Hexose metabolism in pancreatic islets

Glucose-induced and Ca^{2+} -dependent activation of FAD-glycerophosphate dehydrogenase

Joanne RASSCHAERT and Willy J. MALAISSE*

Laboratoire de Médecine Expérimentale, Faculté de Médecine, Université Libre de Bruxelles,
115 Boulevard de Waterloo, B-1000 Brussels, Belgium

A rise in extracellular D-glucose concentration causes in pancreatic islets a preferential stimulation of aerobic, relative to total, glycolysis. The possible participation in such a phenomenon of a glucose-induced and Ca^{2+} -dependent activation of FAD-glycerophosphate dehydrogenase was investigated. In islet homogenates, the activity of the mitochondrial and Ca^{2+} -responsive FAD-glycerophosphate dehydrogenase was about two orders of magnitude lower than that of the cytosolic and Ca^{2+} -insensitive NAD-glycerophosphate dehydrogenase. In islet mitochondria, Ca^{2+} increased the affinity of the FAD-glycerophosphate dehydrogenase for L-glycerol 3-phosphate, but did not affect the maximal reaction velocity. In the presence of 0.1 mM-L-glycerol 3-phosphate, the K_a for Ca^{2+} was close to 0.1 μM . When intact islets were preincubated in the presence of both D-glucose and Ca^{2+} , the activity of FAD-glycerophosphate dehydrogenase measured in intact mitochondria incubated in the presence of 1.2 μM - Ca^{2+} was higher than that recorded under the same conditions in islets preincubated in the absence of D-glucose and/or Ca^{2+} . These findings support the view that, in islets exposed to a high concentration of D-glucose, a Ca^{2+} -induced activation of mitochondrial FAD-glycerophosphate dehydrogenase favours the transfer of reducing equivalents by the glycerol phosphate shuttle, and hence accounts, in part at least, for the preferential stimulation of aerobic glycolysis.

INTRODUCTION

A rise in the extracellular concentration of D-glucose causes, in pancreatic islets, a preferential stimulation of aerobic, relative to total, glycolysis [1]. This phenomenon, which was confirmed in a study of the Pasteur effect in the islets [2], appears attributable to an accelerated transfer of reducing equivalents into the mitochondria as mediated by the glycerol phosphate shuttle [3,4] rather than malate-aspartate shuttle [5]. It represents an essential feature of the metabolic and, hence, secretory response to D-glucose in this fuel-sensor organ [6]. It is perturbed either in the absence of extracellular Ca^{2+} [7] or in certain models of β -cell dysfunction [1,8,9]. The present study provides support for the proposal, recently formulated [10], that the glucose-induced stimulation of the glycerol phosphate shuttle in pancreatic islets may be attributable, in part at least, to activation by Ca^{2+} of the mitochondrial FAD-linked glycerophosphate dehydrogenase. This activation was repeatedly documented in isolated mitochondria from different cell types [11–13]. Previous studies have already documented the presence of this enzyme in islet homogenates or mitochondria [4,9,14,15] and explored changes in its activity in insulinomas [16], aging [17] or hyperthyroidism, with conflicting data on the last issue [18,19].

MATERIALS AND METHODS

Pancreatic islets were isolated by the collagenase method [20] from the pancreas of fed albino rats. Groups of 800–1000 islets were homogenized in a Potter–Elvehjem tube (20 strokes) in 0.4 ml of an iced Hepes/NaOH buffer (5.0 mM, pH 7.2) containing 60 mM-sucrose, 190 mM-mannitol, 15 mM-KCl, 3 mM- KH_2PO_4 , 1.0 mM- MgCl_2 and 0.5 mM-EGTA. After centrifugation for 10 min at 780 g, a sample (0.33 ml) of the supernatant fraction was removed, and the remaining material was mixed with 0.33 ml of the same buffer, again homogenized (20 strokes) and centrifuged for 10 min at 780 g. A sample (0.33 ml) of the supernatant fraction obtained after this second centrifugation

was pooled with that collected after the first centrifugation. To isolate islet mitochondria, this pooled post-nuclear supernatant was then centrifuged at 32000 g for 20 min at 4 °C. The mitochondrial pellet was resuspended in 0.75 ml of the Hepes buffer, and the post-mitochondrial supernatant was further centrifuged for 60 min at 100000 g in order to isolate a post-microsomal supernatant. In this procedure, the recovery of enzymic activity in the post-nuclear supernatant, relative to that measured in the crude homogenate, averaged $84.0 \pm 2.6\%$ ($n = 25$) for lactate dehydrogenase and $56.9 \pm 2.2\%$ ($n = 24$) for glutamate dehydrogenase.

The method used to isolate liver mitochondria was previously described [21]. In this method, the protein content of the post-nuclear supernatant averaged $82.1 \pm 1.9\%$ of that in the crude homogenate, and the activity of glutamate dehydrogenase in the mitochondrial pellet represented $87.0 \pm 4.9\%$ of the paired value recorded in the post-nuclear supernatant ($n = 4$ in both cases).

NAD-glycerophosphate dehydrogenase was assayed in 90 μl of the Hepes buffer (see above) containing the material derived from two to three islets, 0.05–12.0 mM-dihydroxyacetone phosphate, 0.1 mM-NADH, 1.0 mM-KCN, 0.01 mM-rotenone and, when required, 0.45 mM- CaCl_2 . After incubation for 20 min at 37 °C, the reaction was halted by heating for 10 min at 70 °C, and the assay of NAD^+ was performed, as described elsewhere [22], after addition of 45 μl of Tris/HCl buffer (150 mM, pH 8.0) containing L-[U- ^{14}C]glutamate (0.15 mM), ADP (3.0 mM) and bovine liver glutamate dehydrogenase (0.13 unit/sample). Standards of NAD^+ (0.5, 1.0 and 3.0 nmol/sample) were prepared in homogenates of islets heated for 10 min at 85 °C and treated in exactly the same manner. Three features of this assay procedure should be underlined. First, the presence of mitochondrial poisons (KCN and rotenone) in the assay medium was found essential to prevent undesirable generation of NAD^+ from NADH in the absence of dihydroxyacetone phosphate. The latter process yielded a high ‘blank’ reading and affected adversely the reaction velocity in the presence of the triose

* To whom correspondence should be addressed.

phosphate, possibly because of the fall in NADH concentration. Thus, in the presence of 50 μM -NADH such a 'blank' reading averaged 1.16 ± 0.02 pmol/min per islet ($n = 6$) in islet crude homogenates, and was decreased by $91.7 \pm 0.2\%$ in the presence of the mitochondrial poisons. The 'blank' reading was also considerably decreased in the post-microsomal supernatant, in which case it averaged $2.9 \pm 0.3\%$ of the paired value recorded in the post-nuclear supernatant. Second, the high activity of the NAD-glycerophosphate dehydrogenase in islets and the high sensitivity of the radioisotopic procedure used here for the assay of NAD⁺ [22] allowed reliable measurements of enzymic activity to be conducted in the material derived from only two to three islets per sample. Third, it was found important not to use higher concentrations of bovine liver glutamate dehydrogenase in the assay of NAD⁺, to prevent the occurrence of a high basal generation of ¹⁴C-labelled 2-oxoglutarate, which was otherwise recorded in the absence of islet material provided that both dihydroxyacetone phosphate and NADH were present in the assay medium. This finding is likely to reflect a limited contamination of the commercial preparation of bovine liver glutamate dehydrogenase by NAD-glycerophosphate dehydrogenase.

Two procedures were used for the assay of FAD-glycerophosphate dehydrogenase. The first assay was based on the generation of ³HOH from L-[2-³H]glycerol 3-phosphate (0.05–3.0 mM) during a 15–20 min incubation at 37 °C in 75–90 μl of Hepes buffer (see above), with the material derived from 30–50 islets per sample. When the islet material had been sonicated, FAD (50 μM) was also present in the assay medium. The reaction was halted by adding 30 μl of citrate/NaOH buffer (0.4 M, pH 4.9) containing KCN (5 mM), rotenone (10 μM) and antimycin A (10 μM), and the ³HOH was recovered as described elsewhere [23].

The second procedure, used in a limited series of experiments conducted on liver mitochondria, was conducted in a volume of 120 μl in the presence of unlabelled D-glycerol 3-phosphate. The reaction was halted by adding 60 μl of HClO₄ (7.5%, w/v), the samples being then frozen in liquid N₂ and disrupted by mechanical vibration [24]. A sample (50 μl) of the HClO₄ extract was neutralized by addition of 25 μl of a solution of KOH (1.0 M) and Tris (0.2 M). After centrifugation (5000 g, 3 min), samples (20 μl) of the supernatant were mixed with 30 μl of a 2-amino-2-methylpropanol solution (50 mM, pH 9.0) containing NAD⁺ (1.7 mM), Na₂HAsO₄ (1.7 mM), mercaptoethanol (3.3 mM) and rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (0.08 unit/sample), with or without yeast triose phosphate isomerase (1.0 unit/sample). After 30 min incubation at 20 °C, the reaction was halted by heating for 10 min at 70 °C, the NADH forming being measured by a radioisotopic procedure [25].

The assay of glutamate dehydrogenase was conducted, as described elsewhere [24], over 45 min incubation at 20 °C, after mixing the islet extract (50 μl) with Tris/HCl buffer (100 mM, pH 8.0) containing 2-oxo[U-¹⁴C]glutamate (0.5 mM), ADP (2.0 mM) and ammonium acetate (50 mM), with or without NADPH (0.6 mM). The assay of lactate dehydrogenase was performed, as previously reported [26], after mixing the islet extract (50 μl) with Hepes/NaOH buffer (50 mM, pH 7.4) containing L-lactate (20 mM) and NAD⁺ (2 mM). After 20 min incubation at 20 °C, the reaction was stopped by heating for 10 min at 80 °C, and the NADH formed measured by the radioisotopic procedure [25].

Protein was measured by the method of Lowry *et al.* [27], with BSA as standard. The concentration of Ca²⁺ was calculated from that of EGTA and CaCl₂ [28].

In the last series of experiments, groups of 300–400 islets each were preincubated for 60 min in 1.0 ml of a bicarbonate-buffered

medium [20] containing BSA (5 mg/ml) in the absence or presence of Ca²⁺ (1.0 mM), D-glucose (16.7 mM) and/or EGTA (0.25 mM). After this preincubation, the islets were placed in a Potter/Elvehjem tube, washed twice with the iced Hepes/NaOH buffer (see above) and homogenized (20 strokes) in 0.2 ml of the same buffer. After centrifugation for 10 min at 780 g, a sample (0.16 ml) of the supernatant was removed, and remaining material was mixed with 0.16 ml of the same buffer, again homogenized (20 strokes) and centrifuged for 10 min at 780 g. A fraction (0.16 ml) of the supernatant obtained after the second centrifugation was pooled with that collected after the first centrifugation. Samples (50 μl each) of the mitochondrial suspension were then incubated for 10 min at 37 °C in a final volume of 75 μl , in the presence of L-[2-³H]glycerol 3-phosphate (0.1 mM) and, if required, CaCl₂ (0.5 mM).

All results are expressed as means ($\pm$ S.E.M.) together with the number of individual determinations (n). The statistical significance of differences between mean values was assessed by use of Student's *t* test.

RESULTS

NAD⁺-glycerophosphate dehydrogenase

The NAD⁺-glycerophosphate dehydrogenase activity in the post-nuclear supernatant of pancreatic islets averaged, when measured in the presence of dihydroxyacetone phosphate (2.0 mM) and NADH (0.1 mM), 12.2 ± 1.4 pmol/min per islet ($n = 9$). The fraction of this activity recovered in the post-microsomal supernatant was similar to that found for lactate dehydrogenase (Table 1). The activity of the enzyme was not affected by Ca²⁺ (1.2 μM), in either the post-nuclear or the post-microsomal supernatant, with a paired Ca²⁺/no-Ca²⁺ ratio of $100.1 \pm 4.7\%$ ($n = 24$). In the post-nuclear supernatant the K_m for dihydroxyacetone phosphate was close to 7 mM (Fig. 1).

FAD-glycerophosphate dehydrogenase

The measurement of FAD-glycerophosphate dehydrogenase activity was based on the generation of ³HOH from L-[2-³H]glycerol 3-phosphate. Control experiments indicated that, in intact liver mitochondria, the reaction velocity was virtually identical when estimated through either the generation of ³HOH from L-[2-³H]glycerol 3-phosphate or the production of dihydroxyacetone phosphate from unlabelled L-glycerol 3-phosphate (Fig. 2a). The radioactive procedure also yielded comparable results in either intact mitochondria or sonicated mitochondria incubated in the presence of 50 μM -FAD (Fig. 2b). The

Table 1. Subcellular distribution of dehydrogenases activity in pancreatic islets

Percentages of enzymic activity in the mitochondrial pellet or in the post-microsomal supernatant are quoted relative to the paired reading recorded in the post-nuclear supernatant. Abbreviation: N.D., not determined.

Subcellular fraction ...	Post-nuclear supernatant (pmol/min per islet)	Mitochondrial pellet (%)	Post-microsomal supernatant (%)
Dehydrogenase			
Lactate	7.5 ± 1.1 (19)	9.7 ± 1.1 (23)	79.8 ± 2.0 (23)
NAD ⁺ -	12.2 ± 1.4 (9)	17.3 ± 1.7 (15)	83.3 ± 4.0 (18)
glycerophosphate			
Glutamate	6.1 ± 0.5 (23)	42.5 ± 2.7 (25)	4.9 ± 1.4 (26)
FAD-	0.037 ± 0.003 (6)	50.9 ± 3.3 (8)	N.D.
glycerophosphate

Fig. 1. Double-reciprocal plot for the activity of NAD^+ -glycerophosphate dehydrogenase in islet post-microsomal supernatants

Mean values ($\pm$ S.E.M.) are derived from triplicate measurements in a series of seven separate experiments.

data illustrated in Fig. 2 further document that the concentration-dependency towards L-glycerol 3-phosphate and the activation of the enzyme by Ca^{2+} were also comparable in the radioactive or non-isotopic procedure, and in intact or sonicated mitochondria. In these experiments, Ca^{2+} ($1.2 \mu\text{M}$) indeed caused, in intact liver mitochondria, a 4-fold increase in the affinity of the FAD-glycerophosphate dehydrogenase for L-glycerol 3-phosphate, while failing to affect the maximal reaction velocity (Fig. 3).

In pancreatic islets, the activity of FAD-glycerophosphate dehydrogenase averaged, when measured in the presence of 0.1 mM -L-glycerol 3-phosphate but in the absence of Ca^{2+} , 22.4 ± 1.1 ($n = 6$) and 24.0 ± 0.6 ($n = 9$) fmol/min per islet respectively in intact and sonicated mitochondria. Such an activity was much higher than that measured under the same experimental conditions in liver mitochondria. Thus, despite the lower recovery of mitochondrial enzymic activity (e.g. glutamate dehydrogenase) during the subcellular fractionation of islets rather than liver (see above), the reaction velocity in the mitochondrial fraction, when expressed per mg of islet or liver wet weight, was 5.85 ± 0.15 pmol/min in islet mitochondria, as distinct from only 0.71 ± 0.05 pmol/min in liver mitochondria ($n = 11-15$). In the islets, the percentage of activity recovered in the mitochondrial pellet, relative to that present in the post-nuclear supernatant, was not significantly different ($P > 0.1$) for FAD-glycerophosphate dehydrogenase and glutamate dehydrogenase (Table 1). The incomplete recovery of enzymic activity in the mitochondrial pellet is mainly attributable to contamination of the microsomal pellet by light mitochondria [29].

Whether in sonicated material prepared from the islet mitochondrial pellet or in intact mitochondria present in the islet crude homogenate, the affinity of the FAD-glycerophosphate dehydrogenase for L-glycerol 3-phosphate was increased 2-3-fold by Ca^{2+} ($1.2 \mu\text{M}$), with little if any change in maximal velocity (Fig. 4). In these experiments, Ca^{2+} lowered the K_m for L-glycerol 3-phosphate from about 1.4 to 0.6 mM in sonicated

Fig. 2. Relationship between the generation of either dihydroxyacetone phosphate (DHAP) from unlabelled L-glycerol 3-phosphate in intact liver mitochondria (a) or ^3HOH from L-[2- ^3H]glycerol 3-phosphate in sonicated liver mitochondria (b), as shown on the ordinates, and the production of ^3HOH from L-[2- ^3H]glycerol 3-phosphate by intact liver mitochondria, as shown on the abscissae

Mean values are derived from triplicate measurements in five separate experiments. The enzymic activity was measured, in the absence ($\circ$, $\triangle$, $\square$, ∇) or presence ($\bullet$, $\blacktriangle$, $\blacklozenge$, $\blacktriangledown$) of Ca^{2+} ($1.2 \mu\text{M}$), at increasing concentrations of L-glycerol 3-phosphate (∇ , $\blacktriangledown$, $\blacklozenge$, 0.1 mM ; $\triangle$, $\blacktriangle$, 0.3 mM ; $\circ$, $\bullet$, $\square$, 1.0 mM). All enzymic activities are expressed as pmol/min per mg liver wet wt.

mitochondria and from approx. 1.1 to 0.5 mM in intact mitochondria. The reaction velocity, expressed per islet, was about 4 times higher in the crude homogenate than in the sonicated mitochondrial pellet, in fair agreement with the data obtained in the experiments designed to study the subcellular distribution of the enzyme (see above).

At a low concentration of L-glycerol 3-phosphate (0.1 mM), the increase in reaction velocity provoked by increasing concentrations of Ca^{2+} (30 nM – 0.05 mM) yielded an apparent K_a for Ca^{2+} close to $0.1 \mu\text{M}$ (Fig. 5).

In intact islet mitochondria, the generation of ^3HOH from L-[2- ^3H]glycerol 3-phosphate was decreased by $93.8 \pm 0.2\%$ in the presence of rotenone ($10 \mu\text{M}$) and KCN (1.0 mM).

Influence of preincubation with D-glucose and Ca^{2+} on FAD-glycerophosphate dehydrogenase activity

In the last series of experiments, groups of 300–400 islets each were preincubated for 60 min at 37°C in the absence or presence of either D-glucose (16.7 mM) or Ca^{2+} (1.0 mM). The islets were then rapidly washed, homogenized and centrifuged. The post-nuclear supernatant was eventually incubated for 10 min at 37°C in the absence or presence of extramitochondrial Ca^{2+}

Fig. 3. Double-reciprocal plot for the activity of FAD-glycerophosphate dehydrogenase in intact liver mitochondria exposed to L-[2-³H]-glycerol 3-phosphate

The enzymic activity was measured in the absence (●) or presence (○) of Ca^{2+} ($1.2 \mu\text{M}$). Mean values ($\pm$ S.E.M.) are derived from triplicate measurements collected in a series of six separate experiments.

($1.2 \mu\text{M}$), for measurement of ^3HOH production from L-[2- ^3H]glycerol 3-phosphate (0.1 mM).

As illustrated in Table 2, the basal activity of FAD-glycerophosphate dehydrogenase, as measured in the absence of extramitochondrial Ca^{2+} , was not significantly affected by the prior exposure of the islets to D-glucose, whether the preincubation was conducted in the absence or presence of extracellular Ca^{2+} . The basal activity was lower, however, in islets preincubated in the absence rather than in the presence of Ca^{2+} ($P < 0.005$), independently of the absence or presence of D-glucose in the preincubation medium. Pooling all available data, the basal activity in islets preincubated in the absence of Ca^{2+} averaged $77.2 \pm 3.4\%$ ($n = 30$) of that recorded in islets preincubated in the presence of the bivalent cation.

The incorporation of Ca^{2+} ($1.2 \mu\text{M}$) in the final assay medium always significantly increased the activity of FAD-glycerophosphate dehydrogenase (Table 2). Relative to the paired basal value, the extent of such an increase in enzymic activity was not significantly different in the islets preincubated, in the absence of D-glucose, with or without extracellular Ca^{2+} . Pooling the two series of observations, it averaged $44.0 \pm 3.6\%$ ($n = 32$). The mean increase in reaction velocity attributable to the presence of extramitochondrial Ca^{2+} was more marked when the islets had been preincubated in the presence of D-glucose, amounting to $75.6 \pm 9.5\%$ and $66.3 \pm 8.3\%$ ($n = 15$ in both cases) in glucose-stimulated islets preincubated in the presence and absence of extracellular Ca^{2+} respectively. Although the latter values were not significantly different from one another ($P > 0.4$), the difference between glucose-stimulated and glucose-deprived islets, in terms of the relative extent of activation of FAD-glycerophosphate dehydrogenase by extramitochondrial Ca^{2+} , was highly significant ($P < 0.005$) in islets preincubated in the presence of extracellular Ca^{2+} , but was at the limit of statistical

Fig. 4. Double-reciprocal plots for the activity of FAD-glycerophosphate dehydrogenase in either sonicated material prepared from the mitochondrial pellet (a) or intact mitochondria in a crude islet homogenate (b)

The enzymic activity was measured in the absence of Ca^{2+} (●) or in the presence of $1.2 \mu\text{M}$ - Ca^{2+} (○). Mean values ($\pm$ S.E.M., whenever exceeding the size of the mean point) are derived from triplicate measurements collected in three (a) or six (b) separate experiments.

Fig. 5. Effect of increasing concentrations of extramitochondrial Ca^{2+} (logarithmic scale) on the activity of FAD-glycerophosphate dehydrogenase in the islet post-nuclear supernatant incubated in the presence of 0.1 mM -L-[2- ^3H]glycerol 3-phosphate

Mean values ($\pm$ S.E.M.) are derived from 10 ± 2 individual readings recorded in a series of five separate experiments.

Table 2. FAD-glycerophosphate dehydrogenase activity (fmol/min per islet) in post-nuclear supernatants prepared from islets first incubated for 60 min in the absence or presence of D-glucose and/or Ca^{2+}

Preincubation		Incubation		
D-Glucose (mM)	Ca^{2+} (mM)	No Ca^{2+}	1.2 μM - Ca^{2+}	Ca^{2+} /no Ca^{2+} (increment in %)
Nil	Nil	31.9 $\pm$ 2.0 (15)	49.5 $\pm$ 4.4 (16)	47.9 $\pm$ 4.7 (16)
Nil	1.0	41.1 $\pm$ 2.2 (16)	58.1 $\pm$ 3.8 (16)	40.1 $\pm$ 5.4 (16)
16.7	Nil	29.3 $\pm$ 1.8 (15)	48.2 $\pm$ 3.6 (15)	66.3 $\pm$ 8.3 (15)
16.7	1.0	38.2 $\pm$ 2.1 (17)	69.5 $\pm$ 5.2 (15)	75.6 $\pm$ 9.5 (15)

significance ($P < 0.06$) in islets preincubated in the absence of extracellular Ca^{2+} .

As a result of these differences in either basal velocity or Ca^{2+} -induced activation of FAD-glycerophosphate dehydrogenase, the rate of ^3HOH production recorded in the presence of extramitochondrial Ca^{2+} was higher ($P < 0.005$) in islets preincubated in the presence of both D-glucose and Ca^{2+} than the mean value found in islets preincubated in the absence of D-glucose and/or Ca^{2+} , in which case no significant difference was detected between the three sets of results.

DISCUSSION

The present results document the presence in pancreatic islets of the two enzymes involved in the glycerol phosphate shuttle, namely a cytosolic NAD $^{+}$ -linked and Ca^{2+} -insensitive glycerophosphate dehydrogenase and a mitochondrial FAD-linked and Ca^{2+} -responsive glycerophosphate dehydrogenase. The method here used for the assay of the latter enzyme was more specific than that proposed elsewhere [14], since ^3HOH was recovered by evaporation/condensation rather than separated from its precursor by chromatography, in which case the results might be affected by unspecific phosphatase activity. Our measurements confirm that the activity of the FAD-glycerophosphate dehydrogenase is much higher in islets than in liver cells [14]. In pancreatic islets, the activity of the latter enzyme was about two orders of magnitude lower than that of the cytosolic dehydrogenase. Hence the mitochondrial enzyme is likely to play a major role in the regulation of the metabolic flow in the glycerol phosphate shuttle [30].

FAD-linked glycerophosphate dehydrogenase is an outwardly facing enzyme which responds to extramitochondrial rather than intramitochondrial Ca^{2+} concentration [31]. The increase in cytosolic Ca^{2+} concentration occurring in glucose-stimulated islets [32] could thus lead to activation of the enzyme. This in turn could account, in part at least, both for the preferential stimulation of aerobic glycolysis relative to total glycolysis in islets exposed to increasing concentrations of extracellular D-glucose [1] and for the suppression of such a preferential stimulation in islets exposed to a high concentration of the hexose but deprived of extracellular Ca^{2+} [7].

It should be duly underlined that these proposals do not deny the view that the fundamental mechanism for stimulus-secretion coupling in the pancreatic β -cell resides in the closure of ATP-sensitive K^{+} channels, subsequent depolarization of the plasma membrane and eventual gating of voltage-sensitive Ca^{2+} channels as primarily caused, by an increase in the rate of glucose metabolism, and hence in the cytosolic ATP/ADP ratio [33]. The activation by Ca^{2+} of FAD-glycerophosphate dehydrogenase would then represent, like that of pyruvate dehydrogenase [34]

and 2-oxoglutarate dehydrogenase [21], a modality for potentiation or self-enhancement of such a stimulus-secretion coupling.

The experiments including preincubation of intact islets in the absence or presence of D-glucose and/or extracellular Ca^{2+} , and then measurement of FAD-glycerophosphate activity in mitochondria, suggest that a rise in extramitochondrial Ca^{2+} concentration may not represent the sole factor responsible for activation of the enzyme by D-glucose in intact islet cells.

In these experiments, two changes in enzymic activity were observed. First, the basal activity measured in mitochondria incubated in the absence of Ca^{2+} was lower when the islets were preincubated in the absence rather than in the presence of extracellular Ca^{2+} . Second, the activation of the enzyme caused by a rise in the extramitochondrial concentration of Ca^{2+} was more pronounced when the islets were preincubated in the presence rather than in the absence of D-glucose. Such a phenomenon was most obvious when the preincubation medium contained extracellular Ca^{2+} .

Two mechanisms might account for these findings. First, the Ca^{2+} content of the mitochondria at the end of the preincubation period could conceivably affect the Ca^{2+} concentration between the mitochondrial inner and outer membranes during the further incubation of mitochondria [35]. For instance, the prolonged exposure of islets to Ca^{2+} -depleted media may eventually lower the mitochondrial Ca^{2+} content and, through a subsequent change in the Ca^{2+} concentration in the space between the inner and outer membranes, decrease the basal activity of FAD-glycerophosphate dehydrogenase. Likewise, the greater response evoked by extramitochondrial Ca^{2+} in mitochondria from islets preincubated with D-glucose could reflect Ca^{2+} accumulation in the mitochondria during the preincubation period [32]. As a result, full activation of FAD-glycerophosphate dehydrogenase would occur in intact mitochondria through the supply of Ca^{2+} from both the extramitochondrial and intramitochondrial cationic pools.

As a second, alternative or complementary hypothesis, it cannot be ruled out that prior exposure of the islets to a high concentration of D-glucose somehow affects the activity of FAD-linked glycerophosphate dehydrogenase independently of the actual Ca^{2+} concentration in the vicinity of the enzyme. This proposal is consistent with recent observations suggesting that, in pancreatic islets exposed to a given concentration of D-glucose, factors other than the cytosolic Ca^{2+} concentration regulate the rate of aerobic glycolysis [36].

The present findings nevertheless support the view that a Ca^{2+} -induced activation of FAD-glycerophosphate dehydrogenase participates in the preferential stimulation of aerobic glycolysis in islets exposed to high concentrations of D-glucose. The relevance of this concept to the cytophysiology of insulin secretion is reinforced by the recent observation that a site-specific suppression of islet FAD-glycerophosphate dehydrogenase occurs in the model of non-insulin-dependent diabetes found in adult rats injected with streptozotocin during the neonatal period [9].

We thank M. Mahy for technical assistance and C. Demesmaeker for secretarial help. This work was supported by grants from the Belgian Foundation for Scientific Medical Research and Belgian Ministry of Scientific Policy.

REFERENCES

1. Sener, A. & Malaisse, W. J. (1987) *Biochem. J.* **246**, 89–95
2. Malaisse, W. J., Rasschaert, J., Záhner, D. & Sener, A. (1988) *Diabetes Res.* **7**, 53–58
3. Sener, A., Rasschaert, J., Záhner, D. & Malaisse, W. J. (1988) *Int. J. Biochem.* **20**, 595–598

4. Meglasson, M. D., Smith, K. M., Nelson, D. & Erecinska, M. (1989) *Am. J. Physiol.* **256**, E173–E178
5. Malaisse, W. J., Malaisse-Lagae, F. & Sener, A. (1982) *Endocrinology* (Baltimore) **111**, 392–397
6. Malaisse, W. J. (1987) *Med. Sci. Res.* **15**, 65–67
7. Malaisse, W. J. & Sener, A. (1988) *Biochim. Biophys. Acta* **971**, 246–254
8. Sener, A., Blachier, F. & Malaisse, W. J. (1988) *J. Biol. Chem.* **263**, 1904–1909
9. Giroix, M.-H., Rasschaert, J., Bailbe, D., Leclercq-Meyer, V., Sener, A., Portha, B. & Malaisse, W. J. (1991) *Diabetes* **40**, 227–232
10. Malaisse, W. J., Malaisse-Lagae, F., Rasschaert, J., Zähler, D., Sener, A., Davies, D. R. & Van Schaftingen, E. (1990) *Biochem. Soc. Trans.* **18**, 107–108
11. Hansford, R. G. & Chappell, J. B. (1967) *Biochem. Biophys. Res. Commun.* **27**, 686–693
12. Bukowiecki, L. J. & Lindberg, O. (1974) *Biochim. Biophys. Acta* **348**, 115–125
13. Wernette, M. E., Ochs, R. S. & Lardy, H. A. (1981) *J. Biol. Chem.* **256**, 12767–12771
14. MacDonald, M. J. (1981) *J. Biol. Chem.* **256**, 8287–8290
15. MacDonald, M. J. (1982) *Horm. Metab. Res.* **14**, 678–679
16. MacDonald, M. J., Warner, T. F. & Pellett, J. R. (1983) *J. Clin. Endocrinol. Metab.* **57**, 662–664
17. Azhar, S., Ho, H., Reaven, E. P. & Reaven, G. M. (1983) *Metab. Clin. Exp.* **32**, 1019–1021
18. Lenzen, S. & Panten, U. (1983) *Biochem. Med.* **30**, 349–356
19. Matschinsky, F. M., Corkey, B. E., Prentki, M., Meglasson, M. D., Erecinska, M., Shimizu, T., Ghosh, A. & Parker, J. (1989) *Excerpta Med. Int. Congr. Ser.* **800**, 17–26
20. Malaisse-Lagae, F. & Malaisse, W. J. (1984) in *Methods in Diabetes Research* (Larner, R. & Pohl, S. L., eds.), vol. 1, pp. 147–152, John Wiley and Sons, New York
21. Sener, A., Rasschaert, J. & Malaisse, W. J. (1990) *Biochim. Biophys. Acta* **1019**, 42–50
22. Malaisse, W. J. & Sener, A. (1990) *Med. Sci. Res.* **18**, 55–56
23. Levy, J., Herchuelz, A., Sener, A., Malaisse-Lagae, F. & Malaisse, W. J. (1976) *Endocrinology* (Baltimore) **98**, 429–437
24. Malaisse, W. J., Hutton, J. C., Kawazu, S. & Sener, A. (1978) *Eur. J. Biochem.* **87**, 121–130
25. Sener, A. & Malaisse, W. J. (1990) *Anal. Biochem.* **186**, 236–242
26. Zähler, D., Sener, A. & Malaisse, W. J. (1990) *Biochim. Biophys. Acta* **1053**, 125–129
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
28. Hurley, T. W., Becker, J. K. & Martinez, J. R. (1984) *J. Biol. Chem.* **259**, 7061–7066
29. Manuel y Keenoy, B., Levitsky, D. O., Sener, A. & Malaisse, W. J. (1990) *Diabetes Res.* **14**, 139–147
30. Werner, H. V. & Berry, M. N. (1974) *Eur. J. Biochem.* **42**, 315–324
31. Klingenberg, M. (1970) *Eur. J. Biochem.* **13**, 247–252
32. Andersson, T., Betsholtz, C. & Hellman, B. (1982) *Biomed. Res.* **3**, 29–36
33. Malaisse, W. J. & Sener, A. (1987) *Biochim. Biophys. Acta* **927**, 190–195
34. McCormack, J. E., Longo, E. A. & Corkey, B. (1990) *Biochem. J.* **267**, 527–530
35. Wohlrab, H. (1977) *Biochim. Biophys. Acta* **462**, 102–112
36. Malaisse, W. J., Rasschaert, J., Conget, I. & Sener, A. (1991) *Int. J. Biochem.*, in the press

Received 11 February 1991/11 April 1991; accepted 17 April 1991