Interrelationship of Islet Metabolism, Adenosine Triphosphate Content and Insulin Release

By STEPHEN J. H. ASHCROFT, L. CHATRA C. WEERASINGHE and PHILIP J. RANDLE
Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.

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The oxidation of some exogenous substrates and their effects on ATP content and insulin release in mouse pancreatic islets were measured. The ATP concentration of islets incubated without exogenous substrate shows a gradual decrease, which can be prevented by glucose or mannose (20mM) or leucine (2.5mM); D-glyceraldehyde (5mM) is as effective as glucose (5mM); fructose or N-acetylglucosamine (20mM), pyruvate (10mM) and DL-3-hydroxybutyrate (2mM) are less effective; galactose (20mM), acetate (10mM), octanoate (2mM) and succinate (10mM) have no ATP-maintaining ability. Islets oxidize glucose, mannose, glyceraldehyde, leucine and, less readily, N-acetylglucosamine and glucosamine; galactose, however, is poorly metabolized. Mannoheptulose inhibits the oxidation of glucose but not of glyceraldehyde. Insulin release, measured over a 2h incubation, is stimulated by glucose, mannose, leucine, glyceraldehyde or glucosamine but not by fructose or N-acetylglucosamine. The latter, however, potentiates the effects of glucose or glyceraldehyde (5mM) or leucine (2.5mM) on release; the potentiating effects are inhibited by mannoheptulose, which also blocks glucose-, but not glyceraldehyde- or leucine-stimulated release. In the presence of glucose (20mM), metabolic inhibitors depress insulin release and islet ATP content in parallel. However, rates of insulin release and ATP content measured after incubation with various combinations of exogenous substrates do not appear to be correlated. Sulphonylureas stimulate insulin release but decrease islet ATP concentrations. These results provide further evidence of a close association between the metabolic activity of exogenous substrates and their ability to initiate insulin release. Glucoreceptor models are formulated in the light of these observations and discussed.

The nature of the receptor(s) that enable the pancreatic β cell to secrete insulin in response to changes in the extracellular concentration of substrates such as sugars and amino acids has not been clearly defined. Randle *et al.* (1968) suggested that glucose may either interact directly with a receptor that excites the secretory process or that metabolism of the sugar may be coupled to excitation of the secretory process. Early studies *in vitro* suggested that only those sugars which are metabolized may elicit insulin secretion (Grodsky *et al.*, 1963; Coore & Randle, 1964). Support for this suggestion was provided by studies of islet-cell metabolism which showed that there is a close correlation between rates of utilization of glucose and mannose by islets and their effects on insulin secretion; and that agents which selectively inhibit insulin secretory responses to glucose and mannose (mannoheptulose and glucosamine) are inhibitors of glucose and mannose metabolism (Ashcroft *et al.*, 1970, 1972a,b; Jarrett & Keen, 1968). These studies focused attention on the relationship between islet metabolism of glucose and effects of the sugar on insulin release. However, it has been claimed that galactose and mannoheptulose,

which may not be metabolized by islets, may elicit insulin secretion *in vitro* under special conditions (presence of caffeine or theophylline with or without substrates such as pyruvate) (Lambert, 1970; Landgraf *et al.*, 1971). These and other studies have been interpreted in terms of a direct receptor for these sugars and, by inference, as evidence for a direct-receptor mechanism for glucose (Matschinsky *et al.*, 1971).

A detailed study *in vitro* of the specificity of the insulin secretory response to sugars broadened the scope of these considerations (Ashcroft *et al.*, 1972b). With both mouse and rat islets, of a large number of sugars and sugar derivatives tested, only glucose and mannose and, to a small extent, glucosamine elicited insulin release when tested singly at a concentration of 20mM in the presence of caffeine (galactose was not effective). If, however, a sub-stimulatory concentration of glucose (2.5–5mM) was present, then a marked increase in insulin release rate could be elicited by fructose, N-acetylglucosamine or (in the rat only) galactose. It was suggested that these results could be explained on the basis of a glucoreceptor model containing two different classes of subunits: an initiator unit sensitive either to glucose

and mannose directly, or to a metabolite thereof, and a potentiator unit with a broader specificity but dependent on activation of the initiator unit. One attraction of such a model is the possibility that co-operative interactions between the two units could account for the sigmoidicity of the dependence of insulin release rate on extracellular glucose concentration. A low concentration of glucose is required before the insulin response to a potentiator such as *N*-acetylglucosamine can occur. The studies did not exclude the possibility that this glucose provides a source of ATP.

In the present work we have extended these observations by showing that in addition to glucose, L-leucine and D-glyceraldehyde can also permit potentiation of insulin release by *N*-acetylglucosamine or fructose. To investigate the mechanism of this effect, the action of various combinations of initiators and potentiators on insulin release and islet ATP content was studied. Evidence for or against a requirement for glucose metabolism in initiating insulin release was sought by comparing the metabolic and insulin-secretory activity of glucose and glyceraldehyde and the effects of mannoheptulose on these parameters. The ability of the islet to oxidize exogenous substrates was determined and islet concentrations of ATP and insulin release rates were measured under a variety of conditions.

Experimental

Materials

Collagenase was from Boehringer Corp. (London) Ltd., London W.5, U.K. Bovine plasma albumin (fraction V) was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. D-Mannoheptulose was from Pfanstiehl Laboratories, Waukegan, Ill., U.S.A. Firefly lantern extract and D-glyceraldehyde were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Glibenclamide was a gift from Roussel Laboratories Ltd., Wembley Park, Middx., U.K. Tolbutamide was a gift from Burroughs Wellcome and Co., London N.W.1, U.K. Other chemicals were from British Drug Houses Ltd., Poole, Dorset, U.K.

All radiochemicals except D-[U-¹⁴C]glyceraldehyde were supplied by The Radiochemical Centre, Amersham, Bucks., U.K. [U-¹⁴C]Glyceraldehyde was obtained from New England Nuclear, Boston, Mass., U.S.A. and was purified before use by paper chromatography by the method of Antony *et al.* (1969).

Methods

Preparation of islets. Islets were prepared by a collagenase method (Coll-Garcia & Gill, 1969) from 3–4-week-old male white mice starved overnight.

Output of ¹⁴CO₂. The rate of formation of ¹⁴CO₂ from ¹⁴C-labelled sugars by islets was measured by the method of Ashcroft *et al.* (1970). In brief, batches of ten islets were incubated for 2 h at 37°C in 10 µl of bicarbonate medium (Krebs & Henseleit, 1932). After incubation, metabolism was terminated and ¹⁴CO₂ liberated into Hyamine by acidification of the medium. The absorbed ¹⁴CO₂ was counted by liquid-scintillation spectrometry [see Ashcroft *et al.* (1970)].

Insulin release. Batches of six islets were incubated in 0.3 ml of bicarbonate medium containing bovine plasma albumin (1 mg/ml) and other additions as stated. After incubation (2–3 h at 37°C), 0.2 ml of the medium was separated by gentle centrifugation and aspiration, diluted with phosphate-albumin buffer and stored at –20°C until the radioimmunoassay (Hales & Randle, 1963) with mouse insulin as standard (Coll-Garcia & Gill, 1969).

ATP content. When insulin release was measured simultaneously with islet content of ATP, batches of six islets were incubated and 0.2 ml of the medium was separated as described above for insulin assay. After addition of 50 µl of ice-cold HClO₄ (10%, v/v) to the remaining 0.1 ml of medium containing islets, the islets were disrupted by sonication for 10 s at position 2 on a Soniprobe (Dawe Instruments Ltd., London W.3, U.K.). Samples (30 µl) of the islet extracts were assayed for ATP (without neutralization) by the firefly luminescence assay in the Nuclear-Chicago mark 1 liquid-scintillation counter (Stanley & Williams, 1969). ATP standards (0–30 pmol) were made up to contain in the reaction vial the same amount of HClO₄ and bicarbonate medium as in the islet extracts. Routine checks were made that none of the agents whose effects on islet ATP were investigated affected the ATP assay.

When insulin release was not to be measured, batches of six islets were incubated in 50 µl of bicarbonate medium with the additions stated. After incubation, 25 µl of HClO₄ (10%, v/v) were added to each sample and ATP was determined as described above.

Results

Islet content of ATP

Fig. 1 shows the content of ATP in islets incubated for up to 10 h in the presence or absence of external glucose (3.3 or 16.7 mM). In the absence of glucose, islet ATP content declined slowly over the whole period, reaching about 30% of the initial value in 5 h. ATP content was partially maintained for 5 h by 3.3 mM-glucose and completely maintained by 16.7 mM-glucose, although some decrease was evident after this time. Fig. 2 shows the effect of an uncoupler of oxidative phosphorylation (carbonyl cyanide *m*-chloromethoxyphenylhydrazone) on islet ATP.

Fig. 1. Effect of incubation time on islet ATP content in the presence or absence of glucose

Batches of six islets were incubated at 37°C in bicarbonate medium for the time shown in the absence of exogenous substrate (○), or with glucose [3.3 mM (●) or 16.7 mM (▲)]. After incubation islet ATP content was determined as described in the text. Each point plotted is a mean value for four or more batches of islets each assayed in duplicate.

Fig. 2. Effect of an uncoupling agent on islet ATP content

Batches of islets were incubated for the times shown with glucose (3.3 mM) and carbonyl cyanide chloromethoxyphenylhydrazone (0.1 µg/ml). Islet ATP content was determined as described in the text. Each point plotted is the mean value for four or more batches of islets each assayed in duplicate.

Fig. 3. Effect of glucose concentration on islet ATP content

Batches of six islets were incubated at 37°C for 2 h in bicarbonate medium containing glucose at the concentration indicated. Each point plotted is the mean value for four or more batches of islets each assayed in duplicate.

Blocking the synthesis of ATP led to an extremely rapid decline in islet ATP concentration down to about 30% of the initial concentration in 5 min followed by a slower decline over the subsequent 35 min. In a further series of investigations ATP content was measured after 2 h incubation (insulin-release studies involved measurement over 2 h). Fig. 3 shows the effect of glucose concentration on islet ATP at this time. Islet ATP was much higher when glucose was added in the concentration range 0–5 mM; further increase of glucose concentration to 20 mM had a relatively small, although significant, effect. Table 1 shows the ability of other potential respiratory substrates to maintain islet ATP content. Mannose (20 mM) and leucine (2.5 mM) were as effective as 20 mM-glucose whereas 5 mM-D-glyceraldehyde was as effective as 5 mM-glucose. Fructose (20 mM), *N*-acetylglucosamine (20 mM), pyruvate (10 mM) and DL-3-hydroxybutyrate (2 mM) were less effective whereas galactose (20 mM), acetate (10 mM), octanoate (2 mM) and succinate (10 mM) had no detectable effect on islet ATP content. Table 2 shows the effect of various concentrations of D-glyceraldehyde on islet ATP content. Although 5 mM-glyceraldehyde elevated ATP to the concentration achieved with 5 mM-glucose, increasing the glyceraldehyde concentration further up to 40 mM resulted in a progressive decrease in islet ATP

content. Table 3 shows the effects on islet ATP concentration of various combinations of glucose, glyceraldehyde, leucine, fructose, *N*-acetylglucosamine and

Table 1. *Effects of substrates on islet ATP content*

Batches of six to ten islets were incubated for 2–3 h at 37°C in bicarbonate medium containing the additions shown. After incubation the islet ATP content was determined by a firefly assay as described in the text. To combine results of a number of experiments the ATP content is expressed as a percentage of the content of ATP in islets incubated with 20 mM-glucose in the same series of experiments. The numbers of batches of islets are given in parentheses. Results are given as means $\pm$ S.E.M.

Substrate	Concn. (mM)	Islet ATP content (% of content with 20 mM-glucose)
—	—	53 $\pm$ 2 (44)
Galactose	20	48 $\pm$ 2 (16)
Acetate	10	58 $\pm$ 4 (8)
Octanoate	2	46 $\pm$ 4 (16)
Succinate	10	58 $\pm$ 4 (8)
Fructose	20	77 $\pm$ 3 (24)
N-Acetylglucosamine	20	83 $\pm$ 3 (45)
Glucosamine	20	76 $\pm$ 6 (12)
Pyruvate	10	70 $\pm$ 6 (8)
DL-3-Hydroxybutyrate	2	74 $\pm$ 3 (8)
Glucose	5	92 $\pm$ 4 (36)
D-Glyceraldehyde	5	92 $\pm$ 2 (28)
Glucose	20	100 (58)
Mannose	20	98 $\pm$ 5 (8)
Leucine	2.5	96 $\pm$ 5 (12)

mannoheptulose. When glucose (20 mM) or leucine (2.5 mM) was present addition of other substrates had no effect on islet ATP. Mannoheptulose lowered the ATP content with glucose as substrate but not with leucine. Table 4 shows that islet ATP content was decreased by sulphonylureas.

Oxidation of ^{14}C -labelled substrates

Table 5 shows that islets oxidized ^{14}C -labelled glucose, leucine, glyceraldehyde and, less readily, glucosamine and N-acetylglucosamine, whereas galactose was only poorly metabolized. Mannoheptulose inhibited the oxidation of glucose but not that of glyceraldehyde.

Insulin release

The effects on insulin release of various combinations of glucose, leucine, glyceraldehyde, fructose, N-acetylglucosamine and mannoheptulose are shown in Table 3, together with the islet ATP content at the end of incubation in the same experiments. Insulin release was stimulated by glucose (5 or 20 mM), leucine (2.5 mM) or glyceraldehyde (5 mM); fructose or N-acetylglucosamine (20 mM) alone did not increase insulin release. The effects of 5 mM-glucose, 2.5 mM-leucine or 5 mM-glyceraldehyde were, however, markedly potentiated by N-acetylglucosamine. Similar, although less marked, potentiating effects of fructose

Table 2. *Effect of D-glyceraldehyde on islet ATP content*

Batches of islets were incubated for 2 h at 37°C in bicarbonate medium containing D-glyceraldehyde at the concentrations given, with caffeine (5 mM) and albumin (1 mg/ml). After incubation the islet ATP content was determined by a firefly assay method as described in the text. The number of measurements on separate batches of islets are shown in parentheses. Results are given as means $\pm$ S.E.M.

D-Glyceraldehyde concentration (mM)	Islet ATP content (pmol/islet)
0	3.95 $\pm$ 0.13 (44)
5	7.07 $\pm$ 0.14 (45)
10	5.55 $\pm$ 0.35 (12)
20	4.30 $\pm$ 0.55 (12)
40	1.35 $\pm$ 0.57 (12)

were also observed. The potentiating effects of N-acetylglucosamine on insulin release with 5 mM-glyceraldehyde or 2.5 mM-leucine were decreased by mannoheptulose (20 mM), which also blocked glucose-stimulated release. Mannoheptulose did not block insulin release in response to leucine and potentiated the response to glyceraldehyde. At a constant stimulating glucose concentration of 20 mM the rate

Table 3. *Effects of glucose, fructose, N-acetylglucosamine, D-glyceraldehyde, leucine and mannoheptulose on islet ATP content and insulin release*

Batches of six islets were incubated for 2 h at 37°C in bicarbonate medium containing 5 mM-caffeine and 1 mg of albumin/ml with the agents shown in the table. Insulin released into the medium was assayed by radio-immunoassay and islet ATP content was determined by a firefly luciferase method as described in the text. Results are given as means $\pm$ S.E.M., with the number of observations in parentheses.

Concentration in medium (mM)						ATP (pmol/islet)	Insulin release (pg/min per islet)
Glucose	Fructose	N-Acetyl- glucosamine	D-Glycer- aldehyde	Leucine	Manno- heptulose		
—	—	—	—	—	—	3.95 $\pm$ 0.13 (44)	6.81 $\pm$ 0.63 (52)
5	—	—	—	—	—	7.68 $\pm$ 0.24 (36)	41.5 $\pm$ 2.5 (44)
20	—	—	—	—	—	8.10 $\pm$ 0.15 (65)	324 $\pm$ 12 (81)
—	20	—	—	—	—	5.78 $\pm$ 0.21 (32)	8.55 $\pm$ 1.57 (20)
—	—	20	—	—	—	6.73 $\pm$ 0.21 (45)	8.12 $\pm$ 0.99 (45)
—	—	—	5	—	—	7.07 $\pm$ 0.14 (45)	68.9 $\pm$ 4.9 (54)
—	—	—	—	2.5	—	7.77 $\pm$ 0.44 (12)	93.3 $\pm$ 6.9 (37)
5	20	—	—	—	—	7.57 $\pm$ 0.28 (24)	78.3 $\pm$ 5.0 (16)
5	—	20	—	—	—	7.70 $\pm$ 0.42 (16)	138 $\pm$ 45 (4)
5	—	—	5	—	—	7.77 $\pm$ 0.42 (16)	224 $\pm$ 18 (20)
5	—	—	—	2.5	—	8.06 $\pm$ 0.46 (8)	241 $\pm$ 16 (8)
20	—	—	—	—	20	6.02 $\pm$ 0.28 (12)	13.7 $\pm$ 1.6 (16)
20	20	—	—	—	—	7.74 $\pm$ 0.47 (4)	398 $\pm$ 20 (4)
20	—	20	—	—	—	7.42 $\pm$ 0.75 (4)	387 $\pm$ 61 (4)
20	—	—	5	—	—	7.60 $\pm$ 0.25 (4)	331 $\pm$ 40 (4)
20	—	—	—	2.5	—	7.63 $\pm$ 0.40 (4)	400 $\pm$ 65 (4)
—	20	—	5	—	—	7.21 $\pm$ 0.50 (8)	77 $\pm$ 13 (8)
—	—	20	5	—	—	7.06 $\pm$ 0.27 (25)	195 $\pm$ 12 (30)
—	—	—	5	2.5	—	8.09 $\pm$ 0.50 (12)	188 $\pm$ 9 (16)
—	—	—	5	—	20	—	97.5 $\pm$ 6.1 (34)
—	20	—	—	2.5	—	8.99 $\pm$ 0.33 (4)	124 $\pm$ 27 (8)
—	—	20	—	2.5	—	8.85 $\pm$ 0.49 (8)	179 $\pm$ 16 (21)
—	—	—	—	2.5	20	—	118 $\pm$ 15 (12)
—	—	20	5	—	20	6.84 $\pm$ 0.40 (4)	91 $\pm$ 11 (13)
—	—	20	—	2.5	20	6.03 $\pm$ 0.25 (4)	95 $\pm$ 13 (13)
—	20	—	—	—	20	6.08 $\pm$ 0.75 (4)	—
—	—	20	—	—	20	5.53 $\pm$ 0.83 (4)	—

Table 4. *Effects of sulphonylureas on islet ATP and insulin release*

Batches of six islets were incubated with glucose (5.5 mM) and caffeine (5 mM) plus the agents shown for 2 h at 37°C in bicarbonate medium. Insulin released into the medium and islet ATP content were measured as described in the text. Results are given as means $\pm$ S.E.M., with the number of observations in parentheses.

Agent	Islet ATP (pmol/islet)	Insulin release (pg/min per islet)
—	7.40 $\pm$ 0.32	37 $\pm$ 5 (8)
Glibenclamide (0.1 μ g/ml)	4.75 $\pm$ 0.45	110 $\pm$ 14 (4)
Glibenclamide (1 μ g/ml)	3.80 $\pm$ 0.32	109 $\pm$ 12 (4)
Tolbutamide (0.2 mg/ml)	5.25 $\pm$ 0.35	121 $\pm$ 11 (4)

of release of insulin was inhibited by iodoacetamide, rotenone, carbonyl cyanide chloromethoxyphenylhydrazine or phenazine methosulphate and the ATP

content of the islets was also decreased; the ATP concentration and the rate of insulin release showed a significant correlation (Fig. 4). However, rates of

Table 5. Rates of oxidation of ^{14}C -labelled substrates by mouse islets

Batches of ten islets were incubated for 2 h at 37°C in bicarbonate medium containing a ^{14}C -labelled substrate with or without the addition of mannoheptulose as shown. After incubation the $^{14}\text{CO}_2$ evolved was absorbed in Hyamine and counted for radioactivity as described in the text. Results are given as means $\pm$ S.E.M., with the number of observations in parentheses.

^{14}C -labelled substrate	Concn. (mM)	Mannoheptulose (14.3 mM)	$^{14}\text{CO}_2$ formation (pmol of substrate/h per islet)
D-[^{14}C]Glucose	8.3	—	18.6 ± 1.5 (4)
		+	3.7 ± 0.4 (4)
D-[U- ^{14}C]Glyceraldehyde	3.8	—	17 ± 1.1 (5)
		+	19 ± 2.3 (5)
N-Acetyl-D-[^{14}C]glucosamine	9.4	—	4.8 ± 0.2 (5)
		+	4.0 ± 0.2 (5)
D-[^{14}C]Glucosamine	10.3	—	4.2 ± 0.3 (8)
D-[^{14}C]Galactose	10.2	—	0.5 ± 0.08 (4)
L-[^{14}C]Leucine	2.2	—	15.7 ± 1.4 (5)

Fig. 4. Effect of metabolic inhibitors on islet ATP and insulin release

Batches of six islets were incubated at 37°C for 2 h in bicarbonate medium containing glucose (20 mM), caffeine (5 mM), albumin (1 mg/ml) and one of the following inhibitors: Δ , iodoacetamide (0–1 mM); $\bullet$, phenazine methosulphate (0–0.1 mM); $\blacksquare$, rotenone (0–20 ng/ml); ∇ , carbonyl cyanide chloromethoxyphenylhydrazone (0–1 $\mu\text{g}/\text{ml}$). Rates of insulin release and islet ATP content were determined as described in the text. Each point shown is the mean value for four or eight batches of islets each assayed in duplicate.

insulin release and islet ATP content in the presence of various combinations of initiators and potentiators of release (results of Table 2) were not correlated (Fig. 5).

Insulin release was stimulated by sulphonylureas in the presence of glucose (Table 4).

Fig. 5. Effects of various combinations of glucose, fructose, glyceraldehyde, N-acetylglucosamine, leucine and mannoheptulose on islet ATP content and insulin release

Values were taken from Table 3.

Discussion

Islet metabolism

The ATP content of mouse islets incubated for 2 h with 5 mM-glucose is 7.68 pmol/islet; assuming a mean islet volume of 2 nl (Ashcroft *et al.*, 1970), this

is equivalent to approximately 3.9mm. If ATP production is stopped by addition of uncouplers of oxidative phosphorylation or respiratory-chain inhibitors, the islet content of ATP decreases rapidly over a period of about 5min and thereafter declines at a slower rate. If we assume that the initial rate of decrease of ATP concentration after the addition of uncoupler is equal to the rate of ATP turnover at that instant, we may calculate that the rate of ATP synthesis in the islet is approximately 3.3pmol/min per islet. The O₂ uptake rate of mouse islets has been reported to be 1.43pmol/min per islet (Hedekov *et al.*, 1972).

The dependence of islet ATP content on exogenous substrate was investigated by examining a range of potential metabolites to establish which of them the islet was able to utilize for ATP production. As an additional parameter of metabolic activity we measured the rate of ¹⁴CO₂ formation from ¹⁴C-labelled substrates. In general, a good correlation was found between rates of ¹⁴CO₂ production and ATP concentration. Thus we have shown that mouse islets oxidize glucose, mannose and fructose in descending order of rapidity (Ashcroft *et al.*, 1970) and we find here that fructose is considerably less effective than mannose or glucose in maintaining islet ATP content; galactose did not maintain islet ATP content nor was it oxidized at an appreciable rate, in agreement with observations suggesting that this sugar is not significantly metabolized by islet tissue (Hellerström, 1967; Jarrett & Keen, 1968). However, both [¹⁴C]glucosamine and *N*-acetyl[¹⁴C]glucosamine were metabolized to ¹⁴CO₂ although the metabolic pathways involved have not been investigated in this tissue. Islets are also able to metabolize D-glyceraldehyde as shown by ¹⁴CO₂ output and maintenance of ATP content. However, although 5mm-glyceraldehyde was effective in the latter respect, increasing glyceraldehyde concentrations led to a marked depletion of ATP. The reason for this effect was not established; one possibility is that the ability of islet triokinase (presumably present) to phosphorylate glyceraldehyde may exceed the ability of the islet to utilize the triose phosphate so formed for ATP synthesis. The inhibition by mannoheptulose of the oxidation of glucose but not of glyceraldehyde is consistent with the site of action of mannoheptulose being on glucose phosphorylation (Coore & Randle, 1964; Malaisse *et al.*, 1968; Ashcroft *et al.*, 1970).

Insulin release

The characteristics of glucose-stimulated insulin release from mouse islets have been described (Ashcroft *et al.*, 1972b); the response shows a sigmoidal dependence on glucose concentration and is markedly potentiated by caffeine. The response also shows a high degree of specificity; of a large

number of sugars and sugar derivatives tested, only glucose, mannose and, to a much less extent, glucosamine were able to initiate insulin release. To these metabolites the present study adds leucine and glyceraldehyde as initiators of insulin release. The potentiating effect of *N*-acetylglucosamine and fructose on insulin release in the presence of low glucose concentrations was described previously (Ashcroft *et al.*, 1972b). We now extend these observations to show that glyceraldehyde and leucine can substitute for glucose as a basis for potentiation by *N*-acetylglucosamine. It is of interest that mannoheptulose, in addition to its well-known inhibitory action on initiation of insulin release by glucose, also inhibited the potentiation of insulin release by *N*-acetylglucosamine with leucine or glyceraldehyde as initiators, although it did not inhibit glyceraldehyde- or leucine-stimulated release *per se*. Indeed it potentiated glyceraldehyde-stimulated release moderately.

Interrelationships of islet metabolism and insulin release

One aim of the present work was to assess the possibility that interpretation of studies on the specificity of insulin secretory responses may be complicated by ATP lack under certain conditions. For example, does the failure of galactose to stimulate insulin release (Ashcroft *et al.*, 1972b) reflect a discrimination against this hexose by a glucoreceptor, or the fact that incubation with galactose may result in lack of ATP, since galactose is not a good substrate for islet metabolism? One limitation of our results is that we have been unable to devise a method for the accurate determination of ATP turnover (the calculations given above on this parameter can only be regarded as approximations), and our studies therefore relate to the steady-state concentration of ATP in the islet. A number of our results provide some support for the view that the ability of agents to elicit insulin release may depend on their ability to maintain a high islet content of ATP. Thus glucose, mannose, leucine and glyceraldehyde (5mm) were agents that were most potent in eliciting insulin release and these were the agents that best maintained islet ATP. Again when the medium glyceraldehyde concentration was raised above 5mm, both insulin release and islet ATP content were decreased (results not shown). A similar correspondence between islet ATP and insulin release rate was observed when, with 20mm-glucose in the medium, the islet ATP content was lowered by metabolic inhibitors (Fig. 4). However, these experiments did not exclude the possibility that the rate of glucose utilization by the islets, which correlates with the rate of insulin release (Ashcroft *et al.*, 1970, 1972b), may itself be

dependent on the islet ATP concentration; moreover, a number of our observations do not easily fit in with a primary role for ATP concentration in determining the rate of insulin release. Thus, in agreement with Hellman *et al.* (1969), stimulation of insulin release by sulphonylureas was associated with a decrease in islet ATP content. Again, although islet ATP concentration was dependent on the extracellular glucose concentration, almost maximum ATP concentration was achieved by 5 mM-glucose (Fig. 3) whereas insulin release is most sensitive to changes in glucose concentration between 5 and 12 mM (Ashcroft *et al.*, 1972b). Of particular importance for the interpretation of the effects of *N*-acetylglucosamine is the finding that there was no significant difference between the islet ATP content in the presence of

5 mM-glyceraldehyde, 20 mM-*N*-acetylglucosamine, or 5 mM-glyceraldehyde plus 20 mM-*N*-acetylglucosamine, whereas insulin release rates under these three conditions were 8, 69 and 195 pg/min per islet respectively (Table 3). Further, although ATP contents are higher in the presence of well-metabolized substrates (Table 1) there was no correlation between ATP concentration and insulin release rates for the various combinations of stimulators and potentiators tested (Table 3 and Fig. 5). We do not think, therefore, that the ability of an agent to increase the islet ATP content is sufficient to account for the specificity of the effects of the same agents on insulin release.

We have discussed (Ashcroft *et al.*, 1972b) the possible interpretation of the potentiation by *N*-acetylglucosamine of insulin release at low glucose

Scheme 1. *Glucoreceptor models*

(a) The β cell is depicted as possessing initiator sites ($\cdot$) capable of eliciting insulin release when activated, and a potentiator site ($\cdot$), activation of which potentiates the effects of activation of the initiator site. In (b) the primary stimulators of insulin release act through the formation of a trigger metabolite whose action may be potentiated by activation of the potentiator site. The inhibitory effects of mannoheptulose are shown as — $\bullet$ in the Scheme. For further explanation of the models see the Discussion section.

concentrations; the present extension of this observation to show that glyceraldehyde or leucine can substitute for glucose in this regard lends further support to the view that a single-site glucoreceptor model, whether based on a membrane-located receptor capable of binding glucose directly or on an enzyme catalysing a rate-limiting step in glucose metabolism, may be inadequate. The simplest models that we believe encompass the results are shown in Scheme 1. The model shown in Scheme 1(a) retains the essential feature of the regulator-site model but suggests that the receptor molecule may have two distinct types of subunit: (i) an initiator unit capable of binding glucose, mannose glyceraldehyde and leucine and leading to insulin release and (ii) a potentiator unit that binds a wider spectrum of sugars including *N*-acetylglucosamine and other potentiators and can be inhibited by mannoheptulose; in the absence of binding at the initiator site, binding at the potentiator site does not trigger release, but when the initiator site is activated the presence of a potentiator leads, via co-operative interaction between the two units, to a faster rate of insulin release than in its absence. Since mannoheptulose does not inhibit glyceraldehyde- or leucine-stimulated release, the model incorporates an initiator unit having at least three different sites, only one of which is inhibited by mannoheptulose. Glucose is envisaged as capable of activating both units. Scheme 1(b) shows a model incorporating the view that metabolism of the initiators leads to production of an intracellular agent that triggers insulin release; in this case activation of a regulator site by *N*-acetylglucosamine potentiates the initiating action of the trigger metabolite. Mannoheptulose must be assumed to inhibit both metabolism of glucose and also binding of sugars to the regulator site. The present results do not allow us to decide unequivocally between these two models; such a differentiation is likely to be derived from studies of the mechanism of the coupling of excitation to release (Ashcroft & Randle, 1970).

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