The stimulus–secretion coupling of glucose-induced insulin release

Environmental influences on L-glutamine oxidation in pancreatic islets

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(Received 29 June 1981/Accepted 23 October 1981)

L-Glutamine at a near-physiological concentration (1.0 mM) was rapidly taken up and metabolized in rat pancreatic islets. The rate of glutamine deamidation much exceeded that of glutamate conversion into 2-oxoglutarate, the latter conversion being mediated mainly by transamination reactions. The production of $^{14}\text{CO}_2$ from L-[U- ^{14}C]glutamine, which reflected the generation of ATP through the metabolism of exogenous glutamine, appeared to be regulated by the redox state of nicotinamide nucleotides and the ATP content of the islet cells. The influence of environmental factors on glutamine oxidation was examined in order to identify ATP-requiring processes. Glutamine oxidation was decreased in the absence of extracellular Ca^{2+} , under conditions aiming at inhibition of the $(\text{Na}^+ + \text{K}^+)\text{-dependent ATPase}$ and, provided that glucose was present in the incubation medium, by cycloheximide. These findings were interpreted to suggest that the handling of Ca^{2+} by the islet cells, the active transport of univalent cations and the biosynthesis of proinsulin represent three major ATP-consuming processes in this fuel-sensor organ.

The pancreatic β -cell may be considered as a fuel-sensor organ (Malaisse *et al.*, 1979b). Little is known, however, about the energy-requiring processes which utilize the ATP generated by the catabolism of nutrients. In order to explore such an issue, we have here examined the metabolism of L-glutamine, used at a near-physiological concentration (1.0 mM), in isolated pancreatic islets and the influence on glutamine oxidation of various environmental factors likely to affect the energy requirement of the islet cells. The selection of L-glutamine for such a study was based on the knowledge that this amino acid represents one of the fuels utilized by the islets, under physiological conditions, to cover their basal energy expenditure, whether the β -cell is in a resting or stimulated functional state (Malaisse *et al.*, 1980a).

Materials and methods

All experiments were performed with isolated islets removed from fed albino rats and usually incubated in a bicarbonate-buffered solution (Na^+ 139 mM, K^+ 5 mM, Ca^{2+} 1 mM, Mg^{2+} 1 mM, Cl^- 124 mM, HCO_3^- 24 mM) containing bovine albumin (5 mg/ml) and equilibrated against O_2/CO_2 (19:1) (Malaisse *et al.*, 1970). To increase the pH of this

solution, NaCl was partially replaced by equimolar NaHCO_3 , while the gas phase remained unchanged (Malaisse *et al.*, 1971). In certain experiments, a Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/NaOH buffer (25 mM, pH 7.4) was added to the medium, which was equilibrated against air/ CO_2 (19:1). This medium served as a control for experiments performed with the same Hepes buffer, except that NaHCO_3 was omitted and equilibration was against ambient air. Other ionic alterations were: omission of CaCl_2 , and/or addition of EGTA (0.5 mM); omission of KCl; replacement of NaCl (115 mM) by either sucrose (230 mM) or an equimolar amount of LiCl or choline chloride; and replacement of NaCl (115 mM) by an equimolar amount of sodium isethionate.

The content of the islets and incubation medium (treated as a whole) of NH_4^+ (Malaisse *et al.*, 1981), glutamate, 2-oxoglutarate, malate, pyruvate, alanine, oxaloacetate and aspartate (Malaisse *et al.*, 1980a) was measured in groups of 15–30 islets each incubated for 60 min in 0.03–0.06 ml of medium. The islet content of adenine and nicotinamide nucleotides was measured in groups of 10–15 islets each preincubated for 30 min at 37°C in the absence of exogenous nutrient and then incubated for 30 min in the presence or absence of L-glutamine (1.0 mM),

as described by Malaisse *et al.* (1978b). The oxidation of exogenous nutrients (L-[U- 14 C]-glutamine, D-[U- 14 C]glucose) was measured in groups of 12 islets incubated for 30, 60 or 120 min in 0.06 ml of medium (Carpinelli *et al.*, 1980). The conversion of L-[G- 3 H]glutamine into $^3\text{H}_2\text{O}$ was followed in groups of 15 islets incubated for 120 min in 0.02 ml of medium, the $^3\text{H}_2\text{O}$ produced being measured as described by Levy *et al.* (1976). The net uptake of L-[U- 14 C]glutamine was measured in groups of 10 islets each preincubated for 30 min in a nutrient-free non-radioactive medium (0.04 ml) containing sucrose (1.0 mM) and then incubated for 3, 10, 20 or 60 min in the same medium to which was added 0.04 ml of a solution containing L-[U- 14 C]-glutamine and [6,6'-(n)- ^3H]sucrose used as an extracellular marker. The islets were separated from the incubation medium by passing the latter through a layer of di-n-butyl phthalate (Boschero *et al.*, 1977) and the net uptake of L-[U- 14 C]glutamine was calculated after correction for extracellular contamination.

For measuring the activity of glutaminase (EC 3.5.1.2) in islet homogenates, groups of 300–400 islets each were sonicated (2×5 s) in 0.6–0.8 ml of a medium which, in most experiments, had the same composition as the usual incubation medium, except that NaCl (115 mM) was replaced by an equimolar amount of KCl. A portion (25 μl) of this homogenate was mixed with 75 μl of imidazole/HCl buffer (66 mM, pH 7.0) containing L-glutamine. After 20 min incubation at 37°C, the reaction was halted by heating at 90°C for 5 min, and the reaction mixture mixed with 1.0 ml of Tris/acetate buffer (50 mM, pH 8.4) containing NAD $^+$ (0.4 mM), ADP (1.0 mM) and ox liver glutamate dehydrogenase (EC 1.4.1.3; 50 $\mu\text{g}/\text{ml}$; Boehringer, Mannheim, Germany) dissolved in 50% (v/v) glycerol. After a further 30 min incubation at room temperature, the NADH formed was measured by fluorimetry. The reaction velocity was expressed by comparison with standards of glutamate (1–8 nmol/sample) treated in exactly the same manner. In the second step of the procedure, the initial concentration of NH $_4^+$ (generated during the first step in the presence of the islet homogenate) did not exceed 3 μM . After correction for the blank value found in the absence of homogenate or substrate, the amount of NADH formed was proportional to the number of islets (5–25) and the length of incubation (10–20 min), with coefficient of variations of 6.4 and 12.5% respectively.

All results were expressed as means ($\pm$ S.E.M.), together with the numbers of individual observations (n). The statistical significance of differences between mean values was tested by use of Student's t test. The S.E.M. on the sum or difference between mean values was calculated as described by Snedecor (1956).

Results

Glutaminase activity in islet homogenate

In the presence of 10 mM-L-glutamine, glutaminase activity averaged 0.48 ± 0.07 nmol/h per islet ($n = 5$). At increasing concentrations of L-glutamine, the reaction velocities yielded a linear Lineweaver–Burk plot, with K_m 3.5 mM and V_{max} 0.75 nmol/h per islet (Fig. 1). The enzyme activity increased as the pH was raised from 7.0 to 8.5 (Fig. 1). The activity of the enzyme was little affected by K $^+$, a decrease in velocity no greater than $9.6 \pm 3.0\%$ ($n = 3$) being observed when the K $^+$ concentration was decreased from 120 to 2 mM. The glutaminase activity was decreased at low concentrations of HCO $_3^-$, with, in the range 0–24 mM, a mean inhibition of $18.6 \pm 3.5\%$ ($n = 9$; $P < 0.001$) for each 10 mM decrease in HCO $_3^-$ concentration.

Glutamine metabolism in intact islets

As judged from the radioactivity content of islets exposed to L-[U- 14 C]glutamine (1.0 mM), the amino acid rapidly accumulated in the islets, its uptake averaging 5.5 ± 0.4 pmol/islet after 3 min of incubation (Fig. 2).

The influence of exogenous L-glutamine (1.0 mM) on the islet content and/or output of different metabolites is illustrated in Table 1. The glutamine-induced increase in the islet content and/or output of glutamate, 2-oxoglutarate, malate, pyruvate, alanine, oxaloacetate and aspartate amounted to 86.4 ± 5.7 pmol/h per islet. The latter value was not significantly different from the glutamine-induced increment in NH $_4^+$ production (80.8 ± 6.4 pmol/h per islet). This suggests that NH $_4^+$ was generated mainly through the deamidation of L-glutamine, with little if any contribution attributable to the deamination of glutamate. In other words, the conversion

Fig. 1. Glutaminase activity in islet homogenates (a) Lineweaver–Burk plot. (b) Influence of pH on enzyme activity. Values are means ($\pm$ S.E.M.) for two to five individual measurements.

of glutamate into 2-oxoglutarate appeared to be mediated more by transamination reactions than by oxidative deamination. Indeed, glutamine increased the production of alanine and aspartate by 28.4 ± 3.6 pmol/h per islet. These two amino acids correspond to the major 2-oxo acids derived from the metabolism of L-glutamine, i.e. pyruvate and oxaloacetate (Fig. 3).

The production of $^{14}\text{CO}_2$ by islets exposed to L-[U- ^{14}C]glutamine (1.0 mM) occurred linearly with time within 10 min of incubation and, expressed as L-glutamine residues, averaged 17.1 ± 0.9 pmol/h per islet (Table 1). In the presence of antimycin A (0.01 mM), the output of $^{14}\text{CO}_2$ represented no more than $10.8 \pm 1.0\%$ of its control value (Table 2). Such a residual output probably corresponds to antimycin A-insensitive decarboxylation reactions, such as that

catalysed by glutamate decarboxylase (Malaisse *et al.*, 1980a).

The antimycin A-sensitive output of $^{14}\text{CO}_2$ was 76.3 ± 4.5 pmol of $^{14}\text{CO}_2$ /h per islet and exceeded ($P < 0.01$) the value expected from the decarboxylation of 2-oxoglutarate and malate, as computed from the glutamine-induced increments in malate, pyruvate, alanine and aspartate production (58.3 ± 5.2 pmol/h per islet). The difference between these two values probably reflects the oxidation of pyruvate derived from exogenous L-[U- ^{14}C]glutamine (Fig. 3).

Expressed as glutamine residues, the rate of $^3\text{H}_2\text{O}$ formation by islets exposed to L-[G- ^3H]glutamine (1.0 mM) averaged 27.5 ± 2.3 pmol/h per islet (Table 1). In the presence of antimycin A (0.01 mM), the production of $^3\text{H}_2\text{O}$ was decreased to an insignificant value (3.7 ± 2.2 pmol/h per islet; $n = 10$). As judged from the glutamine-induced increments in 2-oxoglutarate, malate, aspartate, pyruvate and alanine content and/or output, and assuming that the production of $^{14}\text{CO}_2$ not accounted for by either the latter increments or antimycin A-resistant processes corresponds to the oxidation of pyruvate derived from exogenous L-glutamine, the rate of $^3\text{H}_2\text{O}$ production should be 32.8 ± 3.5 pmol/h per islet. The latter estimate was not significantly different from the experimental value of 27.5 ± 2.3 pmol/h per islet. There was thus a fair agreement between chemical and radioisotopic data.

Fig. 3 illustrates a simplified scheme for the metabolism of L-glutamine in the islets exposed to the near-physiological concentration (1.0 mM) of the amino acid used in the present series of experiments.

Interrelationship of nutrient oxidation and adenine and nicotinamide nucleotides content in islets exposed to L-glutamine

As indicated in Table 2, menadione, when used at a concentration (0.01 mM) which decreases the islet

Fig. 2. Time-course for the uptake (○) and oxidation (●) of L-[U- ^{14}C]glutamine by pancreatic islets. Values are means ($\pm$ S.E.M.) for six to ten individual measurements.

Table 1. Effect of L-glutamine on the islet content and/or output of metabolites

All metabolites were measured in the islets and their surrounding incubation medium. The statistical significance of differences between control (first column) and experimental data is shown ($^a P < 0.025$; $^b P < 0.005$; $^c P < 0.001$)

	L-Glutamine (mM) ...	Nil	1.0
NH_4^+ (pmol/h per islet)		32.0 ± 3.2 (12)	112.8 ± 5.5 (12) ^c
Glutamate (pmol/h per islet)		10.3 ± 0.7 (14)	57.9 ± 3.7 (14) ^c
2-Oxoglutarate (pmol/h per islet)		0.8 ± 0.2 (7)	1.8 ± 0.2 (7) ^b
Malate (pmol/h per islet)		5.5 ± 0.9 (11)	11.6 ± 1.3 (11) ^c
Pyruvate (pmol/h per islet)		8.5 ± 0.7 (19)	11.8 ± 1.2 (20) ^a
Alanine (pmol/h per islet)		15.1 ± 1.2 (14)	32.3 ± 2.4 (14) ^c
Oxaloacetate (pmol/h per islet)		1.2 ± 0.1 (9)	1.2 ± 0.1 (11)
Aspartate (pmol/h per islet)		17.2 ± 1.7 (11)	28.4 ± 1.5 (11) ^c
L-[U- ^{14}C]Glutamine oxidation (pmol/h per islet)		—	17.1 ± 0.9 (138) ^c
L-[G- ^3H]Glutamine conversion into $^3\text{H}_2\text{O}$ (pmol/h per islet)		—	27.5 ± 2.3 (10) ^c

Table 2. *Effect of metabolic poisons on glutamine oxidation*

All incubations were conducted for 1 h in the presence of L-[U-¹⁴C]glutamine (1 mM). The statistical significance (^a *P* < 0.05; ^b *P* < 0.005; ^c *P* < 0.001) of differences between control (first line) and experimental data was tested by comparison of measurements performed in almost equal numbers within the same experiment(s).

Agent (mm)	L-[U- ¹⁴ C]Glutamine oxidation (pmol/h per islet)
Nil	18.6 ± 1.2 (19)
Antimycin A (0.01)	2.0 ± 0.2 (10) ^c
Menadione (0.01)	23.4 ± 1.7 (20) ^a
Dinitrophenol (0.05)	24.3 ± 1.2 (20) ^b

Fig. 3. *Simplified scheme of glutamine metabolism in islets*

Flow rates (rectangles) are expressed as pmol/h per islet. The glutamine-induced accumulation of metabolites (parentheses) is also expressed as pmol/h per islet. All values in this scheme are within the confidence limits of mean experimental data. This applies also to the glutamine-induced increment in NH₄⁺ production (93 versus 80.8 ± 6.4 pmol/h per islet), ³H₂O formation (30 versus 27.5 ± 2.3 pmol/h per islet, as expressed in glutamine residues) and antimycin A-sensitive ¹⁴CO₂ output (78 versus 76.3 ± 4.5 pmol/h per islet).

content of reduced nicotinamide nucleotides without affecting their ATP content (Malaisse *et al.*, 1978b), increased the rate of L-[U-¹⁴C]glutamine oxidation. Likewise, dinitrophenol (0.05 mM) augmented glutamine oxidation. These findings are consistent with the view that both the redox state of nicotinamide nucleotides and the concentration of adenine nucleotides (e.g. ATP) participates in the

regulation of glutamine oxidative metabolism in the islets.

L-Glutamine exerts little effect on the rate of O₂ consumption in the present system (Hutton & Malaisse, 1980), apparently because its oxidation coincides with a sparing action on the utilization of endogenous nutrients (Malaisse *et al.*, 1980a). In good agreement with such a situation, L-glutamine failed to affect significantly the islet content of reduced or oxidized nicotinamide nucleotides (results not shown) and to prevent the fall in ATP content, ATP/ADP ratio and adenylate charge normally seen when islets are incubated in the absence of exogenous nutrient (Ashcroft *et al.*, 1973). In fact, after 30 min incubation, the adenylate charge {i.e. ([ATP] + 0.5 [ADP]) / ([ATP] + [ADP] + [AMP])} was slightly lower (*P* < 0.025) in the islets exposed to L-glutamine (0.537 ± 0.014; *n* = 18) than in the islets deprived of exogenous nutrient (0.603 ± 0.020; *n* = 15).

Environmental influences on L-glutamine oxidation

The rate of L-[U-¹⁴C]glutamine oxidation was little affected by the presence of D-glucose in the

Fig. 4. *Relationship between the oxidation of D-[U-¹⁴C]glucose and L-[U-¹⁴C]glutamine in islets incubated for 120 min in the presence of glucose (16.7 mM) in various media*

Media: A, deprived of CaCl₂ and containing EGTA; B, containing verapamil; C, NaCl replaced by sucrose; D, deprived of KCl; E, containing ouabain; F, NaCl replaced by sodium isethionate; G, containing Hepes but no bicarbonate; H, pH raised to 8.0; J, containing theophylline. All results are expressed as percentages of the mean control value found within the same experiment(s). Data on glutamine oxidation are taken from Table 3. Except for E, data on glucose oxidation are taken from prior publications.

Table 3. *Environmental influences on glutamine oxidation*

All incubations were conducted for 2 h in the presence of L-[U-¹⁴C]glutamine (1.0 mM) with or without D-glucose. The ionic composition of the incubation medium was altered as described in the Materials and methods section. All results are expressed as percentages of the mean control value found within the same experiment(s) at the same concentration of D-glucose. The statistical significance (^a*P* < 0.05; ^b*P* < 0.025; ^c*P* < 0.01; ^d*P* < 0.005; ^e*P* < 0.001) of differences between control and experimental data was tested by comparison of measurements performed in almost equal numbers within each experiment. Also shown is the significance (*P*) of differences between the values (expressed as %) found in the presence and absence of glucose respectively.

Modification	L-[U- ¹⁴ C]Glutamine oxidation (%)		<i>P</i>
	No glucose	+ Glucose (16.7 mM)	
No Ca ²⁺	80.0 ± 5.2 (15) ^b	80.7 ± 4.3 (15) ^a	
Ca ²⁺ 1.5 mM, EGTA 0.5 mM	96.6 ± 2.7 (26)		
No Ca ²⁺ , EGTA 0.5 mM	63.7 ± 6.1 (22) ^e	67.4 ± 4.7 (16) ^c	
Verapamil 0.02 mM	86.5 ± 6.6 (15)	102.9 ± 5.4 (15)	
Na ⁺ 24 mM, Li ⁺ 115 mM	58.3 ± 2.8 (17) ^e	62.7 ± 3.7 (17) ^e	
Na ⁺ 24 mM, choline 115 mM	76.9 ± 2.8 (26) ^e	77.1 ± 5.1 (17) ^d	
Na ⁺ 24 mM, sucrose 230 mM	73.4 ± 4.8 (17) ^e	85.0 ± 5.8 (16) ^a	
No K ⁺	69.7 ± 3.7 (31) ^a	85.6 ± 4.4 (17) ^a	<0.02
Ouabain 1 mM	80.7 ± 5.4 (15) ^b	78.6 ± 2.8 (17) ^d	
No K ⁺ , ouabain 1 mM	68.8 ± 3.9 (15) ^e	71.5 ± 2.7 (17) ^e	
Cl ⁻ 9 mM, isethionate 115 mM ¹	76.1 ± 6.2 (17) ^b	74.5 ± 6.6 (19) ^c	
Hepes 25 mM, air/CO ₂ (19:1)	94.6 ± 6.0 (18)	119.1 ± 8.6 (20)	<0.05
Hepes 25 mM, 100 % air, no HCO ₃ ⁻	107.3 ± 6.4 (19)	127.6 ± 7.3 (20) ^d	<0.05
pH 8.0	91.4 ± 6.2 (10)	74.0 ± 4.9 (10) ^d	<0.05
Theophylline 1.4 mM	119.4 ± 8.1 (26) ^a	113.6 ± 6.7 (16)	
Cytochalasin B 0.02 mM	101.5 ± 5.6 (18)	100.4 ± 5.7 (16)	
Cycloheximide 0.07 mM	97.4 ± 4.9 (18)	70.3 ± 6.5 (24) ^d	<0.005

incubation medium. Thus, in a large series of experiments (*n* = 74), the output of ¹⁴CO₂ in the presence of glucose (16.7 mM) averaged 91.6 ± 2.5% of the mean control value (100.0 ± 3.2%) found in the same experiment(s) in the absence of glucose (*P* < 0.05).

Table 3 illustrates the influence of various environmental factors on the oxidation of L-[U-¹⁴C]glutamine by islets incubated in the absence or presence of D-glucose (16.7 mM). In the absence of D-glucose, the oxidation of glutamine was inhibited in the absence of Ca²⁺ (with or without EGTA), when NaCl was replaced by LiCl, choline chloride or sucrose, in the absence of K⁺ and/or presence of ouabain, and when Cl⁻ was replaced by isethionate. It was unaffected by EGTA provided that the concentration of Ca²⁺ was unaltered, by verapamil, by an increase of pH from 7.4 to 8.0, and in the presence of cytochalasin B or cycloheximide. It was increased in the presence of theophylline. Comparable results were obtained in the presence of glucose, except that glutamine oxidation was now significantly decreased in the presence of cycloheximide or in alkaline media (pH 8.0). In either the absence or presence of glucose, the removal of HCO₃⁻ failed to affect significantly glutamine oxidation relative to the control value found in media

containing both Hepes and bicarbonate and equilibrated against air/CO₂ (19:1). The omission of K⁺ affected glutamine oxidation more severely (*P* < 0.02) in glucose-deprived than in glucose-stimulated islets.

There was a significant correlation (*P* < 0.01) between the influence of various environmental factors on the oxidation of L-glutamine and D-glucose, both series of measurements being performed in islets stimulated by 16.7 mM-glucose (Fig. 4). There was also a highly significant correlation (*P* < 0.005) between the changes in glutamine oxidation found in the absence of glucose and the changes in glucose oxidation found in the absence of glutamine.

Discussion

In pancreatic islets exposed to L-glutamine at a concentration close to that found (0.5–0.7 mM) in the plasma of normal rats (Eisenstein *et al.*, 1979), the metabolism of the amino acid, as depicted in Fig. 3, would correspond to an O₂ uptake of 75.0 pmol/h per islet, i.e. about 15% of the basal O₂ consumption. However, since glutamine increases little the consumption of O₂ by the islets (Hutton & Malaisse, 1980), its oxidation reflects a sparing

action on the utilization of endogenous nutrients (Malaisse *et al.*, 1980a). The oxidation of glutamine was little affected by glucose, suggesting that glutamine acts as a fuel whether the islet cells are in a resting or an active functional state. The influence of environmental factors on glutamine oxidation was examined here to identify energy-requiring processes in the islet cells.

In either the absence or presence of glucose, the oxidation of glutamine was decreased in the absence of extracellular CaCl_2 and, even more so, in the simultaneous presence of EGTA, although EGTA itself did not affect glutamine oxidation at a normal concentration of Ca^{2+} . Verapamil failed to affect significantly glutamine oxidation. The net uptake of ^{45}Ca by the islets is also much less affected by verapamil than at low concentrations of extracellular Ca^{2+} , especially in the presence of glucose (Malaisse *et al.*, 1977, 1978c). The absence of Ca^{2+} affected glutamine oxidation to almost the same extent as it affects glucose oxidation (Hellman *et al.*, 1974; Malaisse *et al.*, 1978c). Verapamil fails to affect the rate of glycolysis and glucose oxidation in the islets (Malaisse *et al.*, 1977). In the absence of extracellular Ca^{2+} , proinsulin biosynthesis occurs at a near-normal rate, but insulin release is abolished (Pipeleers *et al.*, 1973). However, the release of insulin does not seem to represent a major energy-requiring process in the secretory response (see below). It is conceivable that the absence of extracellular Ca^{2+} affects the oxidation of nutrients by altering the activity of Ca^{2+} -dependent ATPase(s) (Malaisse *et al.*, 1978c). Our experimental data would then suggest that at least 20–30% of the ATP generated by the islets is utilized to ensure the proper handling of Ca^{2+} by the islet cells. This estimate is in fair agreement with that reached from the measurement of Ca^{2+} -dependent ATPase activity in islet homogenates (Owen *et al.*, 1982).

The replacement of NaCl by LiCl , choline chloride or sucrose inhibited glutamine oxidation. The extent of inhibition was similar in the absence or presence of glucose, but more marked when Li^+ was used instead of choline or sucrose. Glucose oxidation is also altered when NaCl is replaced by LiCl , choline chloride or sucrose (Ashcroft *et al.*, 1972a; Hellman *et al.*, 1974; Herchuelz *et al.*, 1980), a phenomenon currently attributed to inhibition of a $(\text{Na}^+ + \text{K}^+)$ -activated ATPase (Ashcroft *et al.*, 1972a; Hellman *et al.*, 1974). However, Na^+ deficiency also affects proinsulin biosynthesis (Pipeleers *et al.*, 1973) and Ca^{2+} handling (Herchuelz *et al.*, 1980). A contributory role for changes in insulin release to the inhibition of nutrient oxidation is not likely, since the replacement of Na^+ by choline fails to suppress the secretory response to glucose (Malaisse *et al.*, 1971).

Ouabain, which may inhibit glucose oxidation

(Ashcroft *et al.*, 1972b; Hellman *et al.*, 1974), decreased glutamine oxidation to the same extent in glucose-deprived or glucose-stimulated islets. This finding is compatible with the view that glucose does not affect the activity of the $(\text{Na}^+ + \text{K}^+)$ -activated ATPase, as suggested by the rate of K^+ inflow into islet cells (Malaisse *et al.*, 1978a). In the K^+ -deprived islets, the decrease in glutamine oxidation was less marked in the presence than in the absence of glucose. This difference could be due to several factors. First, glucose, by decreasing the fractional outflow rate of K^+ (Malaisse *et al.*, 1978a), may protect the islet cells against the depletion of the intracellular K^+ pool(s). Second, in the K^+ -deprived islets, the metabolism of glucose is more severely affected (Henquin & Lambert, 1974; Sener *et al.*, 1980) than that of other nutrients, such as pyruvate or 4-methyl-2-oxopentanoate (Hutton *et al.*, 1980; Sener *et al.*, 1980). This situation, which was ascribed to a specific inhibition of pyruvate kinase (Sener *et al.*, 1980), may account for a lesser decrease in glutamine oxidation, if the catabolism of glucose and that of glutamine are regulated in a complementary fashion. In the simultaneous absence of K^+ and presence of ouabain, a 30% decrease in glutamine oxidation was observed, in both the presence and absence of glucose. This value exceeds that expected from inhibition of the $(\text{Na}^+ + \text{K}^+)$ -activated ATPase, as estimated by comparison of the inflow rate of K^+ into islet cells and their O_2 uptake (Malaisse *et al.*, 1980b). The absence of extracellular K^+ , however, not only affects the active inward transport of this cation, but also severely alters biosynthetic activity (Sener & Malaisse, 1980), the movements of Ca^{2+} (Herchuelz & Malaisse, 1980) and release of insulin (Henquin & Lambert, 1974; Howell & Taylor, 1968; Sener & Malaisse, 1980) in the islet cells. In this respect, it should be kept in mind that the intracellular K^+ content is more markedly decreased in the absence of K^+ (Sener *et al.*, 1980) than in the presence of ouabain (Malaisse *et al.*, 1978a).

The replacement of Cl^- by the impermeant anion isethionate decreased glutamine oxidation by 25%, in either the absence or presence of glucose. The oxidation of glucose by the islets is also decreased by 24% when Cl^- is replaced by isethionate (Somers *et al.*, 1980). Since the influx of permeant anions in secretory granules occurs concurrently with the inward pumping of protons driven by a membrane ATPase (Creutz & Pollard, 1980; Abrahamsson & Gylfe, 1980), the decrease in nutrient oxidation in the presence of isethionate may be somehow related to alteration in the activity of the latter ATPase.

The absence of extracellular HCO_3^- failed to inhibit glutamine oxidation, as it fails to affect glucose oxidation (Henquin & Lambert, 1976; Malaisse *et al.*, 1979a). These results are surprising,

since the absence of HCO_3^- decreases, at least in short-term experiments, the consumption of O_2 by the islets (Hutton & Malaisse, 1980), which indeed possess a HCO_3^- -sensitive ATPase (Sener *et al.*, 1979). The interpretation of these data is complicated by the consideration that a decrease in HCO_3^- concentration may affect the activity of glutaminase, and hence the NH_4^+ content, intracellular pH and further metabolism of glutamate. Thus, in alkaline media (pH 8), the oxidation of glutamine was significantly decreased, at least in the presence of D-glucose.

Theophylline slightly augmented glutamine oxidation, at least in the absence of glucose. Theophylline, although it fails to affect glucose utilization, also slightly augments lactate output from islets incubated at a low concentration (4.2 mM) of glucose (Sener *et al.*, 1976). Thus theophylline could facilitate the metabolism of glutamate derived from either exogenous glutamine or endogenous precursor(s). This is consistent with the fact that theophylline decreases ^{86}Rb fractional outflow rate from islets perfused in the absence of exogenous nutrient (Carpinelli & Malaisse, 1980).

Cytochalasin B, which may cause hypercontraction of the microfilamentous β -cell web (Malaisse & Orci, 1979), did not affect glutamine oxidation. Cytochalasin B also fails to affect utilization of glyceraldehyde or leucine in the islets, whereas its effect on glucose metabolism may be secondary to a specific inhibition of glucose transport into islet cells (McDaniel *et al.*, 1974; Levy *et al.*, 1976). It would thus appear that the contractile activity of the cell web, which controls the access of secretory granules to their exocytotic site (Somers *et al.*, 1979), is not a major energy-consuming process in the islet cells.

The inhibitor of protein synthesis, cycloheximide, failed to affect glutamine oxidation in the absence of glucose, but markedly decreased glutamine oxidation in the presence of glucose. Cycloheximide also inhibits glucose oxidation by the islets according to some (Snyder *et al.*, 1970) but not all authors (Ashcroft *et al.*, 1970). Since glucose dramatically stimulates the biosynthesis of proteins, especially proinsulin, in the islets, our data suggest that such a biosynthetic activity participates to a significant extent in the energy expenditure of glucose-stimulated islet cells.

In conclusion, our data point to three major energy-requiring processes in the islet cells: the handling of Ca^{2+} , the transport of univalent cations, and the biosynthesis of proinsulin and other peptides in glucose-stimulated islets. This list should not be considered as exhaustive. In our opinion, the identification of such ATP-consuming processes and the study of their regulation in intact islets represent an essential step towards elucidating the biochemical organization of this fuel-sensor organ.

This work was supported in part by grants from the Belgian Ministry of Scientific Policy and Belgian Foundation for Scientific Medical Research. J. Schoonheydt and M. Urbain are thanked for technical assistance and C. Demesmaeker for secretarial help. The present paper is the 52nd in a series on the stimulus-secretion coupling of glucose-induced insulin release.

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