitochondrial metabolism have been evaluated, as have other related factors, e.g. ATP/ADP level [20], NADH [21], $[Ca^{2+}]_c$ [22] and recently glutamate [23].

The role of glycolysis in glucose-induced insulin release was evaluated in a previous study in which it was shown that glycolysis alone can maintain glucose-induced insulin release [13]. This is in contrast to our results, which show that glycolysis alone can neither initiate nor maintain glucose-induced insulin release. The importance of glycolytically produced ATP for glucose-induced insulin release was partly based on the observed inhibition of secretion by arsenate [13]. Our results show that, in the presence of arsenate, glucose causes a prompt, initial reduction in pO_2. Arsenate uncouples the reaction catalysed by phosphoglycerate kinase from ATP production, thereby reducing glycolytically produced ATP without affecting glycolytic flux [24]. The fact that both respiration and insulin release are activated as they are in controls despite glycolytic uncoupling is not compatible with glycolytically produced ATP playing a role in the initiation of insulin secretion, and stresses the importance of mitochondrial metabolism for this process. After prolonged exposure to arsenate, oxidative phosphorylation is known to be affected [25], which may explain the gradual return of pO_2 and insulin release within minutes

to levels observed before the rise of glucose concentration.

The proposed role of glycolysis in glucose-induced insulin release was also based on the maintained or slightly augmented release in the presence of CHC [13]. Our result show that in the presence of CHC basal metabolic activity was not affected, but that the glucose-induced initial reduction in the rate of pO_2 was severely reduced with impaired increase in the rate of secretion. Furthermore, the results with CHC indicate that the rate at which glucose-derived metabolites, such as pyruvate but also NADH, are brought to the mitochondria is an important factor in the coupling of metabolism to secretion. By using CHC we allow shuttling of NADH from glycolysis to the mitochondria but no substrate for the Krebs cycle is delivered. Interestingly, oxygen consumption was still enhanced by the rise in glucose concentration, but no sustained insulin secretion was detected. This suggests that the delivery of pyruvate to the mitochondria for ATP production is the major function of glycolysis in β-cell stimulus–secretion coupling. Glycolytically produced NADH is a contributing coupling factor in glucose-induced insulin release, in that the oxidation of the nucleotide elevates mitochondrial Ca^{2+} levels [26]. In an attempt to counteract such a rise in the mitochondrial Ca^{2+} concentration, EGTA was introduced. A rise in the mitochondrial Ca^{2+} concentration affects mitochondrial metabolism in many ways [27], including activation of Ca^{2+}-dependent enzymes in the mitochondria, and thereby enhances mitochondrial metabolism [28]. In support of such a role of a rise in the mitochondrial Ca^{2+} concentration in glucose-stimulated insulin release, we observed that the glucose-induced decline in pO_2 in CHC-treated islets was halved when the Ca^{2+}-chelator EGTA was also added.

A possible explanation for the discrepancy between our results with arsenate and CHC and those of Mertz et al. [13] is that the islets in our study were exposed to the agents approximately 30 min prior to the rise of glucose concentration. Furthermore, whereas Mertz et al. [13] incubated different groups of islets for 60 min in the presence of arsenate at low or high glucose concentrations, we perifused individual islets with media containing arsenate and a low and then a high glucose concentration. Also, our analytical methods [15, 17] allowed time-resolved measurements of the glucose-induced changes in insulin and oxygen tension.

The primary role of mitochondrial metabolism in glucose-induced insulin release was substantiated by our results when mitochondrial metabolism was either blocked by oligomycin or uncoupled by FCCP. Under these conditions glucose caused no increase in insulin release. Blocking the reentry of protons through the F_0/F_1 ATPase with oligomycin reduces mitochondrial ATP production and inhibits the passage of electrons in the respiratory chain and O_2 consumption. Consequently, pyridine nucleotides are kept in a reduced state, which effectively stops substrate handling in both the Krebs cycle and glycolysis. The lack of glucose-induced effects on secretion can therefore be attributed to both the insufficient generation of ATP required for initial phosphorylation of the sugar molecule and later steps in the exocytosis of insulin granules [29] as well as a lack of NAD^+. The latter condition is enhanced by the limited anaerobic metabolism in the pancreatic β-cell due to low levels of lactate dehydrogenase [30]. In fact, lactate production in ob/ob islets is saturated at 3 mM glucose [31]. Unlike oligomycin, FCCP enhances mitochondrial respiration and does not halt glycolysis or intermediate metabolism but diminishes the production of ATP. The compound lowered pO_2 at 3 mM glucose by 13%, which indicates that the energy state is limiting the respiration rate even at this low glucose concentration. Since FCCP does not halt glucose metabolism per se, glycolytically produced ATP and NADH can be formed. The inability of glucose to enhance secretion in the presence of the uncoupler reinforces the role of mitochondrial ATP production and may be explained by the lack of available ATP for the initial phosphorylation steps in glycolysis. This would also explain the gradual restoration of pO_2 observed in the presence of FCCP due to lack of substrate supply from glycolysis. It should also be noted that the dynamics of the glucose-induced decrease in oxygen tension under control conditions in the present study was similar to that obtained when stimulating islets with the mitochondrial fuel ketoisocaproic acid [32]. It might therefore be argued that the changes in pO_2 in the present study reflect the mitochondrial respiration of glucose-derived metabolites.

The energy state prior to the rise of glucose concentration is important for the respiratory and secretory responses. Therefore, we also investigated the effect of increased and decreased consumption of cellular ATP on glucose activation, which was done by modulating the activity of the Na^+/K^+ ATPase, the major consumer of cellular energy [11]. To increase the activity of the ATPase and consequently lower islet ATP content, the cell permeability to sodium ions was increased by gramicidin [33]. In human erythrocytes gramicidin decreased the ATP content by 50% [34]. In the present study gramicidin halved the rate at which pO_2 declined in response to the elevation of the glucose concentration, which was associated with a reduction in the insulin secretory rate. The slower rate of decline in pO_2 in the presence of gramicidin may be explained by a reduced availability of ATP molecules for the initial phosphorylation reactions in glycolysis. To decrease the activity of the ATPase, the Na^+/K^+ exchanger was inhibited by ouabain. The lack of enhanced metabolic and secretory responses could be the result of inhibited cellular respiration due to the Pasteur effect. Indeed, an increase in the ATP/ADP ratio has been reported to inhibit respiration in permeabilized HIT cells [19].

In conclusion, an increase in insulin release in response to a rise in the glucose concentration was only seen when the rise induced a prompt and protracted increase of mitochondrial metabolism. Such activation of

mitochondrial metabolism depended on a good metabolic state prior to the glucose challenge.

Acknowledgements Grants from the Swedish Medical Research Council (72X-14019), the Swedish Diabetes Association, the Novo Nordisk Foundation, the Family Ernfors Foundation, the Marcus and Amalia Wallenberg Foundation, the Göran Gustafsson Foundation, the Magnus Bergvall Foundation, the Swedish Society of Medicine and the Swedish Foundation for Strategic Research supported the study.

References

1. Civelek VN, Deeney JT, Kubik K, Schultz V, Tornheim K, Corkey BE (1996) Temporal sequence of metabolic and ionic events in glucose-stimulated clonal pancreatic β-cells (HIT). Biochem J 315:1015–1019
2. Ashcroft SJ, Sugden MC, Williams IH (1980) Carbohydrate metabolism and the glucoreceptor mechanism. Horm Metab Res Suppl Suppl 10:1–7
3. Matschinsky F (1996) A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes 45:223–241
4. Rizza R, Butler P (1990) Insulin resistance in type II diabetes mellitus. Adv Second Messenger Phosphoprotein Res 24:511–516
5. Goto Y, Suzuki K, Ono T, Sasaki M, Toyota T (1988) Development of diabetes in the non-obese NIDDM rat (GK rat). Adv Exp Med Biol 246:29–31
6. Östensson CG, Khan A, Abdel-Halim S, Guenifi A, Suzuki K, Goto Y, Efendic S (1993) Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. Diabetologia 36:3–8
7. Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311:271–273
8. Rorsman P, Ashcroft FM, Trube G (1988) Single Ca channel currents in mouse pancreatic β-cells. Pflugers Arch 412:597–603
9. Bergsten P, Grapengiesser E, Gylfe E, Tengholm A, Hellman B (1994) Synchronous oscillations of cytoplasmic Ca^{2+} and insulin release in glucose-stimulated pancreatic islets. J Biol Chem 269:8749–8753
10. Wollheim CB, Lang J, Regazzi R (1996) The exocytotic process of insulin and its regulation by Ca^{2+} and G-proteins. Diabetes Rev 4:276–297
11. Stryer L (1995) Biochemistry. WH Freeman, New York
12. Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T, Prentki M (1997) Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in β cells. J Biol Chem 272:18572–18579
13. Mertz RJ, Worley JF III, Spencer B, H JJ, Dukes ID (1996) Activation of stimulus-secretion coupling in pancreatic β-cells by specific products of glucose metabolism. J Biol Chem 271:4838–4845
14. Hellman B (1965) Studies in obese-hyperglycemic mice. Ann NY Acad Sci 131:541–558
15. Bergsten P, Hellman B (1993) Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. Diabetes 42:670–674
16. Revsbech N (1989) An oxygen microsensor with a guard cathode. Limnol Oceanogr 34:474–478
17. Ortsäter H, Liss P, Lund PE, Åkerman KE, Bergsten P (2000) Oscillations in oxygen tension and insulin release of individual pancreatic ob/ob mouse islets. Diabetologia 43:1313–1318
18. Prentki M, Tornheim K, Corkey BE (1997) Signal transduction mechanisms in nutrient-induced insulin secretion. Diabetologia 40 [Suppl. 2]:S32–S41
19. Civelek VN, Deeney JT, Shalosky NJ, Tornheim K, Hansford RG, Prentki M, Corkey BE (1996) Regulation of pancreatic β-cell mitochondrial metabolism: influence of Ca^{2+}, substrate and ADP. Biochem J 318:615–621
20. Nilsson T, Schultz V, Berggren PO, Corkey BE, Tornheim K (1996) Temporal patterns of changes in ATP/ADP ratio, glucose 6-phosphate and cytoplasmic free Ca^{2+} in glucose-stimulated pancreatic β-cells. Biochem J 314:91–94
21. Dukes ID, McIntyre MS, Mertz RJ, Philipson LH, Roe MW, Spencer B, Worley JF 3rd (1994) Dependence on NADH produced during glycolysis for β-cell glucose signaling. J Biol Chem 269:10979–10982
22. Prentki M, Matschinsky FM (1987) Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol Rev 67:1185–1248
23. Maechler P, Antinozzi PA, Wollheim CB (2000) Modulation of glutamate generation in mitochondria affects hormone secretion in INS-1E beta cells. IUBMB Life 50:27–31
24. Duggleby RG, Dennis DT (1974) Nicotinamide adenine dinucleotide-specific glyceraldehyde 3-phosphate dehydrogenase from *Pisum sativum*. Effect of nicotinamide adenine dinucleotide and related compounds on the enzyme-catalyzed arsenolysis of 1,3-diphosphoglyceric acid. J Biol Chem 249:175–181
25. Azzone G, Ernster L (1961) Compartmentation of mitochondrial phosphorylation as disclosed by studies with arsenate. J Biol Chem 236:1510–1517
26. Eto K, Suga S, Wakui M, Tsubamoto Y, Terauchi Y, Taka J, Aizawa S, Noda M, Kimura S, Kasai H, Kadowaki T (1999) NADH shuttle system regulates K(ATP) channel-dependent pathway and steps distal to cytosolic Ca^{2+} concentration elevation in glucose-induced insulin secretion. J Biol Chem 274:25386–25392
27. McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev 70:391–425
28. Rutter GA, Theler JM, Murgia M, Wollheim CB, Pozzan T, Rizzuto R (1993) Stimulated Ca^{2+} influx raises mitochondrial free Ca^{2+} to supramicromolar levels in a pancreatic β-cell line. Possible role in glucose and agonist-induced insulin secretion. J Biol Chem 268:22385–22390
29. Eliasson L, Renstrom E, Ding WG, Proks P, Rorsman P (1997) Rapid ATP-dependent priming of secretory granules precedes Ca^{2+}- induced exocytosis in mouse pancreatic β-cells. J Physiol (Lond) 503:399–412
30. Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, et al. (1994) Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic β-cells. Potential role in nutrient sensing. J Biol Chem 269:4895–4902
31. Tamarit-Rodriguez J, Idahl L-Å, Giné E, Alcazar O, Sehlin J (1998) Lactate production in pancreatic islets. Diabetes 47:1219–1223
32. Ortsäter H, Liss P, Lund PE, Åkerman KE, Bergsten P (1999) Dynamic measurements of oxygen tension in islets of Langerhans. Adv Exp Med Biol 471:367–372
33. Gomez-Poyou A, Gomez-Lejero C (1977) The use of ionophores and channel formers in the study of the function of biological membranes. Curr Topic Bioenerg 6:221–225
34. Engstrom I, Waldenstrom A, Ronquist G (1993) Effects of the ionophore gramicidin D on energy metabolism in human erythrocytes. Scand J Clin Lab Invest 53:247–252