The Effect of Sugars on (Pro)insulin Biosynthesis

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Rates of incorporation of [4,5-³H]leucine into insulin plus proinsulin, designated '(pro)-insulin', and total protein in rat pancreatic islets were measured. Glucose stimulates rates of total protein and (pro)insulin biosynthesis, but (pro)insulin biosynthesis is stimulated preferentially. Mannose and *N*-acetylglucosamine also stimulate (pro)insulin and total protein biosynthesis; inosine and dihydroxyacetone stimulate (pro)insulin biosynthesis specifically. Fructose does not stimulate (pro)insulin biosynthesis when tested alone, but does so in the presence of low concentrations of glucose, mannose or *N*-acetylglucosamine. Many glucose analogues do not stimulate (pro)insulin biosynthesis. Mannoheptulose inhibits synthesis of (pro)insulin and total protein stimulated by glucose or mannose but not by dihydroxyacetone, inosine or *N*-acetylglucosamine; phloretin (9 μ M) inhibits *N*-acetylglucosamine-stimulated (pro)insulin biosynthesis preferentially. The data are in agreement with the view that the same glucose-sensor mechanism may control both insulin release and biosynthesis, and a 'substrate-site' model is suggested. The threshold for stimulation of biosynthesis of (pro)insulin and total protein is lower than that found for glucose-stimulated insulin release; moreover the biosynthetic response to an elevation of glucose concentration is slower than that found for insulin release. The physiological implication of these findings is discussed. Caffeine and isobutylmethylxanthine, at concentrations known to increase islet 3':5'-cyclic AMP and potentiate glucose-induced insulin release, were without effect on rates of glucose-stimulated (pro)insulin biosynthesis.

It has been known for a long time that glucose preferentially stimulates insulin biosynthesis (Howell & Taylor, 1966). A series of similarities between stimulation of insulin release and stimulation of insulin biosynthesis has been demonstrated. Thus the same dependence on glucose concentration was found for the two processes (Morris & Korner, 1970; Lin *et al.*, 1972). It was also shown that insulin biosynthesis was stimulated by mannose, but not by fructose, pyruvate, ribose or xylitol (results in accordance with the effect of these sugars on insulin secretion) and that glucose-induced stimulation of both secretion and biosynthesis could be inhibited by mannoheptulose (Lin & Haist, 1969). The same authors have also demonstrated that caffeine potentiates glucose stimulation of both processes (Lin & Haist, 1973). Insulin secretion as well as insulin biosynthesis is stimulated by leucine (Andersson, 1976). These results seem to show that secretion and biosynthesis share certain control mechanisms.

However, the two processes are not obligatorily coupled, but can under some conditions be dissociated. Stimulated insulin secretion can be observed in the presence of the protein-synthesis inhibitor

cycloheximide (Morris & Korner, 1970) and, conversely, glucose is able to stimulate insulin biosynthesis (but not secretion) in a Ca^{2+} -free medium (Pipeleers *et al.*, 1973b). It has also been demonstrated that glucose stimulates the incorporation of leucine into insulin in foetal rat islets, whereas insulin secretion cannot be evoked by glucose in these islets (Asplund, 1973). In pancreatic islets cultured for 1 week at a low glucose concentration, a high glucose concentration stimulates insulin biosynthesis but not insulin release (Andersson *et al.*, 1976). In addition, different authors (Maldonado *et al.*, 1977; Berne, 1975; Pipeleers *et al.*, 1973a) have demonstrated that the threshold for triggering insulin biosynthesis by glucose is considerably lower than that for insulin secretion.

These dissimilarities between glucose-induced insulin secretion and insulin biosynthesis have prompted the present study. The paper is especially concerned with the specificity of the glucose-sensor towards a series of sugars, which is not well established, but in addition the glucose-concentration-dependence of insulin biosynthesis, the time course of biosynthesis, the effect of different inhibitors

(phloretin and mannoheptulose) and potentiators (e.g. fructose), the relation between insulin biosynthesis and islet total protein synthesis and the possible role of cyclic AMP in insulin biosynthesis have been examined. Finally, the question as to whether the glucose-sensor for insulin biosynthesis is plasma-membrane-bound or whether glucose has to be metabolized in the islets to trigger insulin biosynthesis is discussed.

Experimental

Materials

Fucose, inosine, *N*-acetylglucosamine, collagenase, erythrose, mannoheptulose and bovine plasma albumin (fraction V) were from Sigma (London) Chemical Co., Kingston upon Thames, U.K. Talose was from Koch-Light, Colnbrook, Bucks., U.K. Phloretin was from K & K Laboratories, Plainview, NY, U.S.A. [4,5-³H]Leucine (40–60 Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Freeze-dried anti-insulin serum was from Miles-Yeda Ltd., Stoke Poges, Slough, U.K. Other chemicals were from British Drug Houses, Poole, Dorset, U.K. All sugars are the D-stereoisomer unless stated otherwise.

Methods

Preparation of islets. Islets were prepared by a collagenase method (Coll-Garcia & Gill, 1969) from 200–300 g male rats fed *ad libitum*.

Preparation of insulin-binding affinity column. Anti-insulin globulins were coupled to activated Sepharose 4B as previously described (Ashcroft *et al.*, 1976). Such columns bind both insulin and proinsulin: hence the term '(pro)insulin' has been used throughout to include insulin plus proinsulin. Preproinsulin also binds to antibodies to insulin (Chan *et al.*, 1976), but because of the rapid conversion of preproinsulin in rat islets the radioactivity in preproinsulin is only a minor contribution to the radioactivity bound (Permutt & Routman, 1977).

Measurement of islet (pro)insulin and total protein biosynthesis. The method has been previously described in detail (Ashcroft *et al.*, 1976). Briefly, batches of islets were incubated in 100 μ l of bicarbonate medium (Krebs & Henseleit, 1932) containing albumin (2 mg/ml), 2–4 μ Ci of L-[4,5-³H]leucine and other additions as stated. After incubation for 90 min at 37°C with shaking, the islets were washed with bicarbonate medium containing albumin (2 mg/ml) and non-radioactive leucine (2 mM). Then 250 μ l of 0.1 M-sodium borate buffer, pH 8, containing albumin and 0.5 M-NaCl was added and the islets were disrupted by sonication; 250 μ l of the same buffer was then added. (Pro)insulin in samples of the sonicated preparation was selectively absorbed by

passage through 0.2 ml insulin-binding columns. The columns were washed with the same buffer and then the (pro)insulin was eluted with 1 M-acetic acid containing 3 mg of albumin/ml. The radioactivity in the eluates was determined by liquid-scintillation spectrometry. Incorporation of [³H]leucine into total islet protein was determined by trichloroacetic acid precipitation as previously described (Ashcroft *et al.*, 1976).

In experiments to determine the kinetics of glucose-induced insulin and protein biosynthesis the following protocols were used. To assess the effect of increasing glucose concentration, batches of islets were preincubated for 30 min in 100 μ l of bicarbonate medium containing 2 mM-glucose plus albumin and [³H]leucine as above; 10 μ l of medium containing 2 mM- or 200 mM-glucose was then added and the radioactivity in (pro)insulin and total islet protein determined as described above after a further 0, 10, 30, 50, 70 and 90 min of incubation.

To follow the time course of cessation of a glucose stimulus, islets were preincubated for 60 min in 100 μ l of medium containing 20 mM-glucose, albumin and [³H]leucine. Then 10 μ l of medium, containing the same concentration of albumin and [³H]leucine and either 20 mM-glucose or 20 mM-glucose + 158 mM-mannoheptulose, was added. The radioactivity in (pro)insulin and total islet proteins was determined after a further 20, 40, 60 and 90 min incubation. This final concentration of mannoheptulose (14.3 mM) has been shown to inhibit glucose-stimulated insulin release rapidly (Hellman *et al.*, 1972).

Calculation and expression of results. In all experiments, the incorporation of [4,5-³H]leucine into (pro)insulin and total protein was determined in the presence of 20 mM-glucose. Rates of synthesis of (pro)insulin and protein under other experimental conditions were expressed as a percentage of that in the incorporation with 20 mM-glucose. The ratio of (pro)insulin to total protein synthesis was also calculated and the preferential stimulation of (pro)insulin biosynthesis was expressed by the value of this ratio as a fraction of its value in the presence of 20 mM-glucose in the same experiment; this parameter is the insulin index (Pipeleers *et al.*, 1973a). All results are given as mean $\pm$ s.e.m. for the number of batches of islets stated. The significance of difference from control incubations in the same experiment was assessed with Student's *t* test. No correction has been made to the results to take account of the possible effect of differing ratios of proinsulin to insulin which can affect the radioactivity in (pro)insulin because of the leucine in the connecting peptide. However, it can be calculated that a 100-fold change in the proinsulin/insulin ratio would cause a less than twofold change in the radioactivity in (pro)insulin: moreover, although the proinsulin/insulin ratio is not known for all the conditions tested here, it has been shown that

changes in the rates of biosynthesis of proinsulin over a range of glucose concentrations from zero to 16.7 mM occur without change in the proinsulin/insulin ratio (Malaisse *et al.*, 1974).

Results

Glucose-stimulated synthesis of (pro)insulin and islet

The effect of glucose on synthesis of (pro)insulin and total islet protein is shown in Fig. 1. Both parameters show a sigmoidal dependence on glucose concentration, with a threshold at about 2 mM-glucose and maximum rates at 10 mM-glucose. Over the whole series of experiments, the mean ratio of (pro)insulin to total protein synthesis was 0.212 ± 0.005 ($n = 275$) at 20 mM-glucose, 0.0591 ± 0.0049 ($n = 49$) at 2 mM-glucose and 0.046 ± 0.003 ($n = 159$) in the absence of glucose. These data are in close agreement with those of Permutt & Kipnis (1972), who reported values of 0.218 at 15.3 mM-glucose and 0.061 at 2.8 mM-glucose.

Specificity of stimulation of biosynthesis of (pro)insulin and total protein

The results are given in Tables 1 and 2. Table 1 shows the effects of structural analogues of glucose on

Fig. 1. Dependence on glucose concentration of rates of (pro)insulin and total protein synthesis

Rates of synthesis of (pro)insulin (○) and total protein (●), measured as described in the text, are expressed as a percentage of the rate found at 20 mM-glucose and are given as mean $\pm$ S.E.M. for 4–12 observations.

Table 1. Effect of glucose and glucose analogues on islet (pro)insulin and total protein synthesis

Batches of seven islets were incubated at 37°C for 90 min in Krebs bicarbonate medium containing albumin (2 mg/ml), L-[4,5-³H]leucine (20–40 μ Ci/ml) and the additions (20 mM except where stated otherwise) given in the Table. The incorporation of [³H]leucine into (pro)insulin and total islet protein were measured as described under 'Methods'. Rates of (pro)insulin and protein synthesis are expressed as percentage of the rates found with 20 mM-glucose in the same experiment. The ratio (pro)insulin/protein expresses the fraction of total protein synthesis represented by (pro)insulin synthesis under each condition: the insulin index is the value of this ratio for each condition as a fraction of its value with 20 mM-glucose in the same experiment. Results are given as mean $\pm$ S.E.M. for the number of batches of islets (n) stated. *Significantly greater than control (no addition) batches in the same experiment ($P \leq 0.01$).

Agent	<i>n</i>	(Pro)insulin synthesis	Protein synthesis	(Pro)insulin/Protein	Insulin index
None	159	10.4 $\pm$ 0.8	40.7 $\pm$ 1.7	0.046 $\pm$ 0.003	0.273 $\pm$ 0.078
Glucose	275	100*	100*	0.212 $\pm$ 0.005*	1*
<i>N</i> -Acetylglucosamine	55	54.9 $\pm$ 3.8*	62.4 $\pm$ 2.8*	0.223 $\pm$ 0.010*	0.797 $\pm$ 0.039*
<i>N</i> -Acetylmannosamine	4	5.6 $\pm$ 0.5	27.9 $\pm$ 3.4	0.043 $\pm$ 0.006	0.215 $\pm$ 0.029
Arabinose	3	2.3 $\pm$ 1.1	18.2 $\pm$ 2.7	0.038 $\pm$ 0.012	0.211 $\pm$ 0.005
2-Deoxyglucose	4	5.2 $\pm$ 1.1	19.5 $\pm$ 3.8	0.057 $\pm$ 0.010	0.287 $\pm$ 0.049
Dihydroxyacetone (10 mM)	22	20.1 $\pm$ 2.5*	41.9 $\pm$ 4.4	0.077 $\pm$ 0.009*	0.516 $\pm$ 0.042*
Erythrose	4	0.4 $\pm$ 0.3	6.6 $\pm$ 1.0	0.010 $\pm$ 0.006	0.061 $\pm$ 0.035
Fructose	23	4.1 $\pm$ 0.6	22.2 $\pm$ 1.9	0.036 $\pm$ 0.005	0.218 $\pm$ 0.037
Fucose	3	1.0 $\pm$ 0.4	20.5 $\pm$ 5.5	0.012 $\pm$ 0.002	0.068 $\pm$ 0.032
Galactose	4	4.7 $\pm$ 0.3	40.7 $\pm$ 5.7	0.036 $\pm$ 0.005	0.120 $\pm$ 0.074
Glucosamine	3	5.6 $\pm$ 1.0	37.6 $\pm$ 5.6	0.034 $\pm$ 0.001	0.186 $\pm$ 0.030
L-Glucose	4	1.4 $\pm$ 0.2	22.4 $\pm$ 1.5	0.022 $\pm$ 0.004	0.062 $\pm$ 0.010
Goldthiogluconate	4	3.6 $\pm$ 0.4	25.3 $\pm$ 3.5	0.053 $\pm$ 0.006	0.148 $\pm$ 0.018
Glyceraldehyde (1 mM)	16	15.9 $\pm$ 2.0	39.2 $\pm$ 3.2	0.047 $\pm$ 0.003	0.431 $\pm$ 0.035*
Hypoxanthine (10 mM)	4	9.2 $\pm$ 5.0	24.4 $\pm$ 3.7	0.049 $\pm$ 0.029	0.325 $\pm$ 0.114
Inosine (10 mM)	17	33.1 $\pm$ 3.7*	57.4 $\pm$ 5.1	0.151 $\pm$ 0.011*	0.742 $\pm$ 0.083*
Mannose	18	60.6 $\pm$ 5.0*	76.1 $\pm$ 4.8*	0.221 $\pm$ 0.014*	0.793 $\pm$ 0.049*
Ribose (10 mM)	3	6.8 $\pm$ 1.8	20.5 $\pm$ 1.6	0.048 $\pm$ 0.011	0.320 $\pm$ 0.065
Sorbitol	4	5.3 $\pm$ 2.5	28.1 $\pm$ 2.7	0.062 $\pm$ 0.027	0.177 $\pm$ 0.064
Sucrose	8	4.9 $\pm$ 0.7	29.9 $\pm$ 7.4	0.055 $\pm$ 0.016	0.276 $\pm$ 0.086
Talose	4	6.6 $\pm$ 1.4	50.3 $\pm$ 3.8	0.034 $\pm$ 0.007	0.130 $\pm$ 0.023

islet (pro)insulin and total protein biosynthesis: (pro)insulin biosynthesis was stimulated by 20mM-mannose and -*N*-acetylglucosamine, but not by 20mM-*N*-acetylmannosamine, -arabinose, -2-deoxyglucose, -erythrose, -fructose, -fucose, -galactose, -glucosamine, -L-glucose, -goldthioglucose, -ribose, -sorbitol, -sucrose or -talose. Lower concentrations of ribose were also ineffective (Table 2). (Pro)insulin biosynthesis was also stimulated by 10mM-dihydroxyacetone and inosine, but not by hypoxanthine (Table 1). Whereas glucose, mannose and *N*-acetylglucosamine stimulated both (pro)insulin and total protein synthesis, the stimulatory effects of dihydroxyacetone and inosine were specific for (pro)insulin synthesis.

Potentiation of (pro)insulin biosynthesis

Although without significant effect alone, 20mM-fructose potentiated insulin biosynthesis in the presence of 3mM-glucose, 5mM-mannose or 6mM-*N*-acetylglucosamine. Insulin synthesis in the presence of inosine (4 or 10mM) or dihydroxyacetone (5mM) was not potentiated by fructose (Table 3).

No significant potentiation of (pro)insulin biosynthesis by methylxanthines was found: thus 1mM-isobutylmethylxanthine did not increase (pro)insulin synthesis at 2 or 20mM-glucose, and 5mM-caffeine did not potentiate with 2, 5, 8 or 20mM-glucose (Table 4). (Pro)insulin biosynthesis stimulated by 20mM-glucose was not affected by 10 μ M-adenosine (Table 4).

Table 2. *Effect of ribose on islet (pro)insulin and total protein synthesis*
Details were as for Table 1.

Ribose	<i>n</i>	Proinsulin	Protein	(Pro)insulin	Insulin index
				Protein	
0	7	8.7 $\pm$ 1.3	40.2 $\pm$ 6.5	0.051 $\pm$ 0.013	0.228 $\pm$ 0.046
0.3	4	1.3 $\pm$ 0.7	21.4 $\pm$ 2.0	0.013 $\pm$ 0.007	0.072 $\pm$ 0.036
0.5	4	2.8 $\pm$ 1.1	29.7 $\pm$ 1.4	0.017 $\pm$ 0.006	0.086 $\pm$ 0.035
1	4	2.6 $\pm$ 1.0	26.6 $\pm$ 1.8	0.021 $\pm$ 0.008	0.096 $\pm$ 0.035
2.5	4	4.3 $\pm$ 0.8	28.0 $\pm$ 1.2	0.033 $\pm$ 0.007	0.147 $\pm$ 0.024
5	2	6.0 $\pm$ 0.4	37.6 $\pm$ 1.9	0.043 $\pm$ 0.005	0.168 $\pm$ 0.022
20	2	3.0 $\pm$ 0.9	36.9 $\pm$ 9.6	0.024 $\pm$ 0.013	0.095 $\pm$ 0.050

Table 3. *Effects of fructose on islet (pro)insulin and total protein synthesis*
Details were as for Table 1. *Significantly greater than other two conditions ($P \leq 0.01$).

Expt. no.	Incubation conditions	<i>n</i>	(Pro)insulin synthesis	Protein synthesis	Insulin index
1	20mM-Fructose	18	5.0 $\pm$ 0.7	27.7 $\pm$ 1.8	0.177 $\pm$ 0.020
	5mM-Mannose	20	9.0 $\pm$ 1.2	30.1 $\pm$ 2.3	0.234 $\pm$ 0.036
	20mM-Fructose+5mM-mannose	19	22.5 $\pm$ 2.7*	44.3 $\pm$ 3.8*	0.500 $\pm$ 0.078*
2	20mM-Fructose	4	7.1 $\pm$ 0.7	30.8 $\pm$ 1.6	0.235 $\pm$ 0.031
	3mM-Glucose	4	13.0 $\pm$ 2.4	47.0 $\pm$ 14.3	0.306 $\pm$ 0.049
	20mM-Fructose+3mM-glucose	4	34.3 $\pm$ 8.0*	44.2 $\pm$ 9.7	0.678 $\pm$ 0.104*
3	20mM-Fructose	8	19.4 $\pm$ 1.5	45.7 $\pm$ 6.0	0.458 $\pm$ 0.065
	5mM-Dihydroxyacetone	8	26.2 $\pm$ 3.5	57.0 $\pm$ 7.4	0.502 $\pm$ 0.089
	20mM-Fructose+5mM-dihydroxyacetone	8	29.6 $\pm$ 2.1	43.7 $\pm$ 9.8	0.824 $\pm$ 0.108
4	20mM-Fructose	4	25.6 $\pm$ 3.0	47.4 $\pm$ 8.6	0.531 $\pm$ 0.095
	4mM-Inosine	4	39.3 $\pm$ 11.2	54.3 $\pm$ 15.5	0.671 $\pm$ 0.013
	20mM-Fructose+4mM-inosine	4	41.5 $\pm$ 4.2	59.0 $\pm$ 6.6	0.665 $\pm$ 0.016
5	20mM-Fructose	4	31.0 $\pm$ 6.2	75.6 $\pm$ 21.6	0.485 $\pm$ 0.101
	10mM-Inosine	4	51.4 $\pm$ 3.5	72.3 $\pm$ 6.7	0.715 $\pm$ 0.039
	20mM-Fructose+10mM-inosine	4	44.5 $\pm$ 8.7	69.5 $\pm$ 20.8	0.688 $\pm$ 0.081
6	20mM-Fructose	4	17.3 $\pm$ 1.9	68.5 $\pm$ 10.3	0.263 $\pm$ 0.034
	6mM- <i>N</i> -Acetylglucosamine	4	25.7 $\pm$ 2.1	73.9 $\pm$ 5.7	0.373 $\pm$ 0.027
	20mM-Fructose+6mM- <i>N</i> -acetylglucosamine	4	42.2 $\pm$ 5.0*	79.1 $\pm$ 15.2	0.578 $\pm$ 0.087*
7	20mM-Fructose	4	25.1 $\pm$ 3.6	44.3 $\pm$ 3.8	0.586 $\pm$ 0.104
	9mM- <i>N</i> -Acetylglucosamine	4	81.8 $\pm$ 3.6	55.9 $\pm$ 2.5	1.482 $\pm$ 0.063
	20mM-Fructose+9mM- <i>N</i> -acetylglucosamine	3	93.3 $\pm$ 10.9	64.5 $\pm$ 1.4*	1.462 $\pm$ 0.169

Table 4. *Effects of methylxanthines and adenosine on glucose-stimulated (pro)insulin and protein biosynthesis*
Details were as for Table 1. *n* = 4 in all cases.

Incubation conditions		(Pro)insulin synthesis	Protein synthesis	Insulin index
Glucose concn. (mm)	Other addition			
2	0	4.4 ± 2.0	15.6 ± 2.7	0.188 ± 0.077
2	1 mm-isobutylmethyl- xanthine	3.6 ± 1.2	16.2 ± 3.3	0.138 ± 0.037
20	0	100	100	1
20	1 mm-isobutylmethyl- xanthine	114.0 ± 13.0	94.0 ± 9.0	1.271 ± 0.195
2	0	3.6 ± 0.8	17.2 ± 2.4	0.201 ± 0.018
2	5 mm-cafeine	2.8 ± 1.4	16.6 ± 2.5	0.144 ± 0.081
5	0	39.0 ± 10.0	59.0 ± 6.6	0.574 ± 0.134
5	5 mm-cafeine	18.0 ± 2.4	38.0 ± 2.8	0.413 ± 0.087
8	0	39.0 ± 8.6	50.0 ± 6.2	0.714 ± 0.172
8	5 mm-cafeine	59.0 ± 5.3	78.0 ± 11.4	0.751 ± 0.184
20	0	100	100	1
20	5 mm-cafeine	95.0 ± 13.0	110.0 ± 14.0	0.854 ± 0.046
20	0	100	100	1
20	10 μm-adenosine	86.4 ± 17.9	96.1 ± 11.4	0.904 ± 0.159

Table 5. *Effect of mannoheptulose on stimulation of (pro)insulin and protein biosynthesis*
Details were as for Table 1.

Incubation conditions			(Pro)insulin synthesis	Protein synthesis	Insulin index
Stimulant	Mannoheptulose concn. (mm)	<i>n</i>			
20mm-Glucose	0	4	100	100	1
	14.3	4	8.6 ± 2.0*	39.7 ± 8.4*	0.204 ± 0.018*
20mm-Mannose	0	10	57.0 ± 7.8	68.7 ± 6.7	0.834 ± 0.079
	14.3	10	9.8 ± 1.3*	33.0 ± 4.0*	0.354 ± 0.068*
10mm-Inosine	0	14	40.8 ± 4.6	69.3 ± 5.8	0.593 ± 0.048
	14.3	14	41.7 ± 5.1	60.9 ± 8.0	0.721 ± 0.055
10mm-Dihydroxy- acetone	0	12	16.0 ± 1.4	35.4 ± 4.5	0.530 ± 0.062
	14.3	12	13.8 ± 1.7	24.1 ± 2.3	0.627 ± 0.053
20mm- <i>N</i> -Acetylglucos- amine	0	4	41.8 ± 11.1	66.9 ± 8.2	0.616 ± 0.140
	14.3	4	47.2 ± 7.7	49.3 ± 5.7	0.964 ± 0.140

Inhibition of synthesis of (pro)insulin and total protein

The effects of mannoheptulose and phloretin on synthesis of (pro)insulin and total protein in the presence of glucose, mannose, *N*-acetylglucosamine, dihydroxyacetone or inosine are shown in Tables 5 and 6. Mannoheptulose blocked the stimulatory effects of glucose and mannose, but not the stimulation evoked by *N*-acetylglucosamine, dihydroxyacetone or inosine (Table 5).

Phloretin (0.18mm) had marked inhibitory effects on biosynthesis of both (pro)insulin and total protein in the presence of dihydroxyacetone, inosine, mannose and *N*-acetylglucosamine (but not glucose), but only in the presence of *N*-acetylglucosamine was

there a marked decrease in the insulin index, indicating specificity for (pro)insulin synthesis (Table 6). At 0.009mm, phloretin had no effects on (pro)insulin or total protein synthesis in the presence of glucose, mannose or dihydroxyacetone, but markedly inhibited *N*-acetylglucosamine-stimulated (pro)insulin biosynthesis specifically (Table 6). This concentration of phloretin also inhibited synthesis of (pro)insulin and total protein in the presence of inosine, but the insulin index was not significantly decreased.

Kinetics of changes in synthesis of (pro)insulin and total protein in islets in response to glucose stimulation

The rates at which biosyntheses of (pro)insulin and

Table 6. *Effects of phloretin on stimulation of (pro)insulin and protein synthesis*
Details were as for Table 1.

Incubation conditions			(Pro)insulin synthesis	Protein synthesis	(Pro)insulin	
Stimulant	Phloretin (μM)	<i>n</i>			Protein	Insulin index
Glucose (20mM)	—	8	100	100	0.279 ± 0.031	1
	180	8	147.0 ± 16	120.0 ± 10	0.335 ± 0.033	1.214 ± 0.045
Mannose (20mM)	—	4	74.0 ± 10.8	94.0 ± 7.9	0.247 ± 0.019	0.782 ± 0.073
	180	4	$52.7 \pm 6.1^*$	$54.1 \pm 2.8^*$	0.307 ± 0.016	0.972 ± 0.079
	9	4	75.6 ± 9.2	91.7 ± 8.4	0.254 ± 0.024	0.804 ± 0.084
Dihydroxy-acetone (10mM)	—	4	12.4 ± 1.2	31.8 ± 2.0	0.072 ± 0.010	0.399 ± 0.049
	180	4	$7.2 \pm 0.6^*$	$19.4 \pm 1.8^*$	0.068 ± 0.008	0.375 ± 0.040
	9	4	15.5 ± 0.8	27.7 ± 3.5	0.107 ± 0.016	0.591 ± 0.080
Inosine (10mM)	—	4	19.8 ± 4.6	53.6 ± 6.3	0.111 ± 0.019	0.376 ± 0.058
	180	4	$4.9 \pm 1.2^*$	$30.0 \pm 1.3^*$	0.070 ± 0.019	0.238 ± 0.056
	9	4	$7.6 \pm 1.5^*$	$36.2 \pm 3.1^*$	0.065 ± 0.009	0.219 ± 0.028
<i>N</i> -Acetylglucosamine (20mM)	—	4	53.6 ± 3.5	67.0 ± 3.5	0.288 ± 0.027	0.806 ± 0.066
	180	4	$1.4 \pm 0.3^*$	$8.9 \pm 0.8^*$	$0.072 \pm 0.010^*$	$0.201 \pm 0.024^*$
<i>N</i> -Acetylglucosamine (20mM)	—	3	21.7 ± 5.3	32.0 ± 5.7	0.203 ± 0.020	0.649 ± 0.068
	0.36	3	19.0 ± 2.5	34.3 ± 3.9	0.174 ± 0.007	0.556 ± 0.040
	1.8	3	19.3 ± 5.8	35.4 ± 8.4	0.166 ± 0.011	0.530 ± 0.045
	9	3	$3.9 \pm 0.5^*$	23.8 ± 4.3	$0.053 \pm 0.006^*$	$0.170 \pm 0.020^*$

Fig. 2. *Time-course of glucose-stimulated (pro)insulin biosynthesis*

Islets were preincubated with 2mM-glucose and [^3H]leucine for 30min: at zero time the glucose concentration was then elevated to 20mM and the incorporation of radioactivity into (pro)insulin and total protein was determined at the times shown as described in the text. At each time point the ratio (pro)insulin/total protein was calculated and the data expressed as the fraction of the value of this ratio at 90min in the same experiment. The values are the mean $\pm$ S.E.M. for 11–18 observations in six experiments.

total protein in islets were switched on by a rapid elevation of the extracellular glucose concentration were studied. Islets were first preincubated with [^3H]leucine for 30min at 2mM-glucose and the glucose concentration in the medium was then increased to 20mM. The data were calculated as the

Fig. 3. *Time course of inhibition by mannoheptulose of glucose-stimulated (pro)insulin biosynthesis*

Islets were preincubated for 60min with 20mM-glucose and [^3H]leucine: at time zero, mannoheptulose was added to one series (○) to a final concentration of 14.3mM. The incorporation of radioactivity into (pro)insulin and total protein was measured at the times shown as described in the text. At each time point the ratio (pro)insulin/total protein was calculated and expressed as a fraction of the value of this ratio at the 20min time point, i.e. after 80min total incubation time. The data are plotted as mean $\pm$ S.E.M. for three to five observations.

ratio of (pro)insulin to protein synthesis as a fraction of this ratio at 90min after the increase in glucose concentration. Fig. 2 shows that although a modest increase in this ratio was apparent after 10min at the higher glucose concentration, a lag of some 30min occurred before a maximum value was achieved. In further studies, rates of synthesis of (pro)insulin and total protein were measured at various times after addition of mannoheptulose to islets preincubated for 60min with high glucose. In control islets, the ratio of (pro)insulin to total protein synthesis remained constant, but in islets to which an inhibitory concentration of mannoheptulose was added, a lowering of this ratio was apparent after 20min and a further decrease occurred over the next 70min (Fig. 3).

Discussion

A number of fundamental questions about the control of (pro)insulin biosynthesis remain unanswered. Do the processes of insulin release and biosynthesis share a common glucose-sensor mechanism? Does this mechanism involve metabolism of glucose? Are there intracellular agents, e.g. cyclic AMP or Ca^{2+} , that modulate both processes? To what extent and by what mechanism is (pro)insulin biosynthesis stimulated specifically relative to other islet proteins? Are changes in rates of (pro)insulin biosynthesis of physiological importance? The present study has sought information on these points.

Glucose-sensor(s) for insulin release and biosynthesis

The studies reported here provide support for the view that insulin release and biosynthesis may share a common glucose-sensor mechanism. The specificity of the insulin secretory response to sugars has been reviewed elsewhere (Ashcroft, 1976); the present studies, using a wide range of glucose analogues, have found no sugar or sugar derivatives whose effects on the two processes are qualitatively different. The inability of L-glucose, fructose, galactose and ribose to stimulate (pro)insulin biosynthesis has been previously reported (Lin & Haist, 1969; Pipeleers *et al.*, 1973a; Ashcroft *et al.*, 1976). The stimulation of (pro)insulin biosynthesis by low concentrations of ribose that do not stimulate insulin release (Jain & Logothetopoulos, 1977) could not be confirmed in the present study. One apparent exception is *N*-acetylglucosamine, which was a strong stimulator of (pro)insulin biosynthesis in the absence of glucose, but whose stimulatory effect on insulin release has been reported to require the presence of a sub-stimulatory concentration of glucose (Ashcroft *et al.*, 1973). However, previous studies of stimulation of insulin release by *N*-acetylglucosamine have included caffeine in the incubation medium, and caffeine has been recently found (Williams & Ashcroft, 1978) to

have an unexpected inhibitory action on *N*-acetylglucosamine effects on rat islets; in the absence of caffeine, *N*-acetylglucosamine alone elicits a moderate but significant stimulation of insulin release. The potentiatory effect of fructose, previously shown for insulin release (Ashcroft *et al.*, 1973), is found here to occur also for biosynthesis. Although unable alone to stimulate insulin biosynthesis, 20mM-fructose increased (pro)insulin biosynthesis in the presence of glucose (3mM), mannose (5mM), or *N*-acetylglucosamine (6mM).

The specificity of mannoheptulose as an inhibitor uniquely of glucose and mannose effects on both biosynthesis and release of insulin also supports the concept of a common sensor mechanism. The major difference between the response of the two processes to glucose resides in the sensitivity to glucose concentration. Early studies suggested a similar threshold for glucose for both biosynthesis and release (Morris & Korner, 1970). However, in the present study, in agreement with Maldonado *et al.* (1977), we find a lower threshold for stimulation of biosynthesis by glucose than of release. We do not regard this observation as ruling out a common glucose-sensor, however, since the two processes might have different sensitivities to intracellular coupling factors transmitting the glucose signal into the appropriate cellular response. We conclude, therefore, that there is considerable evidence for a common identity of the glucose-sensor mechanism for both biosynthesis and release of insulin.

What is the nature of the β -cell glucose-sensor? Current evidence (reviewed by Ashcroft, 1976) supports the view that at least part of the effect of glucose on insulin release is mediated by changes in concentration of one or more β -cell metabolites or cofactors in response to changing rates of glucose metabolism. On this 'substrate-site' model (Randle *et al.*, 1968) the glucose receptor is the enzyme(s) catalysing the rate-limiting phosphorylation of glucose. The present results strongly suggest that a similar model may apply to glucose-stimulated insulin biosynthesis. Thus, of the sugars tested, only glucose, mannose and *N*-acetylglucosamine serve as good metabolic substrates for islets (Ashcroft *et al.*, 1973) and elicit insulin synthesis. Moreover dihydroxyacetone and inosine, shown here to stimulate insulin synthesis, have been shown to stimulate insulin release (Hellman *et al.*, 1974a; Capito & Hedeskov, 1976) and to be metabolized by islets (Hellman *et al.*, 1974a; Capito & Hedeskov, 1976), entering glycolysis via triose phosphate and ribose phosphate respectively. Mannoheptulose inhibits the metabolism of glucose and mannose (by inhibiting phosphorylation of the sugars; Ashcroft & Randle, 1970), but not of dihydroxyacetone, *N*-acetylglucosamine or inosine, in parallel with its effects on insulin biosynthesis. The unique sensitivity of *N*-acetylglucosamine-stimulated

insulin biosynthesis to phloretin correlates also with the effects of phloretin on *N*-acetylglucosamine uptake by isolated islets (Williams & Ashcroft, 1978). Higher concentrations of the drug inhibit the response of biosynthesis to other stimulators, perhaps reflecting general inhibition of transport processes. A model incorporating these data is given in Scheme 1.

Regulation of islet synthesis of (pro)insulin and protein

Insulin secretory responses are in general potentiated by agents which increase β -cell cyclic AMP (Hellman *et al.*, 1974b; Howell & Montague, 1973). However, our results do not support an important role for cyclic AMP in regulating islet synthesis of (pro)insulin or protein. Concentrations of caffeine or isobutylmethylxanthine which have been shown to increase islet cyclic AMP and augment stimulated insulin release (Cooper *et al.*, 1973) were without significant effect on synthesis of (pro)insulin or total protein. These results are at variance with some earlier studies: however, among those authors who

have found effects of phosphodiesterase inhibitors on glucose-stimulated (pro)insulin biosynthesis there have been certain disagreements. Thus Lin & Haist (1973) found maximum effect of caffeine on (pro)insulin biosynthesis at 10mM-glucose, whereas Malaisse *et al.* (1974) found potentiation only at glucose concentrations between 4 and 6mM. Maldonato *et al.* (1977) claimed to show some stimulation of (pro)insulin biosynthesis by theophylline: however, the effect was so small (a decrease of K_m for glucose from 4.8 to 4.2mM) that its physiological significance may be questioned. Bone & Howell (1977) found stimulation of insulin biosynthesis by isobutylmethylxanthine at high glucose concentrations: however, the effect on total protein synthesis was not reported, and therefore it is not clear whether a specific effect on insulin synthesis was occurring. The lack of effect of 10 μ M-adenosine on glucose-stimulated (pro)insulin biosynthesis also argues against a major role for cyclic AMP, since adenosine inhibits glucose-stimulated insulin release,

Scheme 1. *Substrate-site model for insulin biosynthesis*

The inhibitory actions ($\ominus$) of phloretin and mannoheptulose on insulin biosynthesis are suggested to be exerted on rate-limiting steps ($\Rightarrow$) namely, transport and phosphorylation respectively. Agents stimulating insulin biosynthesis are suggested to do so by being metabolized to a common product, X, which modulates the biosynthetic process.

an effect ascribed to inhibition of adenylate cyclase (Ismail *et al.*, 1977). Moreover, somatostatin, which has been shown to lower islet 3':5'-cyclic AMP concentration considerably (Efendic *et al.*, 1975; Bent-Hansen *et al.*, 1978), has no effect on (pro)insulin biosynthesis (Olsson *et al.*, 1976).

Since stimulation of (pro)insulin biosynthesis by glucose has been consistently shown to be associated with a general increase in islet protein synthesis, we have considered the possibility that specific stimulation of (pro)insulin biosynthesis might arise by a general stimulation of islet protein synthesis coupled with a more efficient transcription of (pro)insulin mRNA. However, we find that a general stimulation of islet protein synthesis does not accompany stimulation of (pro)insulin biosynthesis by dihydroxyacetone or inosine. These data suggest that those molecules resembling glucose chemically stimulate synthesis of both (pro)insulin and general islet protein, whereas those agents that may act by generating the same metabolic signal as glucose (dihydroxyacetone and inosine) are specific triggers to (pro)insulin biosynthesis. The mechanisms involved are unknown. From studies using actinomycin D, Permutt & Kipnis (1972) concluded that effects of glucose on (pro)insulin biosynthesis involved stimulation of both transcription and translation. Our studies on the kinetics of glucose stimulation of (pro)insulin biosynthesis are consistent with this conclusion. Thus a rapid (less than 10 min) increase in insulin index occurred on raising the glucose concentration from 2 to 20 mM, but the maximum increase in this parameter required at least 30 min. A similar lag was observed by Permutt & Kipnis (1972). We suggest that this delay in attaining maximal insulin index corresponds to mRNA synthesis. The finding that inhibition by mannoheptulose was apparent after 20 min may reflect the rate of mRNA degradation or control at the level of translation. It is unlikely that the rate of cleavage of preproinsulin to proinsulin is a significant determinant of the observed rate of (pro)insulin biosynthesis since this process is probably too rapid to be a rate-limiting step (Steiner *et al.*, 1974).

Physiological significance of glucose-stimulated (pro)insulin biosynthesis

The glucose-concentration dependence of (pro)insulin biosynthesis, contrasting markedly with that of insulin secretion, suggests that changes in blood glucose concentration over the physiological range may have only minor effects on (pro)insulin biosynthesis, which is already about 60% maximally stimulated by blood sugar concentrations in starvation (about 5.5 mM in the rat; Ashcroft *et al.*, 1976). The kinetics of glucose-stimulated (pro)insulin biosynthesis provide a possible rationalization of the

physiological requirements for such a concentration dependence: since (pro)insulin synthesis is switched on by glucose only slowly in comparison with insulin release, if biosynthesis had the same glucose concentration-dependence as release, periods of physiological hyperglycaemia might be too short to elicit sufficient increased (pro)insulin biosynthesis. This suggestion assumes that glucose concentration needs to remain elevated for the lag period of 30 min to stimulate biosynthesis of (pro)insulin: this assumption is open to experimental testing.

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