The Stimulus–Secretion Coupling of Glucose-Induced Insulin Release

DOES GLYCOLYSIS CONTROL CALCIUM TRANSPORT IN THE B-CELL?

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(Received 1 December 1975)

1. The metabolism of glucose and the exchangeable Ca^{2+} pool were measured in rat pancreatic islets, in order to assess the possible significance of glycolysis in the process of glucose-induced insulin release. 2. At high glucose concentration (16.7 mM), glucose was metabolized at the following rate (pmol of glucose residue/h per islet $\pm$ S.E.M.): 131 ± 11 for glucose uptake, 129 ± 8 for glucose utilization, as judged by the conversion of $[5\text{-}^3\text{H}]\text{glucose}$ into $^3\text{H}_2\text{O}$, 60 ± 2 for lactate output and 25 ± 2 for glucose oxidation. 3. The secretory pattern usually correlated with the metabolic data. For instance, the ability of different sugars (glucose, mannose, fructose, galactose, D-glyceraldehyde) to stimulate lactate output closely paralleled their relative insulinotropic capacity. A disparity between metabolic and secretory responses was, however, encountered in the presence of dibutyryl cyclic AMP and theophylline. 4. Despite this contrasting behaviour, the size of the Ca^{2+} -exchangeable pool (net uptake of $^{45}\text{Ca}^{2+}$) was invariably proportional to the rate of lactate output under all experimental conditions examined. It is concluded that glycolysis usually exerts a tight control on the rate constant for Ca^{2+} transport across the B-cell membrane.

The metabolism of glucose in isolated islets of Langerhans is under investigation in a number of laboratories, in order to explore its possible relevance to the process by which the pancreatic B-cell identifies this hexose as a stimulus for insulin release. Because differences can be found between the rates of glucose metabolism and insulin secretion, the idea developed that glucose itself may signal insulin release by interacting with a receptor possibly located at the plasma membrane of the B-cell (Matschinsky *et al.*, 1972; Krahle, 1974). According to some authors, cyclic AMP acts in such a process as a second messenger (Charles *et al.*, 1973; Cerasi, 1975). The aim of the present study is to examine the inverse hypothesis and to assess to what extent the insulinotropic action of different sugars and of drugs known to cause cyclic AMP accumulation in the islets may not be accounted for by changes in the rate of glycolysis. The experimental data suggest that glycolysis exerts a tight control on the transport of calcium across the B-cell membrane.

Experimental

The materials and methods used to study the metabolism of rat pancreatic islets have all previously been reported in detail. The measurement of glucose

accumulation was based on the apparent space of distribution of $[\text{U-}^{14}\text{C}]\text{glucose}$ (16.7 mM; $25 \mu\text{Ci/ml}$) in excess of that of $[6,6'(\text{n})\text{-}^3\text{H}]\text{sucrose}$ (0.1 mM; $50 \mu\text{Ci/ml}$) (McDaniel *et al.*, 1974). Glucose uptake was measured by measuring the fall in glucose concentration in the incubation medium (Malaisse *et al.*, 1974a). Glucose utilization was estimated by measuring the conversion of $[5\text{-}^3\text{H}]\text{glucose}$ into $^3\text{H}_2\text{O}$ (Ashcroft *et al.*, 1972; Levy *et al.*, 1976a). Lactate content of the incubation medium was measured by an enzymic procedure (Malaisse *et al.*, 1976a), and the oxidation of glucose was determined by a radioisotopic technique (Malaisse *et al.*, 1974b). The net uptake of $^{45}\text{Ca}^{2+}$ represents the radioactive content of islets first incubated with $^{45}\text{Ca}^{2+}$ and then submitted to a washing procedure to remove extracellular radioactivity (Malaisse-Lagae & Malaisse, 1971).

Unless otherwise stated, islets were removed from fed rats. Results of the effect of a given agent on a given parameter of islet function, such as those illustrated in Table 2 and Fig. 1, were always obtained within the same experiment(s), all islets in each experiment being derived from the same batch, itself being prepared from the pancreases of three donor rats. For comparison of different metabolic parameters, however, all available results were pooled (Table 1, control values).

Table 1. Glucose metabolism in isolated islets

Results are given as means $\pm$ S.E.M., with the numbers of observations in parentheses and the statistical significance (* P < 0.05; ** P < 0.03; *** P < 0.005; **** P < 0.001) of differences between experimental and appropriate control values. Unless otherwise stated, the islets were removed from fed rats. Other conditions include the use of islets removed from rats starved for 48 h and the addition of mannoheptulose or theophylline to the incubation medium. The control value for glucose uptake is corrected for the rapid accumulation of free glucose (45 ± 3 pmol/islet) occurring within the first 2 min of exposure to the sugar.

Experimental conditions and metabolic parameters	Control value (pmol/h per islet)	Experimental values (% of mean paired control value)			
		4.2	16.7	16.7	16.7
Glucose (mm) ...	16.7	—	+14.3 mm-Mannoheptulose	Starvation	+1.4 mm-Theophylline
Other conditions ...	—	—	—	—	—
Glucose uptake	131 $\pm$ 11 (18)	54.1 $\pm$ 6.0 (7)****	49.3 $\pm$ 16.9 (4)*	60.1 $\pm$ 8.5 (6)***	89.0 $\pm$ 14.1 (3)
Glucose utilization	129 $\pm$ 8 (78)	27.0 $\pm$ 3.3 (16)****	71.8 $\pm$ 5.9 (29)**	—	90.9 $\pm$ 10.2 (16)
Lactate output	60 $\pm$ 2 (102)	44.3 $\pm$ 2.7 (7)****	43.1 $\pm$ 3.5 (6)****	42.6 $\pm$ 3.3 (8)****	91.0 $\pm$ 6.6 (19)
Glucose oxidation	25 $\pm$ 2 (73)	38.7 $\pm$ 5.4 (20)****	37.4 $\pm$ 7.2 (12)*	59.3 $\pm$ 3.7 (23)****	116.4 $\pm$ 14.6 (17)

Fig. 1. Effect of increasing glucose concentrations on glucose utilization (Δ), oxidation ($\bullet$) and lactate output

The metabolism of glucose accounted for by both lactate output and oxidation ($\circ$) was calculated after subtraction of the basal value (no glucose) for lactate output (8 ± 1 pmol of glucose residue/60 min per islet, $n = 6$). Mean values ($\pm$ S.E.M.) are derived from 5–16 individual observations, each metabolic parameter being measured in separate experiments.

Results

Quantitative aspects of glucose metabolism

When islets are first incubated in a glucose-free medium and then exposed for 2 min to glucose (16.7 mm), the space of distribution of glucose in

excess of that of sucrose (2.72 ± 0.17 nl/islet) is close to that of the islet intracellular water content (Malaisse *et al.*, 1976b). The initial rate of glucose entry (45.4 ± 2.8 pmol/islet within the first 2 min of exposure to glucose) is much greater than its subsequent value (2.2 ± 0.2 pmol/min per islet), the latter being estimated by the fall in glucose concentration in the incubation medium (Table 1). More than 95% of the glucose taken up by the islets is converted into triose phosphates (Table 1), the fractional contribution of the sorbitol pathway and pentose shunt to such a process not exceeding 10% (Malaisse *et al.*, 1976b). Oxidation of glucose accounts for approx. 28% of its utilization. The latter ratio is not significantly affected by the glucose concentration of the incubation medium ($r = 0.163$). The curves relating glucose utilization and oxidation to the glucose concentration of the medium both display a sigmoidal pattern (Fig. 1). Most of the glucose utilization is accounted for by lactate production (Table 1), which occurs at a constant rate throughout the 90 min period of incubation used in most of the present experiments (Malaisse *et al.*, 1976b). Because the measurement of lactate output does not depend on the availability of labelled substrate, it was used here as an index of the rate of glycolysis evoked in the islets by different sugars.

Effect of various insulintropic agents on islet glucose metabolism

To examine the possible relationships between glucose metabolism and physiological events in the islets, we first explored the effect on the metabolic parameters of various factors known to affect the secretory activity of the islets. As shown in Table 1, mannoheptulose (14.3 mm) inhibited all parameters of glucose metabolism. A comparable situation was found when the islets were removed from rats starved for 48 h (Table 1).

Table 2. Lactate production by islets from fed rats

Mean values $\pm$ S.E.M. are expressed as pmol/90 min per islet, except in the last group of experiments (*), where a 60 min incubation period was used. Also shown is the number of individual determinations in each group (in parentheses) and the statistical significance (*P*) of differences between mean values (NS, not significant).

Line	Substrate (mM)	Other agent (mM)	Lactate output	<i>P</i>
1	—		23 $\pm$ 1 (11)	
2	Glucose (16.7)		154 $\pm$ 6 (11)	versus 1: <0.001
3	Mannose (16.7)		83 $\pm$ 9 (6)	versus 1: <0.001
4	Fructose (16.7)		54 $\pm$ 8 (6)	versus 1: <0.001
5	—		24 $\pm$ 2 (5)	
6	Galactose (16.7)		27 $\pm$ 7 (5)	versus 5: NS
7	Glucose (1.7)		72 $\pm$ 8 (5)	
8	Glucose (1.7)	Galactose (16.7)	94 $\pm$ 9 (5)	versus 7: NS
9	Glucose (16.7)		169 $\pm$ 7 (5)	
10	Glucose (16.7)	Galactose (16.7)	190 $\pm$ 16 (5)	versus 9: NS
11	Glucose (4.2)*		57 $\pm$ 3 (13)	
12	Glucose (4.2)*	Theophylline (1.4)	67 $\pm$ 3 (13)	versus 11: <0.05
13	Glucose (4.2)*	Dibutyryl cyclic AMP (1.0)	83 $\pm$ 6 (7)	versus 11: <0.005
14	Glucose (16.7)*		122 $\pm$ 7 (19)	
15	Glucose (16.7)*	Theophylline (1.4)	111 $\pm$ 8 (19)	versus 14: NS
16	Glucose (16.7)*	Dibutyryl cyclic AMP (1.0)	129 $\pm$ 17 (8)	versus 14: NS

Fig. 2. Relationship between net uptake of $^{45}\text{Ca}^{2+}$ and lactate output in isolated islets

Net uptake of Ca^{2+} (pg/islet) and lactate output were measured at increasing glucose concentrations (●), in the presence of galactose, mannose and D-glyceraldehyde (▲) and in the simultaneous presence of glucose and either mannoheptulose (■), theophylline (horizontal arrow) or dibutyryl cyclic AMP (vertical arrow). The result obtained with islets from rats starved for 2 days is shown as an open circle. Mean values for both parameters are expressed as percentages of the mean control values found within the same experiment(s) in islets from fed rats exposed to glucose (16.7 mM).

The ability of various sugars to increase lactate output closely paralleled their relative insulinotropic capacities in the present system (Malaisse-Lagae & Malaisse, 1971), according to the following order:

glucose > mannose > fructose > galactose, with galactose failing significantly to increase lactate output, in either the absence or the presence of glucose (Table 2). We have shown previously that, at concentrations of nearly equal insulinotropic potency, glucose (8.3 mM) and D-glyceraldehyde (10.0 mM) yield comparable amounts of lactate and are oxidized at the same rate (Malaisse *et al.*, 1976a), and that starvation fails to affect D-glyceraldehyde metabolism and insulinotropic action (Levy *et al.*, 1976b). The relative insulinotropic capacities of glucose, fructose and sorbitol in the present system also closely paralleled their relative rates of oxidation (Malaisse *et al.*, 1974b).

As shown in Table 1, theophylline (1.4 mM) failed to stimulate glucose uptake, utilization and oxidation in islets exposed to a high glucose concentration (16.7 mM). Theophylline and dibutyryl cyclic AMP also failed to affect lactate output at this high glucose concentration (Table 2). However, at a lower glucose concentration (4.2 mM), dibutyryl cyclic AMP and, to a lesser extent, theophylline significantly enhanced lactate output from the islets (Table 2).

A possible link between glycolysis and insulin release

A possible link between the metabolism of glucose and the secretion of insulin was examined by comparing metabolic (glycolytic flux, ATP concentration) and functional (net uptake of $^{45}\text{Ca}^{2+}$, insulin output) parameters. Fig. 2 illustrates the close correlation ($r = 0.964$; $P < 0.001$) observed under a variety of experimental conditions between the output of lactate, taken as an index of the glycolytic flux, and the net uptake of $^{45}\text{Ca}^{2+}$. The relationship between net

uptake of $^{45}\text{Ca}^{2+}$ and insulin release in the present system has been previously characterized (Malaisse-Lagae & Malaisse, 1971; Brisson *et al.*, 1972).

Discussion

The present values for glucose accumulation (Matschinsky & Ellerman, 1968; Hellman *et al.*, 1971a; McDaniel *et al.*, 1974), uptake (Reese *et al.*, 1973), utilization (Ashcroft *et al.*, 1972; Pace *et al.*, 1975) and oxidation (Ashcroft *et al.*, 1970; Hellerström & Gunnarsson, 1970; Hellman *et al.*, 1971b) and lactate output (Ashcroft *et al.*, 1970; Hedekov & Capito, 1974; Pace *et al.*, 1975) in isolated islets are of the same order of magnitude as those reported in the literature. A detailed and comprehensive analysis of islet metabolism can be found in a review by Hellerström & Brolin (1975).

Two striking features of glucose metabolism in the islets deserve consideration. First, thanks to the rapid equilibration of free glucose on both sides of the cell membrane and to the presence of a phosphorylating activity with a high K_m for glucose (Ashcroft & Randle, 1970; Malaisse *et al.*, 1976c; Matschinsky & Ellerman, 1968), the islets are well equipped to accelerate markedly their glycolytic flux in response to increasing extracellular glucose concentrations. Secondly, lactate output accounts for a relatively large fraction of the total turnover of glucose in the islets.

Teleologically, the first attribute is well suited to provide, through changes in metabolic rate, a sensitive signal for regulation of the glucostat, i.e. the insulin-releasing function of the B-cell. Within the framework of such a view and because the synthesis and release of insulin by the B-cell apparently account for a minor fraction of its total energy expenditure (Hellerström & Brolin, 1975), the process of lactate formation and subsequent output might concomitantly ensure (i) a sufficient reoxidation of NADH to NAD^+ for the maintenance of an appropriate activity of glyceraldehyde 3-phosphate dehydrogenase and (ii) an adequate clearance of C_3 residues formed in excess of those undergoing mitochondrial oxidation to meet the energy requirement of the cell.

Under several experimental conditions, both the metabolic and functional responses of the B-cell to glucose and other sugars are affected in a co-ordinated manner.

One example of such a situation is found in the influence of starvation on islet function. Starvation inhibits both the metabolism and insulinotropic capacity of glucose without affecting the metabolic and secretory responses of the islets to L-glyceraldehyde (Levy *et al.*, 1976b). This starvation-induced anomaly, which is reminiscent of that provoked by mannoheptulose, was traced down to repression of the glucokinase and phosphofructokinase in the islets (Malaisse *et al.*, 1976c).

A second example of close correlation between metabolic and secretory responses is found by comparing the effects of different sugars, including D-glyceraldehyde (Malaisse *et al.*, 1976a). In this respect, the present results on the influence of glucose, mannose, fructose and galactose on lactate output are in good agreement with available data on the oxidation of these sugars (Ashcroft *et al.*, 1970; Hellman *et al.*, 1974a), and their effect on both oxygen consumption (Hellerström, 1967) and ATP content (Ashcroft *et al.*, 1973) in the islets. However, according to Matschinsky & Ellerman (1973) and Pace *et al.* (1975), fructose and mannose are, up to concentrations of about 16.5 mM, much better substrates for lactate production than glucose, and even galactose markedly stimulates lactate output. We have no explanation to offer for the discrepancy between these and the present results.

The effect of theophylline and dibutyl cyclic AMP on islet function represents an example of dissociation between glycolytic flux and secretory activity in the islets. In islets removed from fed rats, the insulinotropic action of these drugs, which are known to increase cyclic AMP concentrations in the islets (Montague & Cook, 1971; Cooper *et al.*, 1973; Hellman *et al.*, 1974b) is most marked at high glucose concentration (Malaisse *et al.*, 1967, 1970; Brisson *et al.*, 1972). Yet, at the high glucose concentration (16.7 mM) used here, both theophylline and, when tested, dibutyl cyclic AMP failed to affect significantly glucose uptake, utilization and oxidation, lactate output and glucose-sensitive events, such as proinsulin biosynthesis (Malaisse *et al.*, 1974a) or net uptake of $^{45}\text{Ca}^{2+}$ (Brisson *et al.*, 1972). The potentiating effect of theophylline and dibutyl cyclic AMP on insulin release evoked by high glucose concentration (16.7 mM) is likely, therefore, to be due to a primarily glucose-independent process, such as an intracellular redistribution of Ca^{2+} (Brisson *et al.*, 1972; Brisson & Malaisse, 1973; Howell & Montague, 1975). However, at a lower glucose concentration (4.2–5.6 mM), close to the threshold value for the stimulating action of glucose on glucose-sensitive physiological events in the B-cell, the increase in lactate output evoked by theophylline and dibutyl cyclic AMP coincides with preferential stimulation of proinsulin synthesis (Malaisse *et al.*, 1974a) and increased net uptake of $^{45}\text{Ca}^{2+}$ (Brisson *et al.*, 1972).

Despite the above-mentioned associations and dissociations between changes in insulin release and metabolic parameters, a unique relationship of proportionality was observed under a great variety of experimental conditions between the rate of glycolysis and the net uptake of $^{45}\text{Ca}^{2+}$ by the islets (Fig. 2). Because our measurements of radioactive Ca^{2+} accumulation in the islets were performed after 90 min incubation in the presence of $^{45}\text{Ca}^{2+}$, they are assumed to reflect the size of the Ca^{2+} -exchangeable

pool(s) in the islets rather than the unidirectional rate of Ca^{2+} influx in the B-cell (Malaisse *et al.*, 1975). In other words, the data illustrated in Fig. 2 suggest that, in the process of carbohydrate-induced insulin release, the rate of glycolysis usually exerts a tight control on the size of the B-cell Ca^{2+} exchangeable pool(s), probably by regulating the transfer rate constant for Ca^{2+} transport across the cell membrane. For reasons given elsewhere (Malaisse *et al.*, 1973, 1975), we believe that this control corresponds, in the B-cell exposed to glucose, to a decreased outward rather than facilitated inward transport of Ca^{2+} across the plasma membrane.

In conclusion, the influence of various sugars and nutritional factors on islet function appears to be compatible, within the limits of the present study, with the idea that glucose-induced accumulation of Ca^{2+} and subsequent release of insulin depend closely on the rate at which the hexose is metabolized by the B-cell. We have observed (Malaisse *et al.*, 1976d) that such a concept could be applied to the differential insulinotropic capacity of α - and β -D-glucose (Grodsky *et al.*, 1974, 1975; Niki *et al.*, 1974).

We thank Mrs. M. Mahy and Mrs. J. Schoonheydt for technical assistance and Mrs. B. Noël for secretarial help. This work was supported in part by grants from the Fonds de la Recherche Scientifique Médicale (Belgium) and a contract of the Ministère de la Politique Scientifique (Belgium) within the framework of the association Euratom, Universities of Brussels and Pisa. J. L. is recipient of a Pfizer Travel Award through the European Association for the Study of Diabetes. This paper is the 23rd of a series.

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