Metabolism and Insulin-Releasing Capabilities of Glucosamine and *N*-Acetylglucosamine in Isolated Rat Islets

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The ability of glucosamine and *N*-acetylglucosamine to stimulate insulin secretion from perfused rat islets and the suitability of these hexoses to be metabolized in a static incubation was studied under various conditions. *N*-Acetylglucosamine alone stimulated insulin release with a threshold of 10 mM, with half-maximal effect at approx. 16 mM, and maximally at 20 mM. With higher concentrations stimulation was slightly diminished. Release caused by 20 mM-*N*-acetylglucosamine was unaffected by 30 mM-mannoheptulose, but was blocked by 2-deoxyglucose or iodoacetate (1 mM). At moderate concentrations (2.75–20 mM), the metabolism of *N*-acetyl[1-³H]glucosamine was similar to that of [1-³H]glucose and secretion rates paralleled the corresponding rates of metabolism with these hexoses. Glucosamine (27.5 mM) alone weakly stimulated insulin secretion, which was unaltered by 30 mM-mannoheptulose but blocked by 2-deoxyglucose or iodoacetate. A lower rate of [1-³H]glucosamine metabolism appeared to account for its weaker stimulatory efficacy. Insulin release caused by 27.5 mM-glucosamine or 27.5 mM-*N*-acetylglucosamine in the presence of basal (2.75 mM) glucose was accurately predicted based on the summed metabolic rates of these compounds. The data strengthen the theory proposing that metabolites or cofactors generated during metabolism are essential for triggering insulin secretion.

N-Acetylglucosamine is a potent stimulator of insulin secretion both *in vivo* and *in vitro* (Lambert *et al.*, 1969; Ashcroft & Crossley, 1975; Ashcroft *et al.*, 1976). The non-acetylated parent compound glucosamine also possesses the ability to stimulate secretion by itself, but it is most effective in the presence of basal glucose or theophylline (Coore & Randle, 1964; Lambert *et al.*, 1969; Landgraf *et al.*, 1971; Ashcroft *et al.*, 1972; Ashcroft & Crossley, 1975). Glucosamine also inhibits glucose-induced insulin release (Coore & Randle, 1964; Lambert *et al.*, 1969). Because of the lack of detailed dose-response studies relating the metabolism of glucosamine with its insulin-releasing capabilities, it remains an unsettled issue as to how this compound stimulates secretion. It has been proposed that the metabolism of glucosamine and *N*-acetylglucosamine, or alternatively their ability to interact with a β -cell receptor system, or both, accounts for their stimulatory efficacy (Landgraf *et al.*, 1971; Ashcroft *et al.*, 1973; Williams & Ashcroft, 1978).

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We have reported a method by which the metabolism of various sugars could conveniently be studied after testing their ability to provoke secretion (Zawalich & Matschinsky, 1977; Zawalich *et al.*, 1977a,b). This method has now been applied to a detailed study of *N*-acetylglucosamine and glucosamine action in isolated islets. The ability of basal glucose and of various metabolic blockers to alter the secretory potency and the metabolism of these compounds was also determined. The results strengthen the theory which incorporates the metabolism of sugars or sugar derivatives as an essential process leading to insulin secretion.

Experimental

Materials

The radioactive compounds D-[1-³H]glucose, D-[5-³H]glucose, D-[1-³H]glucosamine and *N*-acetyl-D-[1-³H]glucosamine were purchased from Amersham-Searle, Arlington Heights, IL, U.S.A. D-Glucosamine, *N*-acetyl-D-glucosamine, D-mannoheptulose, 2-deoxy-D-glucose and iodoacetate (sodium salt) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. D-Glucose was obtained from Mallinckrodt, St. Louis, MO, U.S.A. Collagenase (type IV) was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A.

Measurement of insulin release and metabolism

Fed male Holtzman rats (Holtzman Rat Co., Madison, WI, U.S.A.) weighing 300–400 g were used in all studies. The animals were anaesthetized with 20 mg of Nembutal and islets were isolated by the method of Lacy & Kostianovsky (1967). The detailed methodology for studying the kinetics of insulin release and the composition of the perfusion medium has been published (Zawalich & Matschinsky, 1977; Zawalich *et al.*, 1977a,b). Usually four groups of 50 islets were isolated from the combined pancreases of two rats. Each group was treated separately and constituted an experiment. After loading of the islets into the perfusion chamber, the islets were perfused at a flow rate of 1 ml/min. Insulin released into the medium was assayed in duplicate by radioimmunoassay with human insulin as standards. Figs. 1 and 3 were corrected for the dead space in the perfusion system, which amounted to 3 ml. Since the flow rate was 1 ml/min, this dead space resulted in a 3 min delay of the islets' secretory response to the various stimulants.

In order to measure the capacity of the islets to metabolize the $1\text{-}^3\text{H}$ -labelled derivatives of glucose, glucosamine and *N*-acetylglucosamine, 4–5 μl of tritiated stock solution (initially 4, 7.3 or 3.0 Ci/mmol for the glucose, glucosamine or *N*-acetylglucosamine respectively) was placed in a 10 mm $\times$ 75 mm glass tube and desiccated overnight. (These $1\text{-}^3\text{H}$ -labelled derivatives were used because of the commercial unavailability of derivatives by which total usage could be most accurately assessed.) To this was added 300 μl of the perfusion fluid at the desired sugar concentration. The islets were incubated, after the perfusion, in 125 μl of this solution. In the present experiments, the metabolism of *N*-acetylglucosamine or glucose was measured for 20 min and

that of glucosamine for 30 min unless otherwise indicated. Metabolic rates are expressed as pmol of $^3\text{H}_2\text{O}$ released from the appropriate derivative/h per islet. In the series of experiments involving mannoheptulose or 2-deoxyglucose, these inhibitors were present during the final 20 min of the perfusion and during the entire metabolic phase of the experiment.

In the iodoacetate experiments, the islets were washed with 3 ml of fresh perfusion fluid after termination of the perfusion phase of the protocol and hexose metabolism was measured in the absence of iodoacetate. Significance of differences was examined by Student's *t* test and *P* values less than 0.05 were taken as significant.

Results

Insulin release

N-Acetylglucosamine was a potent stimulator of insulin secretion (Figs. 1a, 1b and 2). Release exceeded basal secretion rates at concentrations of 10 mM and above, was half-maximal at 15–17 mM, and maximal at approx. 20 mM. The secretory rate fell at higher concentrations. The latency of the response was about 5 min with 10 mM-*N*-acetylglucosamine and decreased with increasing concentrations. Glucosamine, after transient suppression of basal insulin secretion, accelerated release at 20, 27.5 or 40 mM (Figs. 1c and 2). At most concentrations tested, the release rate fell at the end of the stimulatory period. Sigmoidal relationships resulted when insulin secretion (the final perfusate sample collected from minutes 45–50 of the perfusion) was plotted against the concentrations (mM) of the two compounds (Fig. 2).

Fig. 1. Insulin release in response to various concentrations of *N*-acetylglucosamine (a, b) or glucosamine (c). Islets were perfused for 30 min in the absence of added substrate and for an additional 20 min at the indicated stimulant concentration. Means, $\pm$ S.E.M. of the last perfusate sample (indicated by bars), of at least four different experiments are given. In plotting the insulin-release profiles, correction is made for the dead space in the perfusion system, which resulted in a 3 min delay of the secretory response. The vertical line indicates the onset of stimulation.

Fig. 2. Concentration-dependence of hexosamine-induced insulin release

Secretion rates calculated from the insulin content of the final perfusate sample (collected from 45 to 50 min) are plotted against concentration (mm) of the stimulant (●, *N*-acetylglucosamine; ▲, glucosamine). An insulin-release value of 20 μ units/min per 100 islets was subtracted from all values shown in Fig. 1 to account for the basal secretion in the absence of any added stimulant.

To characterize further the responsiveness of islet tissue to *N*-acetylglucosamine and glucosamine, experiments were conducted to test the effects of mannoheptulose and basal glucose on these stimulatory compounds. At a concentration which completely suppresses glucose-stimulated secretion (Zawalich *et al.*, 1977a), mannoheptulose did not affect release provoked by 20 mM-*N*-acetylglucosamine (Fig. 3a). Basal glucose (2.75 mM), which by itself has no stimulatory efficacy, significantly ($P < 0.05$) augmented insulin secretion caused by 27.5 mM-*N*-acetylglucosamine (Fig. 3b). Peak release (mean $\pm$ S.E.M.) in the final perfusate sample averaged 216 ± 10 and 156 ± 7 μ units/min per 100 islets in the presence and absence of basal glucose respectively. Mannoheptulose (30 mM) was without effect on the release stimulated by 27.5 mM-glucosamine. Basal glucose significantly ($P < 0.05$) elevated the secretory rate (Fig. 3c).

2-Deoxy-D-glucose, at a 4:1 molar ratio of the deoxy sugar to hexosamine, completely suppressed secretion caused by 15 mM-*N*-acetylglucosamine or 20 mM-glucosamine (results not shown). These high concentrations of 2-deoxyglucose had no adverse effect on release stimulated by 10 mM-4-methyl-2-oxopentanoate (Zawalich *et al.*, 1978). Iodoacetate, (1 mM), present during the entire 30 min prestimulatory period and the 20 min stimulatory period, completely blocked secretion caused by 20 mM-*N*-acetylglucosamine or 27.5 mM-glucosamine (results not shown).

Fig. 3. Mannoheptulose (MH) resistance and glucose (Glc) potentiation of *N*-acetylglucosamine (GlcNAc)- and glucosamine (GlcN)-induced insulin release

Islets were perfused for 30 min in the absence of added substrate and for an additional 20 min with the test substances. At least four different experiments were performed under each condition. The concentrations, given as subscript, are millimolar. The vertical line indicates the onset of stimulation. (a) *N*-Acetylglucosamine (●—●), *N*-acetylglucosamine + mannoheptulose (●---●); (b) *N*-acetylglucosamine (●), *N*-acetylglucosamine + glucose (■); (c) glucosamine (▲—▲), glucosamine + mannoheptulose (▲---▲), glucosamine + glucose (□).

Metabolism of *N*-acetylglucosamine and glucosamine in islet tissue

After termination of the perfusion, the ability of the islets to metabolize the sugar derivatives was examined. The amount of $^3\text{H}_2\text{O}$ released from specifically labelled derivatives served as the indicator of catabolism. Dose-response experiments with $[1\text{-}^3\text{H}]\text{glucose}$ were also performed to allow comparison with the corresponding data obtained with *N*-acetyl $[1\text{-}^3\text{H}]\text{glucosamine}$ and $[1\text{-}^3\text{H}]\text{glucosamine}$. In addition, the amount of $^3\text{H}_2\text{O}$ released from $[1\text{-}^3\text{H}]\text{glucose}$ was compared with that liberated from $[5\text{-}^3\text{H}]\text{glucose}$. $^3\text{H}_2\text{O}$ formation from $[1\text{-}^3\text{H}]\text{glucose}$ is less than that determined with $[5\text{-}^3\text{H}]\text{glucose}$, particularly at the lower concentrations tested (Table 1). The time course of metabolism of the $[1\text{-}^3\text{H}]$ - and $[5\text{-}^3\text{H}]\text{glucose}$ derivatives was also determined. For these studies, a final concentration of 2.75 mM-glucose was used. As shown in Fig. 4, the metabolism of the two derivatives is linear with time. Based on this graph and assuming that *N*-acetyl $[1\text{-}^3\text{H}]\text{glucosamine}$ and $[1\text{-}^3\text{H}]\text{glucosamine}$ are handled similarly to $[1\text{-}^3\text{H}]\text{glucose}$, the ability of the islets to catabolize the hexosamines was measured for 20 or 30 min. The possible time-dependent detrimental effects of these hexosamines, particularly glucosamine, on islet tissue also prompted the use of these shorter incubation periods. The results are ultimately presented as pmol of $^3\text{H}_2\text{O}$ released/h per islet.

As the *N*-acetylglucosamine concentration was increased from 2.75 to 20 mM, $^3\text{H}_2\text{O}$ formation measured from *N*-acetyl $[1\text{-}^3\text{H}]\text{glucosamine}$ increased approx. 7-fold, from 4.7 to 34 pmol/h per islet (Table 2). At higher concentrations of substrate the rate decreased. Mannoheptulose (30 mM) slightly but insignificantly decreased the metabolism of

Fig. 4. Islet catabolism of $[1\text{-}^3\text{H}]$ - or $[5\text{-}^3\text{H}]\text{glucose}$. These islets were initially perfused for 30 min without added substrate and for an additional 20 min with 2.75 mM-glucose. The islets were then removed for the metabolic analysis. $\circ$ — $\circ$, $[1\text{-}^3\text{H}]\text{Glucose}$; $\circ$ --- $\circ$, $[5\text{-}^3\text{H}]\text{glucose}$.

2.75 mM-*N*-acetylglucosamine, but had no effect on the degradation of 20 mM-*N*-acetylglucosamine. 2-Deoxyglucose or iodoacetate markedly impaired the ability of the islets to catabolize *N*-acetylglucosamine (Table 2).

Glucosamine was also metabolized by the enzymic machinery of islet tissue. Although apparently not as suitable a substrate as *N*-acetylglucosamine, $^3\text{H}_2\text{O}$ formation from $[1\text{-}^3\text{H}]\text{glucosamine}$ increased 3-fold as the concentration was increased from 2.75 to 40 mM (Table 2). Iodoacetate and 2-deoxyglucose, but not mannoheptulose, significantly blocked glucosamine metabolism (Table 2). This concentration of mannoheptulose slightly but significantly impaired basal glucose catabolism (Table 3). *N*-Acetylglucosamine or glucosamine significantly altered the metabolism of each other. Yields of $^3\text{H}_2\text{O}$ measured in a 10-fold excess of the non-labelled derivative were significantly lower than the appropriate control (Table 3).

Comparison of substrate and insulin-releasing properties of hexoses

Experiments were conducted to determine if the ability of basal glucose to potentiate *N*-acetylglucosamine- or glucosamine-stimulated insulin secretion could be explained by the summated metabolic rates of these substances. To this end, the metabolism of $[1\text{-}^3\text{H}]\text{glucose}$ (2.75 mM) was measured in the presence of 27.5 mM of each com-

Table 1. Comparison of $^3\text{H}_2\text{O}$ released from $[1\text{-}^3\text{H}]$ - or $[5\text{-}^3\text{H}]\text{glucose}$

After termination of the perfusion, $^3\text{H}_2\text{O}$ formation from the appropriate $[^3\text{H}]\text{glucose}$ derivative was measured for 20 min, except at 27.5 or 40 mM, at which concentrations glucose metabolism was measured for 30 min. The results are expressed as pmol of $^3\text{H}_2\text{O}$ released/h per islet. Results are means $\pm$ S.E.M. of at least four experiments.

Concn. (mM)	$^3\text{H}_2\text{O}$ released (pmol/per islet)	
	$[1\text{-}^3\text{H}]\text{Glucose}$	$[5\text{-}^3\text{H}]\text{Glucose}$
2.75	7.4 $\pm$ 0.4	22.0 $\pm$ 1.4
4.0	11.9 $\pm$ 1.5	30.1 $\pm$ 1.9
5.5	24.5 $\pm$ 2.0	50.5 $\pm$ 4.0
10.0	39.5 $\pm$ 4.0	75.0 $\pm$ 3.5
27.5	68.0 $\pm$ 10.0	107.0 $\pm$ 9.0
40.0	70.0 $\pm$ 9.0	110.0 $\pm$ 10.0

Table 2. *Metabolism of N-acetylglucosamine and glucosamine by isolated rat islets*

After perfusion with the indicated stimulant or stimulant and inhibitor, metabolism was subsequently measured for 20min with *N*-acetylglucosamine (GlcNAc) or 30min with glucosamine (GlcN). In the experiments with mannoheptulose and 2-deoxyglucose these inhibitors were present during the entire metabolic phase. In the iodoacetate experiments, the compound was removed after termination of the perfusion by washing the islets with 3.0ml of fresh perfusion fluid. Results are means $\pm$ S.E.M. for the specified numbers of observations. Abbreviation: N.S., not significant.

Stimulant	No. of observations	Stimulant concn. (mM)	Inhibitor	Inhibitor concn. (mM)	$^3\text{H}_2\text{O}$ released (pmol/h per islet)	
1. GlcNAc	4	2.75			4.7 ± 0.3	
2. GlcNAc	6	2.75	Mannoheptulose	30	4.1 ± 0.4	2 vs. 1, N.S.
3. GlcNAc	4	5.0			5.9 ± 0.4	
4. GlcNAc	4	10			14.0 ± 1.0	
5. GlcNAc	4	15			18.0 ± 0.7	
6. GlcNAc	4	15	2-Deoxyglucose	60	3.8 ± 1.2	6 vs. 5, $P < 0.05$
7. GlcNAc	8	20			34.4 ± 0.9	
8. GlcNAc	8	20	Mannoheptulose	30	33.3 ± 1.5	8 vs. 7, N.S.
9. GlcNAc	4	20			0	
10. GlcNAc	4	27.5	Iodoacetate	1.0	27.0 ± 2.8	
11. GlcNAc	4	40			20.8 ± 4.0	
12. GlcN	4	2.75			6.9 ± 0.6	
13. GlcN	6	2.75	Mannoheptulose	30	5.7 ± 0.9	13 vs. 12, N.S.
14. GlcN	4	10			11.0 ± 2.5	
15. GlcN	4	20			14.2 ± 2.0	
16. GlcN	4	20	2-Deoxyglucose	80	7.0 ± 1.5	16 vs. 15, $P < 0.05$
17. GlcN	4	27.5			19.5 ± 1.8	
18. GlcN	4	27.5	Mannoheptulose	30	20.8 ± 1.5	18 vs. 17, N.S.
19. GlcN	4	27.5			0	
20. GlcN	4	40.0	Iodoacetate	1.0	21.5 ± 2.9	19 vs. 17, $P < 0.05$

Table 3. *Effects of various compounds on $^3\text{H}_2\text{O}$ released from $[1\text{-}^3\text{H}]\text{glucose}$, $N\text{-acetyl}[1\text{-}^3\text{H}]\text{glucosamine}$ ($[1\text{-}^3\text{H}]\text{GlcNAc}$) or $[1\text{-}^3\text{H}]\text{glucosamine}$ ($[1\text{-}^3\text{H}]\text{GlcN}$) by isolated islets*

Glucose or *N*-acetylglucosamine metabolism was measured for 20min. Glucosamine metabolism was measured for 30min. Results are ultimately expressed as pmol of $^3\text{H}_2\text{O}$ released/h per islet. For easier analysis of the data, control data from Table 2 are also shown. At least four experiments were performed under each condition. Results are means $\pm$ S.E.M. Abbreviation: N.S., not significant.

Test sugar	Test sugar concn. (mM)	Competing sugar	Competing sugar concn. (mM)	$^3\text{H}_2\text{O}$ released (pmol/h per islet)	
1. $[1\text{-}^3\text{H}]\text{Glucose}$	2.75	—	—	7.4 ± 0.4	
2. $[1\text{-}^3\text{H}]\text{Glucose}$	2.75	GlcNAc	27.5	6.9 ± 0.3	2 vs. 1, N.S.
3. $[1\text{-}^3\text{H}]\text{Glucose}$	2.75	GlcN	27.5	3.7 ± 0.2	3 vs. 1, $P < 0.05$
4. $[1\text{-}^3\text{H}]\text{Glucose}$	2.75	Mannoheptulose	30	5.0 ± 0.5	4 vs. 1, $P < 0.05$
5. $[1\text{-}^3\text{H}]\text{GlcNAc}$	2.75	—	—	4.7 ± 0.3	
6. $[1\text{-}^3\text{H}]\text{GlcNAc}$	2.75	GlcN	27.5	2.3 ± 0.8	6 vs. 5, $P < 0.05$
7. $[1\text{-}^3\text{H}]\text{GlcNAc}$	27.5	—	—	27.0 ± 2.8	
8. $[1\text{-}^3\text{H}]\text{GlcNAc}$	27.5	Glucose	2.75	28.5 ± 1.5	8 vs. 7, N.S.
9. $[1\text{-}^3\text{H}]\text{GlcN}$	2.75	—	—	6.9 ± 0.6	
10. $[1\text{-}^3\text{H}]\text{GlcN}$	2.75	GlcNAc	27.5	4.4 ± 0.4	10 vs. 9, $P < 0.05$
11. $[1\text{-}^3\text{H}]\text{GlcN}$	27.5	—	—	19.5 ± 1.8	
12. $[1\text{-}^3\text{H}]\text{GlcN}$	27.5	Glucose	2.75	20.7 ± 0.8	12 vs. 11, N. S.

pound. Also, the effect of this basal glucose concentration on the metabolism of 27.5mM-*N*-acetylglucosamine or 27.5mM-glucosamine was determined. *N*-Acetylglucosamine did not interfere with basal glucose metabolism, nor did 2.75mM-glucose interfere with the metabolism of 27.5mM-*N*-acetylglucosamine (Table 3). Glucosamine did,

however, decrease basal glucose metabolism to approx. 50% of control; 2.75mM-glucose did not interfere with the metabolism of 27.5mM-glucosamine. From these results, combined metabolic rates (shown in Table 4) were determined and insulin release was predicted based on experimental data obtained with $[1\text{-}^3\text{H}]\text{glucose}$ alone. As shown in

Table 4. Metabolism and insulin release caused by combinations of glucose and glucosamine or N-acetylglucosamine
Total substrate metabolism was determined from the data presented in Table 3: for glucose + glucosamine, lines 3 + 12, and for glucose + N-acetylglucosamine, lines 2 + 8. Insulin release was predicted from the metabolism and insulin-release data obtained with [1-³H]glucose alone and presented in Fig. 5. Actual insulin release is corrected for basal secretion. Results are means ± S.E.M. for at least four experiments.

Stimulus combination (mM)	Total ³ H ₂ O released (pmol/h per islet)	Insulin release (μunits/min per 100 islets)	
		Predicted	Actual
1. Glucose (2.75), glucosamine (27.5)	24.4	98	92 ± 14
2. Glucose (2.75), N-acetylglucosamine (27.5)	35.4	172	196 ± 15

Fig. 5. Comparison of hexose-induced insulin release and hexose metabolic rates

Insulin release by different concentrations of glucose (○), N-acetylglucosamine (●), glucosamine (▲), or of combinations of 2.75 mM-glucose and 27.5 mM-N-acetylglucosamine (■), or 2.75 mM-glucose and 27.5 mM-glucosamine (□), is plotted as a function of the ³H₂O formation, determined with the 1-³H-labelled derivatives. The solid line connects the points obtained with [1-³H]glucose alone and, for purposes of comparison, metabolic and release data obtained with [5-³H]glucose are shown (dotted line). This graph does not imply that the islets preferentially metabolize [5-³H]glucose. The different rates are presumed to be the end result of the readier labilization of the 5-³H by the islet enzyme systems than the 1-³H. To account for basal insulin secretion, 20 μunits of insulin/min per 100 islets was subtracted from all experimentally obtained insulin values. Data taken from Figs. 1 and 3 form the basis for this graph.

authors have used [5-³H]glucose in determining the usage of the hexose under different conditions (Ashcroft *et al.*, 1972; Zawalich *et al.*, 1977a,b). The appearance of ³H₂O from this derivative appears to measure accurately total glucose usage (defined as the total amount of glucose phosphorylated) by islet tissue. In the present study, the metabolism of N-acetylglucosamine and glucosamine were assessed by using the 1-³H-labelled derivatives, since the 5-³H-labelled compounds are not available. The use of these 1-³H-labelled compounds appears less than ideal, since experiments conducted with both [1-³H]- and [5-³H]-glucose revealed substantial differences in the amount of ³H₂O formed from each compound. Consequently, it would appear that the metabolism of the [1-³H]hexosamines underestimates actual usage of the compounds. Presumably these lower values are the end result of sequestration of the ³H in the one position by metabolic intermediates or end products other than ³H₂O. Despite these obvious shortcomings, it is clear that glucosamine and N-acetylglucosamine are metabolized by islet tissue, and bearing these comments in mind some conclusions do seem warranted.

The present results appear to establish firmly N-acetylglucosamine as a potent independent stimulator of insulin secretion. These results are at variance with a report by Ashcroft *et al.* (1976). They demonstrated an absolute requirement for low glucose (3.3 mM) for N-acetylglucosamine to augment basal secretion. Recently, however, this group has reported that N-acetylglucosamine alone, in the absence of glucose, stimulates secretion and is a suitable substrate for the enzymic machinery of islet tissue (Williams & Ashcroft, 1978). Earlier negative results were apparently due to the presence of caffeine (used to potentiate the insulin-secretory response) in the incubation medium. They demonstrated in that report that caffeine inhibited N-acetylglucosamine oxidation and release. Mannohexose, at molar ratios which completely suppress glucose-stimulated secretion (Zawalich *et al.*, 1977a) had no deleterious effect on N-acetylglucosamine-stimulated secretion or metabolism, whereas a basal glucose concentration significantly augmented its

Fig. 5 and Table 4, insulin release was accurately predicted under these conditions, assuming summated metabolism of the compounds tested.

Discussion

At the outset, it is important to address some of the problems inherent in measuring the metabolism of various carbohydrates. In previous reports, most

secretory response. In contrast, 1 mM-iodoacetate or 60 mM-2-deoxyglucose blocks its secretory ability and metabolic degradation.

Glucosamine also possessed the ability to elevate hormone release. This elevated secretory rate was accompanied by an increased metabolic rate. Mannoheptulose did not alter glucosamine-induced release or metabolism. Glucose, however, augments glucosamine-stimulated secretion. Iodoacetate or 2-deoxyglucose blocked insulin release and metabolism of glucosamine in a parallel fashion.

Little is known about the pathways involved in the initial metabolic transformations of glucosamine or *N*-acetylglucosamine in islet tissue. $^{14}\text{CO}_2$ production from D-[1- ^{14}C]glucosamine and *N*-acetyl[1- ^{14}C]glucosamine has been reported (Ashcroft *et al.*, 1973; Williams & Ashcroft, 1978) and glucosamine increases lactate production from isolated islets (Matschinsky *et al.*, 1975). It may be postulated that after their initial phosphorylation and conversion into fructose 6-phosphate they traverse the glycolytic pathway similarly to glucose. For *N*-acetylglucosamine, after its phosphorylation it is perhaps deacetylated. Since the 1- ^3H -labelled derivatives used in the present study presumably lose most of the ^3H during passage in the tricarboxylic acid cycle (Katz & Wals, 1972), these postulates appear to be the simplest explanations for the observed metabolic results. Some ^3H may also be liberated via the initial steps in the pentose shunt (Katz & Wals, 1972).

The present data combined with previous reports using the same methodology set the stage for discussing the effects of various carbohydrates, their derivatives and various metabolic inhibitors on the secretory function of pancreatic islets.

Stimulants

We previously reported detailed data on glucose, mannose and fructose usage by isolated islets (Zawalich *et al.*, 1977b). We demonstrated that above an apparently critical threshold usage rate, minor changes in metabolic flux were paralleled by dramatic alterations in the secretory rate. Comparison of release and usage rates with mannose or glucose as stimulants supported this interpretation. Further, fructose usage never exceeded this critical threshold value and never stimulated secretion. It could, however, augment secretion provoked by moderate glucose concentrations and this effect could be accurately predicted by assuming additive metabolic effects of the two compounds. A similar phenomenon for *N*-acetylglucosamine and glucosamine is demonstrated in the present paper. Consequently, comparable rates of *N*-acetylglucosamine, glucosamine or glucose metabolism (measured under all conditions with the 1- ^3H -labelled derivative) were accompanied by similar insulin responses. In addition,

release rates in response to basal (2.75 mM) glucose plus *N*-acetylglucosamine or glucosamine could be predicted based on the total metabolism occurring with the combination of substances.

Previous theories attempting to explain carbohydrate-stimulated insulin secretion have involved both initiator and potentiator sites in the pancreatic islets (Ashcroft *et al.*, 1972). This model can now be considerably simplified. Those compounds whose metabolic rate exceeds a critical threshold value (best determined in this system with the appropriate [^3H]glucose derivative) will stimulate insulin secretion and the ultimate secretory rate reached depends on the ability of various islet enzymes (particularly perhaps those controlling initial phosphorylation) to utilize these compounds as substrates. Compounds previously classified as potentiators (i.e. fructose) are also metabolized, although not at rates sufficient to surpass the threshold for stimulated secretion. These compounds can, however, add to the metabolic signal produced by other stimulants, provided that they do not grossly hamper the islet's ability to metabolize the stimulant. Previous studies with glucosamine were difficult to interpret because it also inhibits glucose metabolism (Ashcroft *et al.*, 1970). The present data also appear to explain how glucose potentiates glucosamine release and how, at the same time, its ability to be metabolized is decreased. The metabolism of the glucose that remains intact after glucosamine addition is capable of adding to the insulinogenic signal produced by glucosamine itself.

Inhibitors

In previous reports (Coore & Randle, 1964; Ashcroft *et al.*, 1970; Zawalich *et al.*, 1977a, 1978), mannoheptulose proved to be a powerful inhibitor of glucose or mannose usage and their ability to provoke secretion. However, it lacked the ability to suppress hexose usage completely at equivalent molar ratios. We initially postulated that mannoheptulose was specifically blocking the high- K_m hexokinase present in islet tissue (Matschinsky & Ellerman, 1968; Malaisse *et al.*, 1976a), with little effect on the low- K_m component. The present results with [1- ^3H]glucose lend support to this interpretation. At a high molar ratio of mannoheptulose to glucose (11:1) the metabolism of basal glucose was decreased to 70% of the control value. Mannoheptulose slightly, but insignificantly, decreased the catabolism of low, but not high, concentrations of *N*-acetylglucosamine or glucosamine. Insulin release caused by these latter two compounds was also unaffected. These results support the view initially proposed by Ashcroft's group (Ashcroft *et al.*, 1976; Williams & Ashcroft, 1978) that at least a third hexose-phosphorylating enzyme exists in

islet tissue. This enzyme phosphorylates glucosamine and *N*-acetylglucosamine, with the latter compound being the more suitable substrate. The enzyme is not readily susceptible to mannoheptulose blockage.

That the further glycolytic degradation of these compounds is intimately involved with their ability to provoke secretion is supported by the observation that iodoacetate blocks their ability to stimulate secretion or to be metabolized. We showed that although iodoacetate blocked the ability of glucose, mannose or glyceraldehyde to stimulate secretion, it left intact the response to the keto acid 4-methyl-2-oxopentanoate (Zawalich *et al.*, 1978). Consequently, non-specific poisoning of the β -cells may be eliminated as the explanation of iodoacetate action. Although the specific blockage of glycolysis at the glyceraldehyde 3-phosphate dehydrogenase step, and consequently the failure of hexoses or trioses to generate the ultimate triggering compound, appears to be the simplest explanation for iodoacetate poisoning (Georg *et al.*, 1971; Hellman *et al.*, 1973), ATP depletion may also be crucial. 2-Deoxyglucose, on the other hand, may be affecting islet carbohydrate metabolism and subsequent release by altering transport, phosphorylation or further metabolic degradation of the substrates (Woodward & Hudson, 1954; Wick *et al.*, 1957; Nirenburg & Hoff, 1958).

The simplest explanation for the present and previous reports is that the glycolytic degradation of carbohydrates or their derivatives controls insulin secretion (Grodsky *et al.*, 1963; Hellman *et al.*, 1974; Malaisse *et al.*, 1976b; Zawalich *et al.*, 1977b). The common metabolite(s) or cofactor(s) involved is not known at present.

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