The Effect of *N*-Acylglucosamines on the Biosynthesis and Secretion of Insulin in the Rat

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The effects of *N*-acylglucosamines on insulin release have been studied. *N*-Acylglucosamines stimulated insulin release from rat islets *in vitro* only if a sub-stimulatory concentration of glucose was also present, and this secretory response was abolished by mannoheptulose. In perfused islets the rapidity of the secretory response to *N*-acetyl-D-glucosamine was similar to that observed with D-glucose. Increasing acyl-chain length from *N*-acetyl- to *N*-hexanoyl-D-glucosamine impaired the secretory response; however, *N*-dichloroacetyl-D-glucosamine was a more potent stimulator of release than was *N*-acetyl-D-glucosamine. Polymers of *N*-acetyl-D-glucosamine containing two to six monomers linked α 1–4 did not stimulate insulin release; glucosamine linked to dextran via a propionyl or hexanoyl spacer group was also without insulin-releasing ability. *N*-Acylglucosamines were also effective in eliciting insulin release *in vivo* when injected into conscious rats. At the dose used (86 μ mol), *N*-acetylglucosamine elicited a rapid rise in plasma-insulin concentration; *N*-butyrylglucosamine was less effective, and there was little or no response to *N*-hexanoylglucosamine. The response to *N*-dichloroacetylglucosamine was greater than that to *N*-acetylglucosamine; an increase in plasma insulin concentration could be elicited by *N*-dichloroacetylglucosamine at a dose (17 μ mol) at which neither glucose nor *N*-acetylglucosamine was effective. The secretory response to acetylglucosamine is not mediated by conversion into glucose. Rates of (pro)-insulin biosynthesis by rat islets have been measured. (Pro)-insulin biosynthesis was stimulated by glucose, and this response was abolished by mannoheptulose. *N*-Acetylglucosamine also stimulated (pro)-insulin biosynthesis; this effect of *N*-acetylglucosamine did not require the presence of glucose, and was not abolished by mannoheptulose. It is concluded that there are differences in signal reception and/or transduction for the processes of insulin biosynthesis and release.

N-Acetyl-D-glucosamine has been shown to elicit insulin release from isolated rat or mouse islets of Langerhans if glucose or another initiator of insulin release is also present (Ashcroft *et al.*, 1972, 1973); *N*-acetyl-D-glucosamine has also been shown to be a potent insulin-releasing agent in the rat *in vivo* (Ashcroft & Crossley, 1975). It has been suggested that the effect of *N*-acetyl-D-glucosamine is exerted via an interaction with the pancreatic β -cell glucoreceptor (Ashcroft *et al.*, 1973). The structural requirements for this stimulation of insulin release have now been examined by studying the insulin-releasing activity of acylglucosamines *in vitro* and *in vivo*. In addition, since effects of glucose on insulin release are accompanied by parallel changes in rates of insulin biosynthesis, we have investigated whether *N*-acetyl-D-glucosamine stimulates insulin biosynthesis.

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Experimental

Materials

Collagenase and bovine plasma albumin (fraction V) were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. D-Mannoheptulose was from Pfanstiehl Laboratories, Waukegan, IL, U.S.A. All radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. Other chemicals were supplied by BDH Ltd., Poole, Dorset, U.K. The glucosamine derivatives used were synthesized by methods described below. Polymers of *N*-acetyl-D-glucosamine were a gift from Dr. P. J. R. Phizackerley, Department of Clinical Biochemistry, University of Oxford. Freeze-dried anti-insulin serum (lot no. GP3) was from Miles-Yeda Ltd., Slough, Bucks., U.K.

Methods

Preparation of islets. Islets were prepared by a collagenase method (Collgarcia & Gill, 1969) from

3–4-week-old male rats that were starved overnight.

Batch incubation of islets for measurement of insulin release. Batches of five islets were incubated for 2 h at 37°C in 0.6 ml of bicarbonate medium (Krebs & Henseleit, 1932) containing caffeine (5 mM), albumin (1 mg/ml) and other additions as stated. After incubation the medium was separated by gentle centrifugation (position 2 on MSE Minor bench centrifuge for 1 min) and portions of the medium were diluted with phosphate/albumin buffer and stored at –20°C until assayed for insulin.

Perfusion of islets. Batches of 30–40 islets were perfused essentially as described by Cooper *et al.* (1973), except that the use of a constant-temperature enclosure obviated the need for a water jacket. The perfusion rate was 0.2 ml/min; portions of perfusate were diluted and stored at –20°C until assayed for insulin.

Insulin biosynthesis. Batches of 5–10 islets were incubated in 0.1 ml of bicarbonate medium containing albumin (2 mg/ml), 4 μ Ci of L-[4,5-³H]leucine (58 Ci/mmol) and other additions as stated. After incubation for 90 min at 37°C the tubes were chilled in ice. After centrifugation the medium was removed by aspiration and the islets washed once with 1 ml of bicarbonate medium containing albumin (2 mg/ml) and leucine (2 mM). Portions (250 μ l) of 0.1 M-borate buffer, pH 8, containing albumin (2 mg/ml) and 0.5 M-NaCl were added to each tube, and the islets were disrupted by sonication for 15 s [position 4 on a Soniprobe (Dawe Instruments)]. A further 250 μ l of the same buffer was added to each sample and portions were loaded on to insulin-binding affinity columns (see below). The columns were washed seven times with the same buffer and then the insulin was eluted by three 0.6 ml portions of 1 M-acetic acid containing albumin (3 mg/ml). Methoxyethanol scintillator (15 ml) was added to the acetic acid eluates and the insulin radioactivity counted in a liquid-scintillation spectrometer (Nuclear–Chicago Isocap).

The incorporation of [³H]leucine into islet total protein was determined by adding 50 μ l of the sonicated material to 150 μ l of borate buffer. Trichloroacetic acid (20%, w/v; 20 μ l) was then added. The precipitate was washed once with 10% (w/v) trichloroacetic acid, redissolved in 1 ml of borate buffer, and 0.7 ml was added to 15 ml of methoxyethanol scintillator for measurement of radioactivity. Rates of (pro)-insulin biosynthesis were calculated as a percentage of the rate of total protein synthesis in the presence of 16.7 mM-glucose in the same experiment. The preferential stimulation of (pro)-insulin biosynthesis as opposed to non-insulin proteins was quantified by the 'insulin index' (Pipeleers *et al.*, 1973); this parameter is obtained by dividing the ratio of (pro)-insulin synthesis to total protein synthesis under each experimental condition

by the value of this ratio in the presence of 16.7 mM-glucose in the same experiment.

Preparation of insulin-binding affinity columns. Freeze-dried anti-insulin serum (1 ml; insulin-binding capacity 0.72 unit) was reconstituted in 1 ml of water. The precipitate formed on addition of (NH₄)₂SO₄ to 40% satn. was dissolved in 1 ml of water and dialysed overnight against 2 $\times$ 500 ml of 0.9% NaCl. The dialysed anti-insulin globulins were made up to 15 ml with 0.1 M-NaHCO₃/0.5 M-NaCl solution and shaken with 10 ml of Sepharose 4B activated with CNBr as described in the manufacturer's instructions (Pharmacia, 1974). After coupling, the gel was collected on a glass sinter, washed with 10 ml of 1 M-ethanolamine, pH 8, and then left with a further 100 ml of ethanolamine for 1–2 h. The gel was transferred back to the sinter and washed with 5 $\times$ 100 ml of (i) 0.1 M-sodium acetate, pH 4, containing 1 M-NaCl, (ii) 0.1 M-sodium borate, pH 8.2, containing 1 M-NaCl. Finally the gel was washed with a little 0.1 M-borate without added NaCl and stored at 4°C with an equal volume of gel and buffer.

Columns were made from 1 ml disposable plastic syringes and the gel was retained with nylon net; gel beads (0.2 ml) were added to each column. Preliminary experiments showed that columns of volume 0.2 ml were capable of binding more than 77 munits of insulin. Columns could be re-used after runs if washed afterwards with, first, 1 M-acetic acid containing albumin (3 mg/ml) and then with 0.1 M-borate buffer containing albumin (2 mg/ml) and 0.5 M-NaCl. The use of Sepharose-coupled anti-insulin globulins in the selective extraction of insulin and pro-insulin has been described in detail by Crossley (1974).

Insulin release in vivo. Test sugars were injected into conscious rats via a femoral-vein cannula, and blood samples were subsequently removed from a femoral-artery cannula. Plasma separated by centrifugation was assayed immediately for insulin or stored at –20°C. The kinetics of the insulin-secretory response to glucose and other sugars in this experimental model have been described in detail by Ashcroft & Crossley (1975).

Radioimmunoassay of insulin. Plasma and incubation-media insulin concentrations were measured as previously described (Ashcroft & Crossley, 1975).

Preparation of glucosamine derivatives. *N*-Hexanoyl-D-glucosamine, *N*-monochloroacetyl-D-glucosamine, *N*-trichloroacetyl-D-glucosamine and *N*-2-chloropropionyl-D-glucosamine were prepared by a Schotten–Bauman reaction (Inouye *et al.*, 1956). *N*-Propionyl-D-glucosamine and *N*-butyryl-D-glucosamine were prepared by the method of Ruelius (1951). *N*-Dichloroacetyl-D-glucosamine was prepared by a method described by Shapiro *et al.* (1967).

Glucosamine was linked to Dextran T10 and T80 with aminohexanoic acid as a spacer, and to Dextran

T10 with β -aminopropionic acid as a spacer, by methods based on those described by the manufacturer (Pharmacia, 1974). Dextran (2 g in 120 ml of water) was added to 1 g of CNBr over 30 min, the pH being kept between 10.4 and 10.6. The pH was then allowed to fall to 9.0 and 1.5 g of aminohexanoic acid or aminopropionic acid was added. The mixture was stirred overnight. Unchanged spacer group was removed by dialysis. Glucosamine hydrochloride (1 μ g) was added and the pH was adjusted to 4.5. 1-Ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride (1 g) was added and the reaction allowed to proceed for 6 h. The mixture was dialysed and finally freeze-dried to a fluffy white powder. The amount of *N*-acyl-coupled glucosamine was de-

termined by the Elson-Morgan reaction (Morgan & Elson, 1934).

Results

Insulin release in vitro

The effect of glucosamine derivatives on rates of insulin release from isolated islets is shown in Table 1. Caffeine was present in these experiments to maximize secretory responses. *N*-Acetyl-D-glucosamine (20 mM) was ineffective in the absence of glucose but stimulated insulin release in the presence of a non-stimulating glucose concentration (3.3 mM). 5 mM-*N*-acetyl-D-glucosamine was ineffective. In the presence of 3.3 mM-glucose, *N*-monochloroacetyl-D-

Table 1. *Effects of glucosamine derivatives on insulin release from rat islets*

Batches of five islets were incubated for 2 h at 37°C in bicarbonate medium containing albumin (2 mg/ml) and caffeine (1 mM) with the additions shown. Insulin released into the medium was assayed as described in the Experimental section. The first three lines show the mean ($\pm$ S.E.M.) rates of insulin release in the absence of and in the presence of 3.3 mM- or 20 mM-glucose in these experiments. Each experiment included batches of islets under these conditions: the significance of the effects of the glucosamine derivatives tested was assessed by comparing rates of release in their presence with control rates at the same glucose concentration on the same islet preparation, by using Student's *t* test. N.S., not significant. The subscript numbers in column 1 indicate the number of polymerized units.

Compound	Concn. (mM)	Glucose concn. (mM)	Insulin release [μ units/min per islet (mean $\pm$ S.E.M.)]	No. of observations	Significance of difference from control
—	—	—	0.36 $\pm$ 0.09	30	—
—	—	3.3	0.37 $\pm$ 0.04	40	N.S.
—	—	20	5.82 $\pm$ 0.17	50	<i>P</i> < 0.001
<i>N</i> -Acetyl-D-glucosamine	20	—	0.37 $\pm$ 0.08	10	N.S.
	20	3.3	1.46 $\pm$ 0.23	10	<i>P</i> < 0.01
	5	3.3	0.20 $\pm$ 0.02	5	N.S.
<i>N</i> -Monochloroacetyl-D-glucosamine	20	3.3	1.53 $\pm$ 0.16	5	<i>P</i> < 0.001
<i>N</i> -Dichloroacetyl-D-glucosamine	20	3.3	4.59 $\pm$ 0.54	5	<i>P</i> < 0.001
	5	3.3	2.93 $\pm$ 0.52	5	<i>P</i> < 0.01
	1	3.3	0.55 $\pm$ 0.07	5	N.S.
<i>N</i> -Trichloroacetyl-D-glucosamine	20	3.3	1.09 $\pm$ 0.19	5	<i>P</i> < 0.05
<i>N</i> -Propionyl-D-glucosamine	20	—	0.37 $\pm$ 0.10	5	N.S.
	20	3.3	2.21 $\pm$ 0.14	10	<i>P</i> < 0.001
	5	3.3	0.98 $\pm$ 0.32	5	N.S.
	30	3.3	3.77 $\pm$ 0.33	5	<i>P</i> < 0.001
<i>N</i> -2-Chloropropionyl-D-glucosamine	20	—	0.49 $\pm$ 0.07	5	N.S.
	20	3.3	1.34 $\pm$ 0.11	5	<i>P</i> < 0.001
<i>N</i> -Hexanoyl-D-glucosamine	10	—	0.56 $\pm$ 0.13	5	N.S.
	20	3.3	0.84 $\pm$ 0.15	5	<i>P</i> < 0.001
(<i>N</i> -Acetyl-D-glucosamine) ₂	20*	3.3	0.34 $\pm$ 0.03	5	N.S.
(<i>N</i> -Acetyl-D-glucosamine) ₃	20*	3.3	0.36 $\pm$ 0.07	5	N.S.
(<i>N</i> -Acetyl-D-glucosamine) ₄	20*	3.3	0.33 $\pm$ 0.09	5	N.S.
(<i>N</i> -Acetyl-D-glucosamine) ₆	20*	3.3	0.46 $\pm$ 0.14	5	N.S.
Dextran 80-hexanoylglucosamine	20†	—	0.45 $\pm$ 0.05	5	N.S.
	20†	3.3	0.40 $\pm$ 0.05	5	N.S.
Dextran 10-hexanoylglucosamine	20†	—	0.13 $\pm$ 0.02	5	N.S.
	20†	3.3	0.08 $\pm$ 0.03	5	N.S.
Dextran 10-propionylglucosamine	20†	3.3	0.09 $\pm$ 0.03	5	N.S.
	20†	3.3	0.04 $\pm$ 0.01	5	<i>P</i> < 0.001

* Equivalent to 20 mM-*N*-acetyl-D-glucosamine monomer.

† Equivalent to 20 mM-glucosamine residues.

glucosamine and *N*-trichloroacetyl-D-glucosamine (20mM) were of similar potency to 20mM-*N*-acetyl-D-glucosamine. However, *N*-dichloroacetyl-D-glucosamine was a considerably more effective stimulator of insulin release: in the presence of 3.3 mM-glucose, 5mM-*N*-dichloroacetyl-D-glucosamine gave a greater stimulation of insulin release than 20mM-*N*-acetyl-D-glucosamine. *N*-Propionyl-D-glucosamine and 2-chloropropionyl-D-glucosamine stimulated insulin release in the presence of 3.3 mM-glucose but not in its absence. *N*-Hexanoyl-D-glucosamine (10mM) did not stimulate insulin release significantly in the presence or the absence of glucose, but a slight stimulation was observed with 20mM-*N*-hexanoyl-D-glucosamine in the presence of 3.3 mM-glucose.

None of the polymers of *N*-acetylglucosamine stimulated insulin release in the presence of 3.3 mM-glucose. The dextran-linked glucosamines were also ineffective.

The glucose-dependency of effects of acetylglucosamines on insulin release is further illustrated in Fig. 1, which shows a typical perfusion experiment with *N*-acetylglucosamine. In the presence of 3.3 mM-glucose, insulin release rises rapidly in response to 20mM-*N*-acetylglucosamine; the increased rate of

Fig. 1. Potentiation of insulin release by *N*-acetyl-D-glucosamine in perfused rat islets

Batches of 30 islets were perfused at 37°C with bicarbonate medium containing albumin (2 mg/ml) and caffeine (5 mM). The flow rate was about 0.2 ml/min. Collection of the perfusate at 2 min intervals was commenced after an equilibration period of 40 min. The composition of the perfusion medium was changed at time 50 min as follows: (a) 3.3 mM-glucose → 20 mM-glucose; (b) 3.3 mM-glucose → 3.3 mM-glucose + 20 mM-*N*-acetylglucosamine; (c) no added sugar → 20 mM-*N*-acetylglucosamine. Perfusate samples were diluted and assayed in duplicate for insulin as described in the Experimental section.

insulin release is sustained, although it is less than with an equimolar concentration of glucose. No stimulation is observed in the absence of glucose. Similar results are obtained with *N*-propionylglucosamine (S. J. H. Ashcroft & J. R. Crossley, unpublished work).

Insulin release in vivo

The characteristics of the insulin-secretory response to intravenous injection of *N*-acetylglucosamine have been described (Ashcroft & Crossley, 1975). These earlier studies showed that arterial-plasma insulin concentrations attain a maximum within 1 min after venous injection of *N*-acetylglucosamine and return to basal values within 40 min for doses within the range 0.17–1.4 mmol; the response is abolished by mannoheptulose and is not mediated by conversion of the *N*-acetylglucosamine into glucose. In view of the rapid clearance of *N*-acetylglucosamine from the blood (Ashcroft & Crossley, 1975) and the close reproducibility of the insulin responses of an animal to two consecutive injections of *N*-acetylglucosamine, the following experimental design was developed. Two blood samples were taken before injection of *N*-acetylglucosamine (86 μmol) and further samples were taken 1, 2.5, 5 and 10 min after injection. The animals were then left for 1–2 h and then a similar procedure was used with an equivalent amount of another acylglucosamine. This allowed verification of a secretory response to *N*-acetylglucosamine in each animal. No significant increases in amounts of blood glucose occurred during these experiments. The results are shown in Table 2. As previously reported (Ashcroft & Crossley, 1975), *N*-acetylglucosamine is a potent insulin-releasing agent *in vivo*. The present study demonstrates that other acylglucosamines share this ability, and the specificity is similar to that observed *in vitro* (see above). Thus the response to *N*-butyrylglucosamine was less marked than that to *N*-acetylglucosamine, and there was little or no response to this dose of *N*-hexanoylglucosamine. The response to *N*-dichloroacetylglucosamine was, however, greater than to *N*-acetylglucosamine. The potency of *N*-dichloroacetylglucosamine is further demonstrated by the results in Table 3; injection of 17 μmol of *N*-dichloroacetylglucosamine gave a consistent increase in plasma insulin concentration. This dose of glucose or *N*-acetylglucosamine was ineffective (results not shown).

Insulin biosynthesis

The incorporation of [³H]leucine into immunoreactive insulin will be referred to as (pro)-insulin biosynthesis, since no attempt was made to quantify the relative incorporation into insulin and pro-insulin. Table 4 shows the effects of glucose, *N*-acetyl-

Table 2. *Effects of N-acetyl-D-glucosamine on insulin release in vivo*

Two blood samples were taken from each of 21 rats before injection of 86 μ mol of *N*-acetyl-D-glucosamine. Further blood samples were taken 1, 2.5, 5 and 10 min after injection. The animals were left for 1–2 h and then received a second injection as detailed in the Table. Again, blood samples were taken before and after the second injection. Blood-glucose concentrations were essentially unchanged throughout these experiments. Results are given as means $\pm$ S.E.M. for the number of rats (*n*) given.

Time (min)	Plasma insulin concentration (μ units/ml)					
	... -10	0	1	2.5	5	10
First injection: 86 μ mol of <i>N</i> -acetyl-D-glucosamine (<i>n</i> = 21)	11.3 $\pm$ 1.9	16.7 $\pm$ 2.5	117 $\pm$ 17.4	40.2 $\pm$ 6.2	18.1 $\pm$ 2.5	18.6 $\pm$ 3.3
Second injection: 86 μ mol of						
(i) <i>N</i> -acetyl-D-glucosamine (<i>n</i> = 4)	17.8 $\pm$ 1.9	16.2 $\pm$ 2.8	179 $\pm$ 25.6	45.7 $\pm$ 8.4	17.5 $\pm$ 2.3	18.3 $\pm$ 4.3
(ii) D-glucose (<i>n</i> = 3)	10.2 $\pm$ 6.1	10.5 $\pm$ 5.5	85.0 $\pm$ 20.1	21.2 $\pm$ 4.2	8.9 $\pm$ 4.4	12.2 $\pm$ 6.9
(iii) <i>N</i> -dichloroacetyl-D-glucosamine (<i>n</i> = 2)	12.6 $\pm$ 2.6	14.3 $\pm$ 5.3	327 $\pm$ 38.8	297 $\pm$ 26.0	161 $\pm$ 18.5	55.8 $\pm$ 19.3
(iv) <i>N</i> -propionyl-D-glucosamine (<i>n</i> = 1)	24.5	38.6	303	108	81.0	35.0
(v) <i>N</i> -2-chloropropionyl-D-glucosamine (<i>n</i> = 1)	17.7	12.1	250	82.5	11.9	6.3
(vi) <i>N</i> -butyryl-D-glucosamine (<i>n</i> = 6)	18.0 $\pm$ 5.6	21.3 $\pm$ 6.3	35.0 $\pm$ 11.3	61.0 $\pm$ 19.4	32.2 $\pm$ 8.2	36.3 $\pm$ 13.2
(vii) <i>N</i> -hexanoyl-D-glucosamine (<i>n</i> = 4)	6.9 $\pm$ 5.9	5.3 $\pm$ 3.1	1.5 $\pm$ 1.5	16.3 $\pm$ 11.6	1.9 $\pm$ 1.9	7.4 $\pm$ 7.1

Table 3. *Effect of N-dichloroacetylglucosamine on insulin release in vivo*

The plasma insulin concentration after intravenous injection of 17 μ mol of *N*-dichloroacetyl-D-glucosamine was measured in three rats at the times shown. Blood glucose concentrations were constant throughout the experiments. Values for plasma insulin concentration less than 2 μ units/ml are recorded as '0'.

Time (min)	Plasma insulin concn. (μ units/ml)								
	... -10	0	1	2.5	5	10	20	40	60
Expt.									
1	4.9	8.3	122.4	46.3	14.5	17.7	19.0	11.3	9.5
2	0	2.2	52.9	15.4	3.1	5.5	7.4	0	0
3	9.7	9.2	262.9	91.1	22.9	4.6	4.5	1.6	14.0

glucosamine and mannoheptulose on rates of protein and (pro)-insulin biosynthesis in isolated rat islets. The rates of (pro)-insulin synthesis are expressed as a percentage of the rate of total protein synthesis at 16.7 mM-glucose in the same experiment. The results show that like D-glucose (but not L-glucose), *N*-acetyl-D-glucosamine produces a marked stimulation of insulin biosynthesis. This effect of *N*-acetyl-D-glucosamine does not require the presence of glucose. Mannoheptulose blocks the stimulation of insulin synthesis by glucose but not that elicited by *N*-acetyl-D-glucosamine. The effects of glucose and *N*-acetyl-D-glucosamine represent preferential stimulation of the synthesis of insulin as distinct from non-insulin proteins; in the absence of substrate the insulin index was 0.32 ± 0.05 ; this was increased by glucose to 1.00 and by *N*-acetylglucosamine to 1.17 ± 0.10 .

Discussion

Stimulation of insulin release by glucosamine derivatives

The present results show that a number of acyl-D-glucosamines share with *N*-acetyl-D-glucosamine the ability to elicit insulin release in the presence of a sub-stimulatory concentration of glucose. The kinetics of this potentiation *in vitro* have not been previously reported. Perfusion of rat islets (Fig. 1) demonstrates that the secretory response to 20 mM-*N*-acetylglucosamine or *N*-propionylglucosamine occurs as rapidly as that to 20 mM-glucose; however, the insulin-release rate attained was not as high as that with glucose. The insulin-secretory response to *N*-acetylglucosamine *in vivo* has previously been shown to be rapid (Ashcroft & Crossley, 1975); the present results demonstrate

Table 4. *Effects of N-acetyl-D-glucosamine, glucose and mannoheptulose on (pro)-insulin and protein biosynthesis in rat islets*

Batches of five islets were incubated at 37°C for 90 min in bicarbonate medium containing L-[4,5-³H]leucine (58 Ci/mol) and the additions shown. The islets were washed and disrupted by sonication. The incorporation of radioactivity into pro-insulin and insulin was measured by using an insulin-binding affinity column and incorporation into total protein by precipitation with trichloroacetic acid. Rates of (pro)-insulin biosynthesis are expressed as a percentage of the rate of total protein synthesis in the presence of 16.7 mM-glucose in the same experiment. The insulin index (ratio of (pro)-insulin to total protein synthesis) is expressed as a fraction of the mean control value (at 16.7 mM-glucose) taken as unity.

Incubation conditions	No. of observations	(Pro)-insulin synthesis (mean±S.E.M.)	Total protein synthesis (mean±S.E.M.)	Insulin index (mean±S.E.M.)	Significance of differences in insulin index from control value at a glucose concn. of 16.7 mM
No substrate	4	3.9±0.6	60.4± 8.5	0.32±0.05	<i>P</i> <0.001
3.3 mM-D-glucose	8	7.5±0.7	81.9±12.1	0.52±0.06	<i>P</i> <0.001
16.7 mM-D-glucose	13	20.0±1.4	100.0± 9.2	1.00±0.00	—
20 mM-N-acetyl-D-glucosamine	14	31.7±2.3	149.0±21.2	1.17±0.10	N.S.
20 mM-N-acetyl-D-glucosamine+3.3 mM-glucose	4	27.6±0.4	90.7± 9.9	1.52±0.21	N.S.
14.3 mM-D-mannoheptulose	6	3.7±0.6	58.0± 4.4	0.31±0.06	<i>P</i> <0.001
16.7 mM-D-glucose+14.3 mM-D-mannoheptulose	4	5.0±0.4	48.8± 2.4	0.38±0.07	<i>P</i> <0.001
20 mM-N-acetyl-D-glucosamine+14.3 mM-D-mannoheptulose	6	24.5±6.9	120.0±15.7	0.94±0.19	N.S.
20 mM-L-glucose	4	4.0±0.3	88.4±22.3	0.28±0.05	<i>P</i> <0.001

that other acylglucosamines have marked rapid effects on insulin release *in vivo*.

Elongation of the acyl chain appears to impair the ability of *N*-substituted glucosamines to elicit insulin release. Thus *N*-hexanoylglucosamine *in vitro* had only a small effect of insulin release (Table 1), and *in vivo* both *N*-butyrylglucosamine and *N*-hexanoylglucosamine were less effective than *N*-acetylglucosamine in increasing plasma insulin concentrations (Table 2). Chlorinated derivatives of *N*-acetylglucosamine were also tested. Although mono- and tri-chloroacetylglucosamine had similar insulin-releasing ability compared with *N*-acetylglucosamine, the dichloroacetylglucosamine was markedly a more potent stimulator. This was evident *in vitro* (Table 1), where 5 mM-dichloroacetylglucosamine was a more effective potentiator of release than was 20 mM-*N*-acetylglucosamine, and *in vivo* (Tables 2 and 3), where intravenous injection of as little as 5 mg of *N*-dichloroacetylglucosamine consistently elicited a rise in plasma insulin concentration. Unfortunately, although this was not the case with other acylglucosamines, *N*-dichloroacetylglucosamine proved toxic in larger amounts. Injection of 70 mg resulted in rapid death of the animals.

The mechanism of the effect of *N*-acetylglucosamines on insulin release has not been established, although we have suggested (Ashcroft *et al.*, 1973) that it involves combination with a potentiator site on the β -cell glucoreceptor. The present results provide further evidence on the specificity of this site; An important point is whether the *N*-acylglucosamines

have to enter the β -cell and be metabolized before they elicit release. For this reason, in the present study we tested the effect of high-molecular-weight derivatives of glucosamine, which are unlikely to be able to enter the β -cells. However, neither the dextran-linked glucosamines nor the α 1-4-linked polymers of *N*-acetylglucosamine were effective in causing insulin release (Table 1). Although clearly negative results of this kind cannot be regarded as conclusive evidence that entry of acylglucosamines into the β -cell is required for insulin release to be elicited, they are at least consistent with this possibility. In this connexion it may be relevant that the acylglucosamines that were able to elicit insulin release are all substrates for an *N*-acetylglucosamine kinase prepared from rat liver, and their affinity for this enzyme decreases with increase in the length of the acyl side chain (S. J. H. Ashcroft & J. R. Crossley, unpublished observations). Preliminary experiments also demonstrate the existence of such an enzyme in extracts of rat islets, although technical difficulties have so far precluded characterization of its substrate specificity (S. J. H. Ashcroft & J. R. Crossley, unpublished observations). The ability of islets to metabolize *N*-acetylglucosamine has been reported (Ashcroft *et al.*, 1973).

Effect of N-acetylglucosamine on insulin biosynthesis

Consistent with previous studies (Lin & Haist, 1969; Morris & Korner, 1970; Pipeleers *et al.*, 1973), our data show that glucose is a potent and specific

stimulator of insulin biosynthesis. From a comparison of the ability of various carbohydrates to stimulate or inhibit insulin biosynthesis with their effects on calcium uptake and subsequent insulin release by the β -cell, Pipeleers *et al.* (1973) conclude that both the biosynthetic and secretory processes are dependent on the same glucose-sensing device. The inhibition of glucose-stimulated insulin biosynthesis by mannoheptulose is good evidence for this postulate. Such a joint control does not of course imply that the two processes are obligatorily coupled; that this is not so is shown by the inhibition of release but not biosynthesis in the absence of extracellular Ca^{2+} (Pipeleers *et al.*, 1973; Lin & Haist, 1973) and by the inhibition of biosynthesis by cycloheximide without profoundly altering release (Morris & Korner, 1970). The present results, however, have shown that parallel effects of carbohydrates on insulin biosynthesis and release are not always observed (Table 4). Thus whereas the secretory response to *N*-acetyl-D-glucosamine requires the simultaneous presence of glucose, insulin biosynthesis is stimulated by *N*-acetylglucosamine in the absence of glucose, and is not further stimulated by the addition of 3.3 mM-glucose. Moreover, although mannoheptulose abolished the effect of *N*-acetylglucosamine-plus-glucose on insulin release, *N*-acetylglucosamine-stimulated insulin biosynthesis was not decreased by mannoheptulose. Such a dichotomy between release and biosynthesis might indicate that the two processes differ at the level of signal reception. This would imply a model with a biosynthesis glucoreceptor distinct from the release glucoreceptor. The former would have a mannoheptulose-sensitive glucose site and a separate *N*-acetylglucosamine site not affected by mannoheptulose; the release glucoreceptor would require an initiator site for glucose and a potentiator site for *N*-acetylglucosamine. In the absence of firm evidence for it, such a model, although plausible, is unattractively complex.

An alternative would be to preserve the same glucoreceptor for biosynthesis and release but to postulate that the interaction of glucose with the β -cell either as a regulator of a membrane-bound glucoreceptor or as a substitute for metabolic transfor-

mation leads to the production of two distinct intracellular signals (S1 and S2). Signal S1 is sufficient to trigger insulin biosynthesis but both S1 and S2 signals are required for insulin release. *N*-acetylglucosamine, either directly or via its metabolism, can cause the production of signal S1 and then elicit biosynthesis, but glucose is required to produce signal S2, in order for *N*-acetylglucosamine to stimulate insulin secretion. A possible role for signal S2 would be to modulate the β -cell Ca^{2+} metabolism.

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