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β-Cell Recognition of Stereoisomers of D-Glucose

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Summary. The ability of all eight D-aldohexose steroisomers to stimulate insulin release and biosynthesis was compared with their ability to serve as a metabolic substrate for isolated islets of Langerhans as judged by formation of lactate. Insulin release and synthesis were stimulated by glucose or mannose but not by allose, altrose, gulose, idose, galactose or talose. No potentiary effects of allose, altrose, gulose, idose, or talose were found on insulin release in the presence of 4 mmol/l glucose nor did these sugars inhibit insulin release in the presence of 20 mmol/l glucose. Lactate formation was increased above values found in the absence of added substrate by 20 mmol/l D-glucose or mannose, but not by allose, altrose, gulose, galactose or talose. The results support the substrate-site hypothesis for the recognition of sugars as stimuli of insulin release and synthesis.

Key words: Insulin release, insulin biosynthesis, islet lactate output, glucoreceptor, D-aldohexose stereoisomers.

Sugars are involved in three major recognition events in the pancreatic β-cell: i) as metabolic substrates; ii) as secretagogues for insulin release; iii) as stimulators of insulin biosynthesis. Detailed studies have been carried out [1–3] on the specificity towards sugars of these processes. Evidence reviewed recently [4, 5] indicates that is is the ability of a hexose to undergo metabolic transformation in the β-cell that underlies its stimulatory action on insulin release, the 'substrate-site' hypothesis [6]. This concept has been extended to the control of insulin biosynthesis [7]. In the present study we have further tested this model by investigating the effects of all eight D-aldohexose stereoisomers on insulin release and biosynthesis together with their ability to serve as substrates for islet glycolysis to lactate.

Materials and Methods

Materials

D-allose, D-altrose, D-gulose, D-idose L(+) lactic acid, collagenase (Type 1), bacterial luciferase, FMN and bovine albumin (Fraction V) were from Sigma (London) Chemical Co., Kingston-on-Thames, Surrey, U. K. D-talose was from Koch-Light, Colnbrook, Bucks., U. K. Lactate dehydrogenase, glutamate pyruvate transaminase, NAD^+ and NADH were from Boehringer Corp. (London) Ltd., London, W. 5, U. K. L-{4,5-^{3}H} leucine (50 Ci/mmol) was from the Radiochemical Centre, Amersham, Bucks., U. K. Tetradecyl aldehyde was from Ralph N. Emmanuel Ltd., Wembley, U. K. Other chemicals were from British Drug Houses, Ltd., Poole, Dorset, U. K. Rat insulin standard was a gift from Dr. A. J. Moody, Novo Research Institute, Batsvaerd, Denmark.

Methods

Preparation of Islets: Islets were prepared by a collagenase method [8] from male albino Wistar rats fed ad libitum on standard laboratory diet.

Insulin Release: Batches of 5 islets were incubated for 2 h at 37° in 0.3 ml Krebs-Ringer bicarbonate medium [9] containing albumin (2 mg/ml) and the sugar indicated. Following incubations, islets were sedimented by centrifugation (1 min at position 1 on an MSE bench centrifuge, approx. 100 g). Insulin released into the medium was determined by a charcoal radioimmunoassay [10] using rat insulin as standard.

(Pro)insulin Biosynthesis: Batches of 7 islets were incubated for 90 min at 37° in 0.1 ml Krebs-Ringer bicarbonate buffer containing albumin (2 mg/ml), L-{4,5-^{3}H} leucine (4 μCi; 50 Ci/mmol) and the additions stated. The incorporation of {^{3}H} -leucine into insulin plus proinsulin (designated '(pro)insulin') was measured using insulin-binding affinity columns for (pro)insulin and trichloracetic acid-precipitation for total protein as previously

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Table 1. Effects of D-glucose stereoisomers on insulin release

Sugar	Insulin release (μU/Islet per h)					
	0 glucose		4mM glucose		20mM glucose	
	−sugar	+sugar	−sugar	+sugar	−sugar	+sugar
Allose	19.5 ± 4.3 (10)	25.5 ± 4.4 (14)	40.6 ± 10.3 (5)	42.7 ± 11.8 (5)	343.5 ± 28.3 (15)	364.7 ± 33.5 (5)
Altrose	18.1 ± 0.3 (5)	14.8 ± 1.5 (15)	17.6 ± 3.2 (10)	13.6 ± 1.9 (10)	207.6 ± 15.4 (15)	172.9 ± 15.7 (10)
Glucose		21.6 ± 2.5 (30)		26.7 ± 2.6 (54)		215.1 ± 12.5 (70)
Mannose	18.1 ± 0.3 (5)	107.3 ± 8.2 (5)[a]	–	–	–	–
Gulose	22.0 ± 7.9 (5)	24.3 ± 6.0 (5)	45.2 ± 11.8 (5)	32.9 ± 9.6 (5)	228.7 ± 17.2 (10)	169.0 ± 7.7 (5)
Idose	22.0 ± 7.9 (5)	24.4 ± 5.7 (15)	23.0 ± 4.2 (10)	32.8 ± 4.8 (10)	218.4 ± 19.9 (15)	196.4 ± 22.8 (10)
Galactose	22.7 ± 3.1 (20)	20.2 ± 2.7 (20)	28.4 ± 4.9 (15)	39.2 ± 7.1 (15)	133.0 ± 12.9 (20)	122.6 ± 10.8 (15)
Talose	18.1 ± 0.3 (5)	15.7 ± 2.2 (10)	20.2 ± 5.7 (9)	23.3 ± 5.5 (10)	200.5 ± 13.4 (15)	179.4 ± 19.5 (10)

Batches of 5 islets were incubated for 2 h with the steroisomers alone or in the presence of 4 or 20 mmol/l glucose. Results are given as mean ± SEM for the numbers of islet batches stated in parentheses. All sugars were the D-stereoisomer. Data for glucose are the mean values over the whole series of experiments

[a] Significantly different from control incubations at the same glucose concentration in the same experiments ($P \leq 0.001$)

Table 2. Effects of D-glucose stereoisomers on insulin biosynthesis

Stereoisomer	n	(Pro)insulin synthesis	Protein synthesis	$10^3 \times \frac{\text{Proinsulin}}{\text{protein}}$	Insulin index
None	24	4.2±0.5	43.5±6.4	49±6	0.112±0.011
Glucose	24	100[a]	100[a]	438±20[a]	1[a]
Allose	8	2.1±0.4	24.9±2.5	40±6	0.086±0.011
Altrose	8	3.3±0.5	22.6±2.9	73±9	0.155±0.015
Mannose	4	63.3±15.6[a]	66.8±14.9	388±78[a]	0.967±0.077[a]
Gulose	8	4.6±0.7	29.8±3.9	72±8	0.157±0.016
Idose	8	3.3±0.3	31.3±3.7	55±5	0.115±0.013
Galactose	4	1.9±0.1	27.8±1.8	28±2	0.069±0.006
Talose	8	2.3±0.3	23.7±3.1	51±6	0.111±0.018

Batches of 7 islets were incubated for 90 min with L-{4,5-^{3}H}leucine and the sugars shown, 20 mmol/l. Rates of (pro)insulin and total protein synthesis are expressed as a percentage of the rates found with 20 mmol/l glucose in the same experiment. The ratio (pro)insulin/protein expresses the fraction of total protein synthesis represented by (pro)insulin under each condition. The insulin index is the value of this ratio for each condition as a fraction of its value with 20 mmol/l glucose in the same experiment. Results are given as mean ± SEM for the numbers of batches of islets stated (n). All sugars were the D-stereoisomers

[a] Significantly greater than control (no addition) batches in the same experiment $P \leq 0.01$

described in detail [11]. Rates of (pro)insulin and total protein biosynthesis were expressed as a percentage of the rates found with 20 mmol/l glucose in the same experiment. The ratio of (pro)insulin to total protein synthesis was also calculated under each condition and the value of this ratio as a fraction of its value with 20 mmol/l glucose in the same experiment, the 'insulin index' [12], was used to express the preferential stimulation of (pro)insulin synthesis.

Lactate Output: Batches of 8–10 islets were incubated in 25 μl of Krebs-Ringer bicarbonate buffer containing the additions stated. After incubation the islets were sedimented by centrifugation (1 min at position 4 on an MSE bench centrifuge approx. 600 g). Lactate in the incubation medium was assayed by conversion to pyruvate using NAD^+ and the enzymes lactate dehydrogenase and glutumate pyruvate transaminase [13]: the NADH thus formed was measured using bacterial luciferase [14, 15]. Incubation medium, 10 μl, was added to 15 μl of 2-amino-2-methyl-propan-1-ol, 100 mmol/l, pH 9.9 containing NAD^+ (0.4 mmol/l), sodium glutamate (2.4 mmol/l), lactate dehydrogenase (75 μg/ml) and glutamate-pyruvate transaminase (75 μg/ml). The reaction was allowed to proceed for 1 h at room temperature. Reaction medium 8 μl, was then added to 1 ml phosphate buffer, 100 mmol/l, pH 7.5 containing 5 mmol/l mercaptoethanol. Immediately prior to assay, 1 ml of the same buffer containing 40 μg FMN was added followed by 20 μl of tetradecyl aldehyde (saturated solution in methanol) and 10 μl of bacterial luciferase (4 mg/ml in H_2O). Luminescence was measured a fixed time after addition of the luciferase using an Isocap liquid scintillation spectrometer with the photomultipliers out of coincidence; counts were recorded for 0.2 min. Standard curves were constructed using lactate standards (0–400 pmol) carried through the same procedure, and NADH standards were also included in each luminescence assay. Blank samples containing incubation media but no islets were also taken through the entire incubation and assay procedure. To avoid contamination with lactate, gloves were worn throughout these experiments and all glassware was rinsed with hot tap water and then twice-distilled water just before use.

Presentation of Results: All results are expressed as mean ± SEM for the number of batches of islets stated. The significance of differences from control values obtained in the same experiments was assessed using Student's t-test.

Table 3. Effects of D-glucose stereoisomers on islet lactate output

Stereoisomer	n	Lactate output pmol/islet per h
None	35	10.1±0.9
Glucose	31	47.2±2.8[a]
Allose	8	11.3±0.9
Altrose	7	12.3±1.0
Mannose	8	39.1±4.2[a]
Gulose	7	11.6±1.8
Galactose	8	5.8±1.5
Talose	8	11.0±1.2

Batches of 8–10 islets were incubated for 2 h with the sugars stated. Results are given as mean ± SEM for the number of batches of islets stated (n). All sugars were the D-stereoisomers
[a] Significantly greater than control (no addition) incubations in the same experiments ($P \leq 0.001$)

Results

Insulin Release: Of the eight D-aldohexose stereoisomers only glucose and mannose elicited insulin release above the basal values found in the absence of added sugar (Table 1). No potentiatory effects of allose, altrose, gulose, idose, galactose or talose were found when tested on insulin release in the presence of 4 mmol/l glucose, nor did these sugars inhibit insulin release stimulated by 20 mmol/l glucose.

(Pro)insulin Biosynthesis: Specific stimulation of (pro)insulin biosynthesis relative to islet protein biosynthesis, as indicated by an increased insulin index, was elicited by glucose or mannose (Table 2). No effects of the other D-aldohexose stereoisomers on (pro)insulin or protein synthesis were found.

Lactate Output: Islets incubated in the absence of added sugar produced lactate at a rate of 10.1 pmol/islet per h. The rate of lactate output was increased substantially by 20 mmol/l glucose or mannose, but not by equimolar allose, altrose, gulose, galactose or talose (Table 3). Results with idose are not included, because although some lactate formation was observed this could be accounted for by the approx. 5% contamination of this sugar with glucose: lactate output (pmol/islet/h) was 22.4 ± 1.5 with 20 mmol/l idose and 24.0 ± 2.2 with 1 mmol/l glucose (n = 8).

Discussion

It has previously been shown that glucose and mannose are well metabolized by pancreatic islets, whereas galactose and talose are not [2, 16, 17]. Moreover, insulin release is also stimulated by glucose or mannose whereas allose, altrose, galactose and talose are ineffective as either initiators or potentiators of insulin release [2, 17]. Glucose and mannose stimulate insulin biosynthesis but galactose and talose do not [7]. We havenow extended these observations to include the metabolic activity, as assessed by lactate production, and the effects on insulin release and biosynthesis of all eight D-aldohexose stereoisomers.

The results show that recognition of these isomers by the glucosesensors(s) regulating insulin biosynthesis and release parallels their recognition as metabolic substrates; they thus support the 'substrate-site' hypothesis as applied both to insulin release and to insulin biosynthesis. On this model pancreatic β-cell sugar recognition has its basis in metabolic discrimination. This discrimination may involve three distinct stages: the plasma membrane, sugar phosphorylation, and subsequent transformation of sugar phosphate to elicit changes in concentration of key metabolite(s) or co-factor(s). Thus the sugar transport system(s) of the β-cell restricts to the extracellular space such sugars as L-glucose [18]. Other sugars may enter the β-cell but fail to be phosphorylated, e. g. 3-o-methylglucose, galactose [2, 16, 19]. Although for glucose and mannose phosphorylation by glucokinase may be the rate-limiting discriminatory step [20], this need not be so for other sugars. For *N*-acetyl-D-glucosamine which elicits insulin release and biosynthesis, evidence has been presented that entry into the β-cell via the glucose transporter may be the rate-limiting step, and subsequent phosphorylation occurs not via glucokinase but by a non-rate-limiting reaction catalysed by a specific *N*-acetylglucosamine kinase [21]. Other sugars such as 2-deoxyglucose may enter the β-cell and be phosphorylated but fail to be further metabolized [16].

The combination of these discriminatory events results in the rather strict specificity towards sugars displayed by the β-cell. Only at carbon-2 does there appear to be latitude towards the configuration and nature of the substituent. Thus mannose, which differs from glucose in the orientation of the -OH group on carbon-2, is well metabolized by islets as judged by various parameters [2, 16, 22] and elicits insulin biosynthesis and release. Fructose, the 2-ketohexose isomer of glucose, is metabolized, albeit slowly in comparison with glucose [2, 16, 22] and stimulates insulin biosynthesis and release provided that a low concentration of glucose is also present [1, 7]. *N*-acetylglucosamine in which the -OH group on carbon-2 is replaced by the acetamido group (CH_3CONH-) stimulates insulin release and biosynthesis and is metabolized by islets [11, 21].

From the point of view, therefore, of producing hypoglycemic glucose analogues of therapeutic value in diabetics with residual β-cell function, modification of glucose at the 2-carbon may thus be the most hopeful approach. Indeed in the rat, *N*-acetylglucosamine is a potent hypoglycemic agent in vivo [10] and this action is greatly enhanced by the introduction of chlorine substituents into the acetyl group [11]. Detailed studies on the secretory and biosynthetic responses of the human β-cell are required to ascertain the relevance of these considerations to man.

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References

1. Ashcroft, S. J. H., Bassett, J. M., Randle, P. J.: Insulin secretion mechanisms and glucose metabolism in isolated islets. Diabetes **21** (Suppl. 2), 538–545 (1972)
2. Ashcroft, S. J. H., Weerasinghe, L. C. C., Randle, P. J.: Interrelationships of islet metabolism, adenosine triphosphate content and insulin release. Biochem. J. **132**, 223–231 (1973)
3. Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., Täljedal, I.-B.: The pancreatic β-cell recognition of insulin secretagogues. Comparisons of glucose with glyceraldehyde isomers and dihydroxyacetone. Arch. Biochem. Biophys. **162**, 448–457 (1974)
4. Ashcroft, S. J. H.: The control of insulin release by sugars. Ciba Found. Symp. **41**, 117–139 (1976)
5. Ashcroft, S. J. H.: The use of glucose analogues in the elucidation of the mechanisms of insulin release and biosynthesis. Febs 11th Meeting **42** (Symposium Al), 227–236 (1978)
6. Randle, P. J., Ashcroft, S. J. H., Gill, J. R.: Carbohydrate metabolism and the release of hormones. In: Carbohydrate metabolism and its disorders. Dickens, F., Randle, P. J., Whelan, W. J. (eds.), Vol. 1, p. 427. London: Academic Press 1961
7. Ashcroft, S. J. H., Bunce, J., Lowry, M., Hansen, S. E., Hedeskov, C. J.: The effect of sugars on (pro)insulin biosynthesis. Biochem. J. **174**, 517–526 (1978)
8. Coll-Garcia, E., Gill, J. R.: Insulin release by isolated pancreatic islets of the mouse incubated in vitro. Diabetologia **5**, 61–66 (1969)
9. Krebs, H. A., Henseleit, K.: Untersuchungen über die Harnstoffbildung im Tierkörper. Hoppe Seylers Z. Physiol. Chem. **210**, 33–62 (1932)
10. Ashcroft, S. J. H., Crossley, J. R.: The effects of glucose, N-acetylglucosamine, glyceraldehyde and other sugars on insulin release in vivo. Diabetologia **11**, 279–284 (1975)
11. Ashcroft, S. J. H., Crossley, J. R., Crossley, P.: The effect of N-acylglucosamines on the biosynthesis and secretion of insulin in the rat. Biochem. J. **154**, 701–707 (1976)
12. Pipeleers, D. G., Marichal, M., Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release. Glucose-regulation of insular biosynthetic activity. Endocrinology **93**, 1001–1011 (1973)
13. Zawalich, W. S., Matschinsky, F. M.: Sequential analysis of the releasing and fuel function of glucose in isolated perifused pancreatic islets. Endocrinology **100**, 1–8 (1977)
14. Ashcroft, S. J. H., Capito, K., Hedeskov, C. J.: Time-course studies of glucose-induced changes in glucose 6-phosphate and fructose 1:6 diphosphate content of mouse and rat pancreatic islets. Diabetologia **9**, 299–302 (1973)
15. Kerbey, A. L., Radcliffe, P. M., Randle, P. J.: Diabetes and the control of pyruvate dehydrogenase in rat heart mitochondria by concentration ratios of adenosine triphosphate/adenosine diphosphate, of reduced/oxidized nicotinamide adenine dinucleotide and of acetyl coenzyme A/coenzyme A. Biochem. J. **164**, 509–519 (1977)
16. Ashcroft, S. J. H., Hedeskov, C. J., Randle, P. J.: Glucose metabolism in mouse pancreatic islets. Biochem. J. **118**, 143–154 (1970)
17. Macdonald, M. J., Ball, D. H., Patel, T. N., Lauris, V., Steinke, J.: Studies of insulin release and rat pancreatic islet metabolism with diastereoisomers of D-glucose. Biochim. Biophys. Acta **385**, 188–193 (1975)
18. Hellman, B., Sehlin, J., Täljedal, I.-B.: Evidence for mediated transport of glucose in mammalian pancreatic β-cells. Biochim. Biophys. Acta **241**, 147–154 (1971)
19. Hellman, B., Sehlin, J., Täljedal, I.-B.: Transport of 3-0-methyl-D-glucose into mammalian pancreatic β-cells. Pflügers Arch. **340**, 51–58 (1973)
20. Ashcroft, S. J. H., Randle, P. J.: Enzymes of glucose metabolism in normal mouse islets. Biochem. J. **119**, 5–15 (1970)
21. Williams, I. H., Ashcroft, S. J. H.: N-acetylglucosamine and the substratesite hypothesis for the control of insulin biosynthesis and secretion. FEBS Lett. **87**, 115–120 (1978)
22. Sener, A., Levy, J., Malaisse, W. J.: The stimulus-section coupling of glucose-induced insulin release. Does glycolysis control calcium transport in the β-cell? Biochem. J. **156**, 521–525 (1976)

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