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Hexose metabolism in pancreatic islets. Feedback control of D-glucose oxidation by functional events

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A rise in extracellular D-glucose concentration in pancreatic islet cells causes a greater relative increase in the oxidation of pyruvate and acetyl residues than in glycolysis. A possible explanation for such an unusual situation was sought in the present study. The preferential stimulation of mitochondrial oxidative events was found to display a sigmoidal dependency on hexose concentration, and an exponential time course during prolonged exposure of the islets to a high concentration of D-glucose. The preferential stimulation of mitochondrial oxidative events was abolished in islets incubated in the presence of cycloheximide and absence of Ca^{2+}, in which case the oxidation of D-[6-^{14}C]glucose was more severely inhibited than that of D-[3,4-^{14}C]glucose. Likewise, the inhibitor of protein biosynthesis and the absence of Ca^{2+} affected the oxidation of L-[U-^{14}C]leucine preferentially, relative to that of L-[1-^{14}C]leucine, in islets exposed to a high, but not a low, concentration of the amino acid. These results demonstrate that in pancreatic islets it is possible to dissociate both glycolysis from mitochondrial oxidative events and the oxidation of acetyl residues from their generation rate. Moreover, the experimental data suggest that nutrient-responsive and ATP-requiring functional processes exert a feedback control on mitochondrial respiration in this fuel-sensor organ.

Introduction

It was recently reported [1] that a rise in D-glucose concentration from 2.8 to 16.7 mM preferentially stimulates, in normal rat pancreatic islets, mitochondrial oxidative events, such as the transfer of reducing equivalents, probably as mediated by the glycerol phosphate shuttle [2], the decarboxylation of pyruvate and the oxidation of acetyl-CoA in the Krebs cycle. This preferential stimulation of mitochondrial oxidative events was confirmed by comparing the effect of hypoxia upon the production of $^{3}H_2O$ from D-[5-^{3}H]glucose and that of [^{14}C]lactate and $^{14}CO_2$ from D-[U-^{14}C]glucose [3], and could be reproduced by stimulating with D-fructose islets exposed to a low concentration of D-glucose [4]. Such a preferential stimulation by hexoses of mitochondrial oxidative processes represents a rather unusual situation. Thus, the opposite response occurs in either glucose-insensitive but normal secretory cells, e.g., parotid cells [2], or in tumoral islet cells in which a rise in D-glucose concentration from 2.8 to 16.7 mM inhibits, through a Crabtree effect, D-[U-^{14}C]glucose or D-[6-^{14}C]glucose oxidation as well as O_2 uptake [5]. Because the cytosolic ATP/ADP ratio apparently plays a key role in coupling metabolic to cationic events in the process of

Abbreviation: d.f., degrees of freedom.

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nutrient-stimulated insulin release [6–8], the preferential stimulation of mitochondrial oxidative events in normal islets may represent a favourable feature of this fuel-sensor organ [1].

In the present study, we have further explored the unusual situation found in normal islet cells, with emphasis on the concentration-response relationship and the time course for the preferential stimulation of mitochondrial oxidative events, its simulation in leucine-stimulated islets and its possible dependency on glucose-responsive and ATP-requiring functional events. For the latter purpose, the metabolism of glucose was investigated in islets exposed to cycloheximide and/or deprived of extracellular Ca^{2+} in order to inhibit glucose-induced proinsulin biosynthesis and/or insulin release [9,10]. Our results suggest that the preferential action of D-glucose upon mitochondrial oxidative events in the islet cells may indeed be attributable, to a large extent, to the stimulation by the hexose of ATP-requiring functional processes.

Materials and Methods

All experiments were performed with islets isolated by the collagenase procedure [11] from the pancreas of fed albino rats. The methods used to measure the production of ^3H_2O from D-[5-^{3}H]glucose [3], the oxidation of ^{14}C-labelled nutrients [12], the formation of ^{14}C-labelled lactate, pyruvate and amino acids from D-[U-^{14}C]glucose [13], and the generation of ^{14}C-labelled 2-*keto*-isocaproate from L-[1-^{14}C]leucine [14] are described in the references cited.

In some experiments, the utilization of D-[5-^{3}H]glucose and oxidation of D-[6-^{14}C]glucose were measured simultaneously in the same samples. For this purpose, groups of 20 islets each were incubated in 40 μl of our usual bicarbonate-buffered medium [11] containing both D-[5-^{3}H]glucose and D-[6-^{14}C]glucose in a small tube, which was placed in a larger tube itself placed in a counting vial containing 0.5 ml of 0.1 M HCl and sealed with a rubber stopper. The vials were gassed for 5 min with a mixture of O_2/CO_2 (19/1, v/v). After incubation, 20 μl of a citrate-NaOH buffer (0.4 M, pH 4.9) containing 10 μM antimycin A, 10 μM rotenone and 5.0 mM KCN and 250 μl of hyamine hydroxide were injected into the incubation medium and the larger tube, respectively. After 60 min incubation at room temperature, the large tube was removed and examined for its radioactive content ($^{14}CO_2$). The small tube was placed back into the counting vial, which was immediately sealed and further incubated for 22 h at room temperature for the recovery of ^3H_2O [3]. Double-channel counting indicated the absence of cross-contamination.

All results are expressed as the mean (±S.E.) together with the number of individual determinations (n) or degrees of freedom (d.f.). The S.E. on differences or ratios between mean values was calculated as described elsewhere [15,16]. The statistical significance of differences was assessed by use of a Student's t-test. Although mean absolute values for distinct metabolic variables, as derived from separate experiments, are usually presented in the tables and figures, the statistical comparisons quoted in the text are always restricted to data collected within the same experiment(s).

Results

Concentration-response relationship and time course for D-glucose utilization and oxidation

During 120 min of incubation, the production of ^3H_2O from D-[5-^{3}H]glucose and that of $^{14}CO_2$ from D-[6-^{14}C]glucose both increased in a sigmoidal manner when the hexose concentration was raised from 2.8 to 16.7 mM. The ratio of $^{14}CO_2/^3H_2O$ production was twice as high at 16.7 than at 2.8 mM D-glucose, and itself displayed a sigmoidal concentration-response relationship on increasing the hexose concentration (Fig. 1, upper left panel). At a high concentration of D-glucose (16.7 mM), the production of ^3H_2O occurred at a fairly constant rate (data not shown), but that of $^{14}CO_2$ displayed an exponential-like time course (Fig. 1, lower right panel). At the high glucose level, the ratio of $^{14}CO_2/^3H_2O$ production, thus, progressively increased during incubation (Fig. 1, upper right panel).

Effect of cycloheximide and Ca^{2+} deprivation on glucose metabolism

In order to investigate the possible relevance of functional events to the regulation of glucose

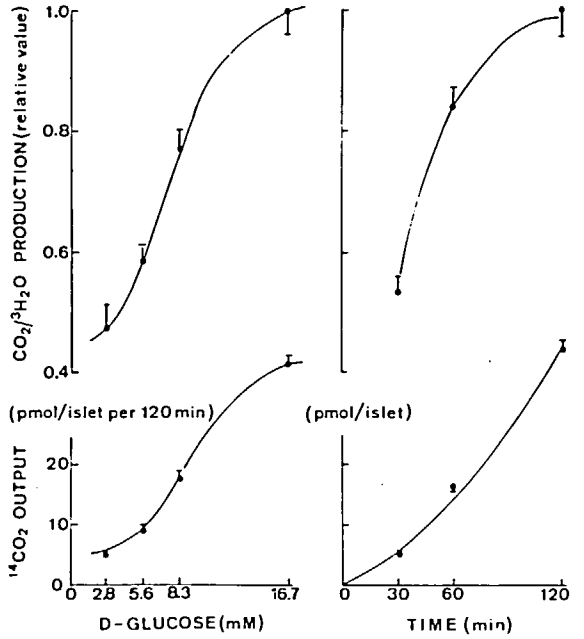

Fig. 1. Concentration-response relationship (left) and time course (right) for the formation of $^{14}CO_2$ from D-[6-^{14}C]glucose (lower panels) and for the ratio between $^{14}CO_2$ output and ^3H_2O production from D-[5-^{3}H]glucose (upper panels). The experiments were conducted over a fixed period of incubation (120 min, left) or at a fixed concentration of D-glucose (16.7 mM, right). Mean values (±S.E.) refer to 18 (left) or 24 (right) individual determinations, the ratio between $^{14}CO_2$ output and ^3H_2O production being expressed relative to the mean value recorded within the same experiment(s) after 120 min incubation in the presence of 16.7 mM D-glucose. Such a reference value averaged 30.5 ± 1.2%.

metabolism, we first examined the behaviour of islets concomitantly exposed to cycloheximide (50 μM) and deprived of Ca^{2+}, the incubation medium containing no $CaCl_2$, which was replaced by an equimolar amount of $MgCl_2$ (final Mg^{2+} concentration, 2.0 instead of 1.0 mM). The results of these experiments are illustrated in Fig. 2.

In the control experiments performed in the absence of cycloheximide and at normal concentrations of both Ca^{2+} (1.0 mM) and Mg^{2+} (1.0 mM), a rise in D-glucose concentration from 2.8 to 16.7 mM augmented to a lesser relative extent the production of ^3H_2O from D-[5-^{3}H]glucose than the oxidation of either D-[3,4-^{14}C]glucose or D-[6-^{14}C]glucose. The 16.7 mM/2.8 mM ratio averaged 2.17 ± 0.10 (d.f. = 74) for ^3H_2O production, 6.33 ± 0.31 for the oxidation of D-[3,4-^{14}C]glucose (d.f. = 71) and 6.05 ± 0.21 for the oxidation of D-[6-^{14}C]glucose (d.f. = 70). Relative to the oxidation of D-[3,4-^{14}C]glucose, which informs on the decarboxylation of pyruvate, the oxidation of D-[6-^{14}C]glucose, which informs on the oxidation of acetyl residue in the Krebs cycle, was not affected by the rise in hexose concentration, averaging 36.9 ± 2.1% (n = 20) and 34.4 ± 1.6% (n = 20) at 2.8 and 16.7 mM D-glucose, respectively.

At a low concentration of D-glucose (2.8 mM), the catabolism of the hexose was little affected by the presence of cycloheximide and the nominal absence of Ca^{2+}, the ratio of experimental/control values averaging 0.98 ± 0.06 (d.f. = 80) for ^3H_2O production, 0.99 ± 0.05 (d.f. = 82) for the oxidation of D-[3,4-^{14}C]glucose and 0.87 ± 0.05 (d.f. = 81) for the oxidation of D-[6-^{14}C]glucose. The ratio of D-[6-^{14}C]glucose oxidation to D-[5-^{3}H]glucose utilization failed to be significantly affected by the presence of cycloheximide and the nominal absence of Ca^{2+}. It averaged 10.1 ± 0.8 and 9.7 ± 0.4% (n = 21 in both cases) in control and experimental islets, respectively. At a high concentration of D-glucose (16.7 mM), the utilization of D-[5-^{3}H]glucose remained not significantly affected by the presence of cycloheximide and the nominal

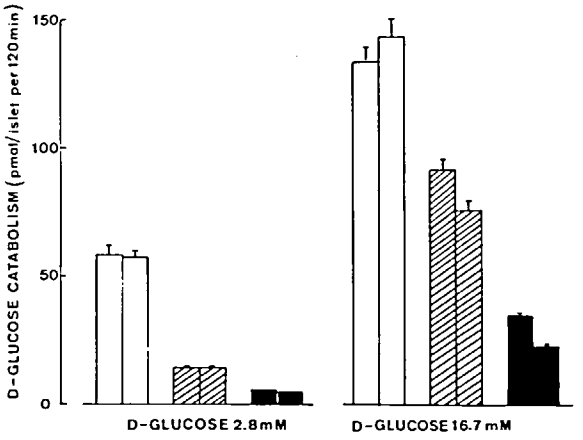

Fig. 2. D-Glucose catabolism in pancreatic islets exposed to a low or high concentration of hexose. Mean values (±S.E.) are derived from 40–43 individual determinations and refer to the generation of ^3H_2O from D-[5-^{3}H]glucose (open columns), oxidation of D-[3,4-^{14}C]glucose (hatched columns) and oxidation of D-[6-^{14}C]glucose (filled columns). In each pair, the column on the left illustrates the control values and that on the right indicates the results collected in the presence of cycloheximide and the nominal absence of Ca^{2+}.

TABLE I

EFFECT OF ENVIRONMENTAL AGENTS ON THE UTILIZATION OF D-[5-^{3}H]GLUCOSE AND OXIDATION OF D-[3,4-^{14}C]GLUCOSE OR D-[6-^{14}C]GLUCOSE

Expt. No.	D-Glucose (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	EGTA (mM)	Cycloheximide (mM)	D-[5-^{3}H]Glucose (pmol/120 min per islet)	D-[3,4-^{14}C]Glucose	D-[6-^{14}C]Glucose
1	2.8		2.0		0.05	47.5 ± 4.3 (26)		6.1 ± 0.4 (24)
	2.8		1.0	0.25	0.05	43.2 ± 4.6 (26)		6.8 ± 0.5 (23)
2	16.7		2.0		0.05	125.1 ± 11.0 (20)		24.5 ± 1.8 (20)
	16.7		1.0	0.25	0.05	111.2 ± 9.5 (20)		18.9 ± 1.2 (19)
3	16.7	1.0	1.0				91.2 ± 3.3 (14)	34.4 ± 1.7 (15)
	16.7		1.0	0.25	0.05		65.8 ± 3.5 (15)	15.0 ± 1.3 (15)

absence of Ca^{2+}, the ratio of experimental/control values amounting to 1.07 ± 0.07 (d.f. = 83). However, both the oxidation of D-[3,4-^{14}C]glucose and that of D-[6-^{14}C]glucose were significantly decreased ($P < 0.001$) by the presence of cycloheximide and the nominal absence of Ca^{2+} in the islets exposed to the high concentration of D-glucose. The relative extent of such an inhibition was more marked ($P < 0.005$) in the case of D-[6-^{14}C]glucose (−34.5 ± 3.7%; d.f. = 79) than D-[3,4-^{14}C]glucose oxidation (−17.6 ± 4.6%; d.f. = 81).

In the next series of experiments, the islets exposed to cycloheximide were incubated either in the nominal absence of Ca^{2+} and presence of 2.0 mM Mg^{2+}, as in the previous set of experiments, or in media deprived of $CaCl_2$ and enriched with EGTA (0.25 mM), in which case the $MgCl_2$ content (1.0 mM) of the incubation medium was not altered. As shown in Table I (experiments 1 and 2), the production of ^3H_2O from D-[5-^{3}H]glucose was not significantly different under these two experimental conditions, whether a low or high D-glucose concentration was used. Likewise, at the low hexose concentration (2.8 mM), the oxidation of D-[6-^{14}C]glucose was not significantly different in the nominal absence of Ca^{2+} or presence of EGTA, respectively. However, at a high D-glucose concentration (16.7 mM), the oxidation of D-[6-^{14}C]glucose was significantly lower ($P < 0.001$) in the presence of EGTA than in the nominal absence of Ca^{2+}. In the presence of EGTA and cycloheximide, the relative extent of inhibition in D-[3,4-^{14}C]glucose and D-[6-^{14}C]glucose oxidation, at the high hexose concentration, averaged 28.0 ± 5.1% (d.f. = 27) and 56.5 ± 5.9% (d.f. = 28), respectively (Table I, Expt. 3). These two values, in addition to being significantly different from one another ($P < 0.001$), were both significantly higher than the corresponding degree of inhibition recorded in the first series of experiments performed in the nominal absence of Ca^{2+} (see above). The changes evoked by the presence of cycloheximide and absence of Ca^{2+} at the various steps of D-glucose catabolism in islets exposed to a high concentration of the hexose are illustrated in Fig. 3. It should be stressed that the absolute rate of D-[6-^{14}C]glucose oxidation remained higher at 16.7 mM than at 2.8 mM, even in the presence of cycloheximide and absence of Ca^{2+} (Fig. 1 and Table I).

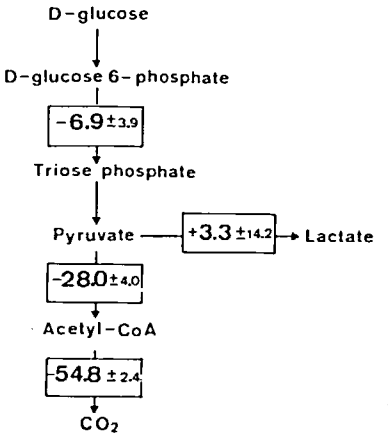

Fig. 3. Schematic view of the percent changes evoked by the presence of cycloheximide and absence of Ca^{2+} in the metabolism of D-glucose by pancreatic islets exposed to a high concentration of the hexose (16.7 mM).

TABLE II

EFFECT OF CYCLOHEXIMIDE AND/OR THE ABSENCE OF Ca^{2+} UPON THE OXIDATION OF D-[6-^{14}C]GLUCOSE (16.7 mM)

Ca^{2+} (mM)	EGTA (mM)	Cycloheximide (mM)	D-[6-^{14}C]Glucose oxidation (pmol/120 min per islet)	Inhibition (%)
1.0			34.4 ± 1.3 (16)	nil
1.0		0.05	29.6 ± 0.8 (16)	13.9 ± 2.3
	0.25		21.3 ± 1.2 (16)	38.2 ± 3.4
	0.25	0.05	16.1 ± 1.2 (16)	53.3 ± 3.5

In the last set of experiments in this series, we assessed the respective roles of the presence of cycloheximide and absence of Ca^{2+} in the control of D-[6-^{14}C]glucose oxidation by islets exposed to a high concentration of the hexose (16.7 mM). The results indicate that the presence of cycloheximide caused a significant reduction ($P < 0.001$) in D-[6-^{14}C]glucose oxidation (Table II). The absence of Ca^{2+} also inhibited D-[6-^{14}C]glucose oxidation. The latter inhibition was more pronounced than that caused by cycloheximide ($P < 0.001$). The inhibition was even greater ($P < 0.005$) in both the presence of cycloheximide and absence of Ca^{2+}, these two environmental factors acting in a simple additive manner (Table II).

Production of ^{14}C-labelled lactate, pyruvate and amino acids

In order to complete the evaluation of D-glucose metabolism, we measured the production of [^{14}C]lactate and that of ^{14}C-labelled pyruvate and amino acids, considered as a whole, in islets exposed to D-[U-^{14}C]glucose (Table III). In the control media, a rise in D-glucose concentration from 2.8 to 16.7 mM increased the production of both [^{14}C]lactate and ^{14}C-labelled pyruvate and amino acids. The 16.7 mM/2.8 mM ratio for the production of ^{14}C-labelled pyruvate and amino acids (2.46 ± 0.21; d.f. = 22) was somewhat higher ($P < 0.02$) than that for the formation of [^{14}C]lactate (1.83 ± 0.11; d.f. = 22). The rise in hexose concentration indeed decreased ($P < 0.05$) the production ratio of [^{14}C]lactate/^{14}C-labelled pyruvate and amino acids from 5.22 ± 0.40 to 4.05 ± 0.41 (d.f. = 22 in both cases). It should be stressed that the 16.7 mM/2.8 mM ratios for the production of either [^{14}C]lactate or ^{14}C-labelled pyruvate and amino acids were significantly lower ($P < 0.001$) than those recorded for the oxidation of D-[3,4-^{14}C]glucose or D-[6-^{14}C]glucose (see above).

At the low concentration of D-glucose (2.8 mM), the presence of cycloheximide and absence of Ca^{2+} failed to affect significantly the production of either [^{14}C]lactate or ^{14}C-labelled pyruvate and amino acids (Table III), the ratio of experimental/control values averaging, respectively. 0.99 ± 0.09 and 0.90 ± 0.16 (d.f. = 26 in both cases). At the high concentration of D-glucose (16.7 mM), the production of [^{14}C]lactate failed to be affected by the presence of cycloheximide and absence of

TABLE III

EFFECT OF CYCLOHEXIMIDE AND THE ABSENCE OF Ca^{2+} UPON THE PRODUCTION OF [^{14}C]LACTATE AND THAT OF ^{14}C-LABELLED PYRUVATE AND AMINO ACIDS FROM D-[U-^{14}C]GLUCOSE

D-Glucose (mM)	Ca^{2+} (mM)	EGTA (mM)	Cycloheximide (mM)	pmol of glucose equivalent/120 min per islet	
				[^{14}C]lactate	^{14}C-labelled pyruvate and amino acids
2.8	1.0			41.7 ± 3.2 (14)	8.2 ± 1.2 (14)
2.8		0.25	0.05	40.8 ± 2.8 (14)	7.4 ± 0.9 (14)
16.7	1.0			74.4 ± 6.1 (14)	20.4 ± 4.8 (14)
16.7		0.25	0.05	76.5 ± 9.4 (14)	15.2 ± 3.9 (14)

Ca^{2+} (ratio of experimental/control values: 1.03 $\pm$ 0.16; d.f. = 26). However, the production of ^{14}C-labelled pyruvate and amino acids appeared to be decreased, the ratio of experimental/control value averaging 0.73 $\pm$ 0.23 (d.f. = 26). The significance of the latter change became apparent by the finding that, in islets exposed to 16.7 mM D-glucose, the production ratio of [^{14}C]lactate/^{14}C-labelled pyruvate and amino acids was increased ($P < 0.05$) by the presence of cycloheximide and absence of Ca^{2+} from a control value of 4.05 $\pm$ 0.41 to 6.64 $\pm$ 1.06 (d.f. = 22 in both cases). In the presence of cycloheximide and absence of Ca^{2+}, the production of ^{14}C-labelled pyruvate and amino acids remained significantly higher, however, at 16.7 than at 2.8 mM D-glucose, the 16.7 mM /2.8 mM ratio averaging 2.06 $\pm$ 0.30 (d.f. = 22; $P <$ 0.005).

L-Leucine catabolism

In control media, a rise in L-leucine concentration from 1.0 to 20.0 mM increased almost 3-fold (2.78 $\pm$ 0.09; d.f. = 24) the rate of 2-keto[1-^{14}C]isocaproate generation (Fig. 4). Relative to the generation rate of the 2-keto acid from L-[1-^{14}C]leucine, the oxidation of the amino acid averaged 67.2 $\pm$ 3.1% and 54.5 $\pm$ 4.3% in the presence of 1.0 and 20.0 mM L-leucine, respectively ($n = 13$ in both cases). The 20.0 mM/1.0 mM ratio for the oxidation of L-[1-^{14}C]leucine and L-[U-^{14}C]leucine averaged, respectively. 2.26 $\pm$ 0.12 (d.f. = 36) and 2.53 $\pm$ 0.24 (d.f. = 41). The ratio of L-[U-^{14}C]leucine/L-[1-^{14}C]leucine oxidation was not significantly affected by the rise in amino acid concentration, with an overall mean value of 34.7 $\pm$ 8.4% (d.f. = 28).

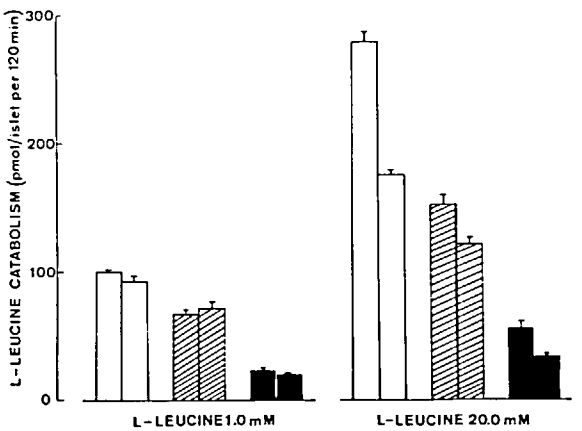

Fig. 4. L-Leucine catabolism in pancreatic islets exposed to a low or high concentration of the amino acid. Mean values (±S.E.) are derived from 13–16 individual determinations and refer to the generation of 2-ketoisocaproate from L-[1-^{14}C]leucine (open columns), oxidation of L-[1-^{14}C]leucine (hatched columns) and oxidation of L-[U-^{14}C]leucine (filled columns). In each pair, the column to the left illustrates the control values, and that to the right indicates the results collected in the presence of cycloheximide (50 μM) and EGTA (0.25 mM) and absence of Ca^{2+}.

The presence of cycloheximide and absence of Ca^{2+} failed to affect significantly the generation of 2-keto[1-^{14}C]isocaproate, the oxidation of L-[1-^{14}C]leucine and that of L-[U-^{14}C]leucine in islets exposed to a low concentration of the amino acid (1.0 mM), the ratio of experimental/control values averaging, respectively, 0.93 $\pm$ 0.06 (d.f. = 22), 1.07 $\pm$ 0.09 (d.f. = 22) and 0.85 $\pm$ 0.08 (d.f. = 28). However, at a higher concentration of L-leucine (20.0 mM), the catabolism of the amino acid was significantly impaired by the presence of cycloheximide and absence of Ca^{2+} ($P < 0.01$). Thus, the ratio of experimental/control values averaged 0.63 $\pm$ 0.05 (d.f. = 22) in the case of the generation of 2-keto[1-^{14}C]isocaproate, 0.80 $\pm$ 0.06 (d.f. = 22) in the case of L-[1-^{14}C]leucine oxidation and 0.60 $\pm$ 0.10 (d.f. = 28) in the case of L-[U-^{14}C]leucine oxidation. Relative to the mean corresponding control value, the percent inhibition was almost twice as high for L-[U-^{14}C]leucine than for L-[1-^{14}C]leucine oxidation ($P < 0.005$; d.f. = 27). As a result of these changes, the 20.0 mM/1.0 mM ratios in L-[1-^{14}C]leucine and L-[U-^{14}C]leucine oxidation represented, in the presence of cycloheximide and absence of Ca^{2+}, only 74.7 $\pm$ 4.6% and 69.9 $\pm$ 4.6%, respectively, of the mean control value found within the same experiments (d.f. = 22 and 28; $P < 0.005$). Nevertheless, even in the presence of cycloheximide and absence of Ca^{2+}, the oxidation rate of both L-[1-^{14}C]leucine and L-[U-^{14}C]leucine remained much higher in islets exposed to 20.0 mM rather than 1.0 mM L-leucine.

Discussion

The present results confirm that a rise in extracellular D-glucose concentration causes, in islet cells, a preferential stimulation of mitochondrial

oxidative events, including the transfer of reducing equivalents generated by glycolysis into the mitochondria, the decarboxylation of pyruvate and the oxidation of acetyl residues in the Krebs cycle [1]. As judged from the ratio of D-[6-^{14}C]glucose oxidation to D-[5-^{3}H]glucose utilization, this preferential stimulation displays a sigmoidal dependency on hexose concentration and an exponential time course during prolonged exposure to a high concentration of D-glucose. Such a concentration dependency and time course are reminiscent of those characterizing the functional response of the B-cell to D-glucose. Indeed, the stimulation of proinsulin biosynthesis, cationic fluxes and insulin release by D-glucose also displays a sigmoidal concentration dependency and a progressively increased magnitude during prolonged exposure to the hexose [9,17–20].

The preferential stimulation of mitochondrial oxidative events was suppressed in the presence of cycloheximide and/or absence of Ca^{2+}. The relative extent of inhibition was apparently modulated by the availability of extracellular Ca^{2+}, being more pronounced in the presence of EGTA than in the nominal absence of Ca^{2+}. Although a primary effect of Ca^{2+} deprivation upon the activity of selected enzymes should not be ruled out, the present data suggest that the inhibition of mitochondrial oxidative events cannot be ascribed solely to such an effect. Indeed, the presence of cycloheximide, which inhibits protein synthesis in the islet cells [9], decreased D-[6-^{14}C]glucose oxidation even in islets maintained at a normal extracellular Ca^{2+} concentration. Moreover, at a low concentration of D-glucose (2.8 mM), Ca^{2+} deprivation failed, as a rule, to affect significantly D-glucose catabolism, especially the ratio between D-[6-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization. The present results are compatible, therefore, with the concept that ATP-requiring functional processes, stimulated by D-glucose, exert a feedback control upon mitochondrial respiration in the islet cells [15,21]. Further work is required, however, to elucidate the precise mechanism of such a feedback control and its relative dependency on distinct, albeit interrelated, glucose-responsive and ATP-requiring functional events, such as the biosynthesis of proinsulin [9], the active pumping of Ca^{2+} by suitable Ca^{2+}-ATPases [22] and the contractile activity of the microfilamentous cell web [23].

The present results, when considered in the framework of current knowledge on the regulation of D-glucose catabolism in islet cells [24], affords two further pieces of information.

First, it is shown that the regulation of glycolysis can be dissociated from that of mitochondrial oxidative events. Thus, in islets exposed to a high concentration of D-glucose, the presence of cycloheximide and absence of Ca^{2+} failed to affect significantly the production of $^{3}H_2O$ from D-[5-^{3}H]glucose, but inhibited severely the oxidation of D-[3,4-^{14}C]glucose or D-[6-^{14}C]glucose. This suggests that the rate of glycolysis may be mainly regulated by the extracellular concentration of D-glucose according to a mass action phenomenon [24,25], whereas mitochondrial oxidative events are also tightly controlled by the balance between ATP formation and hydrolysis. In this respect, it should be underlined that the presence of cycloheximide and absence of Ca^{2+} did not abolish the capacity of D-glucose to cause a concentration-dependent increase in $^{14}CO_2$ production from either D-[3,4-^{14}C]glucose or D-[6-^{14}C]glucose. This could, conceivably, reflect the fact that the presence of cycloheximide an absence of Ca^{2+} failed to suppress completely all glucose-responsive and ATP-requiring functional processes in the islet cells. Alternatively, a rise in D-glucose concentration may increase, to a limited extent, the mitochondrial production of $^{14}CO_2$ from exogenous ^{14}C-labelled D-glucose, independently of any major change in the rate of ATP utilization. In any case, the dissociated regulation of glycolytic and mitochondrial oxidative events, respectively, does not invalidate, but rather reinforces, the concept that the stimulation of functional events by D-glucose is causally linked to an acceleration of hexose catabolism in the islet cells [17,26], the postulated participation of a feedback control mechanism being perfectly compatible with the fuel hypothesis for insulin release [21].

The second piece of information concerns the identification of a novel regulatory step in D-glucose catabolism in islet cells. In previous work, we have already stressed the view that the catabolism of D-glucose in normal islet cells is not ruled solely at the level of hexose phosphorylation by

hexokinase and glucokinase, but also involves more distal regulatory steps such as the channelling of hexose phosphates into distinct metabolic pathways [27,28], the activation of phosphofructokinase [25] and the respective contribution of aerobic and anaerobic glycolysis to the formation of pyruvate from triose phosphate [1,3]. The operation of the latter regulatory mechanism is confirmed in the present study, since a rise in D-glucose concentration from 2.8 to 16.7 mM augmented the fractional contribution of aerobic glycolysis to the total rate of pyruvate generation from $26.5 \pm 6.5\%$ to $42.8 \pm 4.5\%$ ($P < 0.05$). The present data reveal, however, the existence of a further regulatory mechanism located at an even more distal site, namely at the site of entry of acetyl residues into the Krebs cycle. Indeed, at a high concentration of D-glucose (16.7 mM), the presence of cycloheximide and absence of Ca^{2+} decreased the oxidation rate of acetyl residues, relative to their rate of generation from pyruvate, from a control value of $36.8 \pm 1.9\%$ to $22.7 \pm 1.8\%$ ($n = 15$ in both cases; $P < 0.001$). It should be stressed that the operation of this regulatory mechanism was only disclosed in the experiments conducted in the presence of cycloheximide and absence of Ca^{2+}. Thus, under normal environmental conditions, the ratio of D-[6-^{14}C]glucose/D-[3,4-^{14}C]glucose oxidation appears to be unaffected by a rise in hexose concentration, as judged from results from both a prior report [1] and the present study.

The present results enable the extension to L-leucine-stimulated islets of the concept of a feedback control by functional processes of mitochondrial oxidative events. Indeed, the presence of cycloheximide and absence of Ca^{2+} failed to affect L-leucine catabolism at a non-insulinotropic concentration of the amino acid (1.0 mM), but significantly inhibited the conversion of L-leucine to 2-ketoisocaproate and its further oxidation at a 20-fold higher, and hence stimulatory [29], concentration of the amino acid. The contrasting results obtained at low and high concentrations of L-leucine argue against a primary interference with the transport of the amino acid into islet cells. At the high concentration of L-leucine, the decrease in the generation rate of 2-ketoisocaproate, as provoked by the presence of cycloheximide and absence of Ca^{2+}, may reflect a reduced rate of 2-ketoglutarate formation from L-glutamate as catalyzed by glutamate dehydrogenase. Indeed, the provision of α-ketoglutarate, through the latter reaction, apparently regulates, in pancreatic islets, the rate of 2-ketoisocaproate generation from L-leucine [30].

There was a striking analogy between the oxidation of D-[6-^{14}C]glucose relative to that of D-[3,4-^{14}C]glucose and the oxidation of L-[U-^{14}C]leucine relative to that of L-[1-^{14}C]leucine. Thus, in control media, a rise in either D-glucose or L-leucine concentration failed to affect significantly the D-[6-^{14}C]glucose/D-[3,4-^{14}C]glucose or L[U-^{14}C]leucine/L-[1-^{14}C]leucine oxidation ratios. However, at a high concentration of these nutrients, the presence of cycloheximide and absence of Ca^{2+} inhibited more severely the oxidation of D-[6-^{14}C]glucose and L-[U-^{14}C]leucine than that of D-[3,4-^{14}C]glucose and L-[1-^{14}C]leucine, respectively. This indicates that the environmental factors under consideration affected, to a greater extent, the oxidation in the Krebs cycle of acetyl-CoA residues derived from either pyruvate or 2-ketoisocaproate than the provision of such residues or their precursors through the reactions catalyzed by either pyruvate dehydrogenase or banched-chain α-ketoacid dehydrogenase. Such a situation could reflect the known analogy in the regulation of the latter two mitochondrial enzymes in islet or other cell types [31,32].

In conclusion, the present results strongly suggest that nutrient-responsive and ATP-requiring processes exert in islet cells a feedback control on mitochondrial oxidative events. Further work is in progress to elucidate the mechanism of such a feedback control. Meanwhile, our findings should serve as a warning in the interpretation of metabolic, especially oxidative, data collected in models of B-cell dysfunction characterized by a decreased functional response to D-glucose. Indeed, in such models, a preferential impairment of hexose oxidation, rather than utilization [33], could represent, in part at least, the consequence rather than the cause of the secretory defect.

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References

1 Sener, A. and Malaisse, W.J. (1987) Biochem. J. 246, 89–95.
2 Sener, A., Rasschaert, J., Zähner, D. and Malaisse, W.J. (1988) Int. J. Biochem. 20, 595–598.
3 Malaisse, W.J., Rasschaert, J., Zähner, D. and Sener, A. (1988) Diabetes Res. 7, 53–58.
4 Sener, A., Blachier, F. and Malaisse, W.J. (1988) Turk. Biomed. Res. J., in press.
5 Sener, A., Blachier, F. and Malaisse, W.J. (1988) J. Biol. Chem. 263, 1904–1909.
6 Cook, D.L. and Hales, C.N. (1984) Nature 311, 271–273.
7 Ashcroft, F.M., Harrison, D.E. and Ashcroft, S.J.H. (1984) Nature 312, 446–448.
8 Malaisse, W.J. and Sener, A. (1987) Biochim. Biophys. Acta 927, 190–195.
9 Pipeleers, D.G., Marichal, M. and Malaisse, W.J. (1973) Endocrinology 93, 1001–1011.
10 Malaisse, W.J., Hutton, J.C., Sener, A., Levy, J., Herchuelz, A., Somers, G. and Devis, G. (1978) J. Membr. Biol. 38, 193–208.
11 Malaisse-Lagae, F. and Malaisse, W.J. (1984) in Methods in Diabetes Research (Larner, R. and Pohl, S.L., eds.), pp. 147–152, John Wiley & Sons, New York.
12 Carpinelli, A.R., Sener, A., Herchuelz, A. and Malaisse, W.J. (1980) Metab. Clin. Exp. 29, 540–545.
13 Sener, A., Malaisse-Lagae, F. and Malaisse, W.J. (1988) Eur. J. Biochem. 170, 447–452.
14 Hutton, J.C., Sener, A. and Malaisse, W.J. (1980) J. Biol. Chem. 255, 7340–7346.
15 Sener, A., Malaisse-Lagae, F. and Malaisse, W.J. (1982) Biochem. J. 202, 309–316.
16 Sener, A., Malaisse-Lagae, F., Dufrane, S.P. and Malaisse, W.J. (1984) Biochem. J. 220, 433–440.
17 Malaisse, W.J., Sener, A., Herchuelz, A. and Hutton, J.C. (1979) Metab. Clin. Exp. 28, 373–386.
18 Kipnis, D.M. and Permutt, M.A. (1972) Israel J. Med. Sci. 8, 224–232.
19 Welsh, M., Scherberg, N., Gilmore, R. and Steiner, D. (1986) Biochem. J. 235, 459–467.
20 Malaisse, W.J., Van Obberghen, E., Devis, G., Somers, G. and Ravazzola, M. (1974) Eur. J. Clin. Invest. 4, 313–318.
21 Malaisse, W.J., Sener, A., Herchuelz, A., Valverde, I., Hutton, J.C., Atwater, I. and Leclercq-Meyer, V. (1980) Horm. Metab. Res. (suppl.) series 10, 61–66.
22 Owen,.A., Sener, A. and Malaisse, W.J. (1983) Enzyme 29, 2–14.
23 Somers, G., Blondel, B., Orci, L. and Malaisse, W.J. (1979) Endocrinology 104, 255–264.
24 Sener, A. and Malaisse, W.J. (1984) Experientia 40, 1026–1035.
25 Malaisse, W.J., Malaisse-Lagae, F. and Sener, A. (1982) Diabetologia 23, 1–5.
26 Malaisse, W.J. (1983) Diab. Métab. 9, 313–320.
27 Malaisse, W.J., Giroix, M.-H. and Sener, A. (1985) J. Biol. Chem. 260, 14630–14632.
28 Sener, A. and Malaisse, W.J. (1988) Arch. Biochem. Biophys. 261, 16–26.
29 Malaisse, W.J., Hutton, J.C., Carpinelli, A.R., Herchuelz, A. and Sener, A. (1980) Diabetes 29, 413–437.
30 Sener, A., Malaisse-Lagae, F. and Malaisse, W.J. (1983) J. Biol. Chem. 258, 6693–6694.
31 Paxton, R., Harris, R.A., Sener, A. and Malaisse, W.J. (1988) Horm. Metab. Res. 20, 317–322.
32 Harris, R.A. and Paxton, R. (1985) Fed. Proc. 44, 305–315.
33 Portha, B., Giroix, M.-H., Serradas, P., Welsh, N., Hellerström, C., Sener, A. and Malaisse, W.J. (1988) Diabetes, in press.