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The Pancreatic β-Cell Recognition of Insulin Secretagogues

XII. Insulin Release in Response to Halogenated Hexosamines

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SUMMARY

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The effects of *N*-iodoacetyl-2-amino-2-deoxy-D-glucose and various *N*-bromoacetylglycosylamines on the release of insulin from microdissected pancreatic islets of non-inbred *ob/ob* mice were studied. *N*-Bromoacetyl-β-D-glucosylamine (10 mM) initiated insulin release in the absence of D-glucose and, at concentrations of 2.5–10 mM, but not 20 mM, potentiated insulin release in response to 10 mM D-glucose. The potentiating, but not the initiating, action was significantly inhibited in the presence of mannoheptulose. *N*-Bromoacetyl-β-L-glucosylamine or *N*-bromoacetyl-β-D-galactosylamine had no effect in the absence of D-glucose. However, 2.5–20 mM concentrations of the L-glucose derivative and 1.25–5.0 mM concentrations of the D-galactose derivative potentiated the effect of 10 mM D-glucose; at 20 mM the D-galactose derivative inhibited the D-glucose-induced insulin release. *N*-Iodoacetyl-2-amino-2-deoxy-D-glucose (0.1–10 mM) did not initiate or potentiate insulin release but, at a concentration of 10 mM, inhibited the effect of D-glucose. The results support our hypothesis that alkylation of thiol groups in the β-cell plasma membrane leads to potentiation of D-glucose-induced insulin release if glycolysis is not simultaneously inhibited by the thiol reagent. If a regulatory site ("direct receptor") for the D-glucose molecule plays a role in stimulus recognition, *N*-iodoacetyl-2-amino-2-deoxy-D-glucose may be valuable in attempts to label and isolate it.

INTRODUCTION

Three types of data suggest that D-glucose is recognized as an insulin secretagogue by being metabolized in pancreatic β-cells. First, among several sugars tested, only those which are actively metabolized in the pancreatic islets are consistently capable of initiating insulin release (1–4). Second, close correlations have been demonstrated between rates of insulin release and glucose metabolism in the islets (2–5), although there seems to be an unresolved discrepancy between the metabolic and insulin-releasing activities of D-glucose anomers (6). Third, D-glyceraldehyde (3, 7) and 1,3-dihydroxyacetone (7) are also capable of initiating insulin release, presumably because these com-

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pounds are phosphorylated in the β-cells and feed into glycolysis at the triose phosphate step. However, these observations do not rule out the possibility that stimulus recognition is dependent on the interaction of the unmetabolized glucose molecule with some regulatory site or "direct receptor." Although there is little evidence for such a site, several authors have discussed its possible existence (3, 8–13) and some have even rejected the idea that glycolysis is essential for the initiation of insulin release by D-glucose (9, 10).

We observed that several thiol-blocking agents stimulate insulin release, probably through their reaction with relatively exposed thiol groups in the β-cell plasma membrane (14–16). These effects can be explained in several ways and do not compel us to assume that membrane thiol groups are essential for the secretagogic D-glucose recognition. However, we were struck by the fact that thiol groups have been suggested to play a role in the function of receptors in other cells, e.g., the receptors responsible for adrenergic stimulus recognition (17, 18). If thiol groups were involved in the function of a D-glucose receptor in the β-cell plasma membrane, this could perhaps be demonstrated with the aid of glucose analogues incorporating alkylating functions and capable of reacting with thiols. *N*-Iodoacetyl-2-amino-2-deoxy-D-glucose, *N*-bromoacetyl-β-D-glucosylamine, *N*-bromoacetyl-β-L-glucosylamine, and *N*-bromoacetyl-β-D-galactosylamine were therefore synthesized and tested for effects on the release of insulin from microdissected pancreatic islets of non-inbred *ob/ob* mice. These islets contain more than 90% β-cells and are known to release insulin in response to D-glucose but not in response to L-glucose or D-galactose.

MATERIALS AND METHODS

The following compounds were synthesized and had the same analytical properties as previously described (19): *N*-bromoacetyl-β-D-glucosylamine (Fig. 1), *N*-acetyl-β-D-glucosylamine, *N*-bromoacetyl-β-D-galactosylamine, and *N*-acetyl-β-D-galactosylamine. *N*-Bromoacetyl-β-L-glucosylamine, m.p. 178° (decomposition), $[\alpha]_D^{20}$ +13°, and *N*-acetyl-β-L-glucosylamine, m.p. 267° (decomposition), $[\alpha]_D^{20}$ +20°, were prepared from L-glucosylamine as above (19, 20) *N*-Iodoacetyl-2-amino-2-deoxy-D-glucose (anomeric configuration unspecified), m.p. 184° (decomposition), was prepared by acylating 2-amino-2-deoxy-D-glucose in methanolic solution with iodoacetic anhydride (Fig. 1). All compounds gave satisfactory CHN analyses and were homogeneous by thin-layer chromatography on silica gel G plates (developing solvent, acetone–methanol, 10:1 by volume).

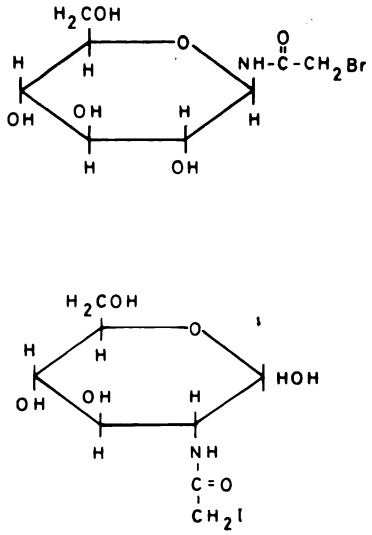

FIG. 1. *Structures of N-bromoacetyl-β-D-glucosylamine (top) and N-iodoacetyl-2-amino-2-deoxy-D-glucose (bottom)*

All *N*-bromoacetylglycosylamines were highly reactive toward L-cysteine (Table 1); although *N*-iodoacetyl-2-amino-2-deoxy-D-glucose was not included in these tests, the compound must also be assumed to react with thiol groups, in view of the well-known behavior of iodoacetate and iodoacetamide. At the concentrations used in the insulin release measurements, none of the synthesized reagents affected the insulin assay. By means of a coupled hexokinase-glucose 6-phosphate dehydrogenase assay, it was verified that the insulin-releasing sugar analogues did not contribute to the D-glucose concentration in the incubation media. In those control experiments, appropriate glucose standards were

prepared in media containing *N*-bromoacetyl-β-D-glucosylamine.

Pancreatic islets were microdissected freehand (21) from adult *ob/ob* mice taken from a non-inbred colony in our laboratory. Microdissection and subsequent incubations were performed in Krebs-Ringer bicarbonate buffer equilibrated with O_2–CO_2 (95:5) and containing 1 mg of bovine serum albumin (fraction V, Sigma Chemical Company) per milliliter as basal medium. Insulin release was studied both by incubating islets in closed vials and determining insulin in samples of medium taken at the end of incubation (22), and by microperifusing islets in an apparatus allowing determinations of the dynamics of insulin release with time (23). Details of the experiments are given in the legends to figures and tables. After incubation at 37° the islets were frozen in melting isopentane, freeze-dried overnight (−40°, 0.1 Pa), and weighed on a quartz fiber balance. Insulin was assayed radioimmunologically, using crystalline mouse insulin as reference. Free and antibody-bound insulin were separated by precipitation with 81% ethanol (24). ^{125}I-Labeled insulin was obtained from Farbwerke Hoechst, Frankfurt am Main, Germany. Other reagents were of analytical grade.

RESULTS

N-Acetyl-β-L-glucosylamine and *N*-acetyl-β-D-galactosylamine did not significantly affect insulin release in the absence or presence of D-glucose (not shown). *N*-Acetyl-β-D-glucosylamine also had no significant effect (Fig. 2).

When added to a D-glucose-free medium, 2.5 mM *N*-bromoacetylglycosylamine had no effect on insulin release (Table 2). *N*-Bromoacetyl-β-D-glucosylamine, 10 mM, caused a significant secretory response; 10 mM *N*-bromoacetyl-β-L-glucosylamine or 10 mM *N*-bromoacetyl-β-D-galactosylamine raised the mean values for insulin release to a lesser extent and without statistical significance.

Table 2 shows that all three *N*-bromoacetylglycosylamines were capable of potentiating D-glucose-induced insulin release, although there were striking differences in the dose-response relationships. In combination with a submaximally stimulating D-glucose concentration of 10 mM, significant potentiation was observed with a 2.5 mM concentration of each *N*-bromoacetylglycosylamine. At the same D-glu-

TABLE 1

Reaction of N-bromoacetylglycosylamines with L-cysteine

L-Cysteine (1 mM) and 10 mM test compound were incubated at 37° in Krebs-Ringer bicarbonate buffer, pH 7.4, for 5 or 60 min. The incubation media were then analyzed for free L-cysteine, using 6,6'-dithiodinicotinic acid as chromogenic reagent for thiol groups. Results are given as the mean values of three determinations. BA- = *N*-bromoacetyl-.

Test compound	Free L-cysteine after reaction	
	5 min	60 min
	mM	*mM*
None (control)	1.00	0.96
BA-D-glucosylamine	0.37	0.01
BA-L-glucosylamine	0.32	0.00
BA-D-galactosylamine	0.32	0.00

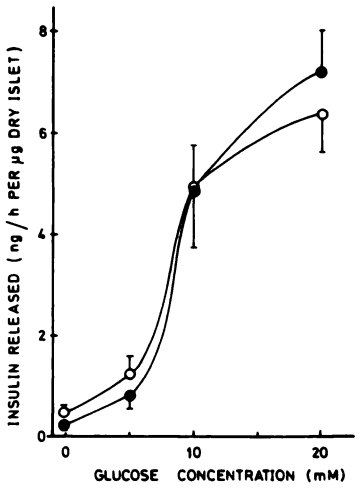

FIG. 2. *Effect of N-acetyl-β-D-glucosylamine on dose-response curve for D-glucose-induced insulin release*

After preliminary incubation for 40 min in glucose-free basal medium, islets were incubated for 60 min in basal medium containing D-glucose as indicated. Control incubations (○) were performed without further additions to the medium, whereas in test incubations (●) the medium also contained 10 mM *N*-acetyl-β-D-glucosylamine. Values are means ± standard errors of seven different experiments.

TABLE 2

Effects of N-bromoacetylglycosylamines on insulin release

After preliminary incubation for 40 min in basal medium containing no (series I and II) or 20 mM (series III) D-glucose, islets were incubated for 60 min in basal medium containing D-glucose and *N*-bromoacetylglycosylamine as indicated. Amounts of insulin released during the 60-min period are presented as the mean values ± standard errors for the numbers of experiments shown. In addition to the values recorded for each group of islets, the means ± standard errors of differences between parallel test and control incubations are given. BA- = *N*-bromoacetyl-. Statistical significances were judged by *t*-test on paired observations.

BA-glycosylamine	No. of expts.	Insulin release	
		Test	Test minus control
		ng/hr/μg dry islets	
Series I: no D-glucose			
None (control)	7	0.16 ± 0.04	
2.5 mM BA-D-glucosylamine	7	0.15 ± 0.02	−0.01 ± 0.04
2.5 mM BA-L-glucosylamine	7	0.12 ± 0.02	−0.04 ± 0.04
2.5 mM BA-D-galactosylamine	7	0.20 ± 0.01	0.04 ± 0.04
None (control)	11	0.53 ± 0.11	
10 mM BA-D-glucosylamine	11	1.05 ± 0.19	0.52 ± 0.17[a]
10 mM BA-L-glucosylamine	11	0.76 ± 0.20	0.23 ± 0.20
10 mM BA-D-galactosylamine	11	0.85 ± 0.15	0.32 ± 0.19
Series II: 10 mM D-glucose			
None (control)	7	2.20 ± 0.45	
2.5 mM BA-D-glucosylamine	7	4.63 ± 0.49	2.43 ± 0.32[b]
2.5 mM BA-L-glucosylamine	7	4.76 ± 0.80	2.56 ± 0.44[c]
2.5 mM BA-D-galactosylamine	7	5.88 ± 0.84	3.68 ± 1.05[a]
None (control)	11	3.71 ± 0.47	
10 mM BA-D-glucosylamine	11	8.91 ± 1.09	5.20 ± 1.05[b]
10 mM BA-L-glucosylamine	11	7.34 ± 0.73	3.63 ± 0.93[c]
10 mM BA-D-galactosylamine	11	4.99 ± 0.68	1.28 ± 0.91
Series III: 20 mM D-glucose			
None (control)	8	5.08 ± 0.60	
10 mM BA-D-glucosylamine	8	5.32 ± 0.78	0.24 ± 0.84
10 mM BA-L-glucosylamine	8	16.91 ± 1.41	11.83 ± 1.23[b]
10 mM BA-D-galactosylamine	8	2.41 ± 0.34	−2.67 ± 0.49[b]

[a] $p < 0.02$.
[b] $p < 0.001$.
[c] $p < 0.01$.

cose concentration, either the D-glucose or L-glucose derivative at 10 mM also caused significant potentiation, whereas 10 mM *N*-bromoacetyl-β-D-galactosylamine had no significant effect. The maximum insulin secretory response to D-glucose is obtained with 20 mM D-glucose (15). At this high D-glucose concentration, 10 mM *N*-bromoacetyl-β-L-glucosylamine potentiated the effect of D-glucose, while 10 mM *N*-bromoacetyl-β-D-glucosylamine had no effect and 10 mM *N*-bromoacetyl-β-D-galactosylamine was inhibitory.

In view of the results in Table 2, the effects of various *N*-bromoacetylglycosylamine concentrations were studied in more detail at a D-glucose concentration of 10 mM. Figure 3 shows that 2.5–10 mM, but not 20 mM, *N*-bromoacetyl-β-D-glucosylamine markedly potentiated the D-glucose-induced insulin release. *N*-Bromoacetyl-β-L-glucosylamine differed from *N*-bromoacetyl-β-D-glucosylamine in also being capable of potentiating insulin release at the highest concentration of the derivative (20 mM). Low concentrations of *N*-bromoacetyl-β-D-galactosylamine potentiated the D-glucose-induced insulin release with a maximum around 2.5 mM D-galactose derivative. At higher concentrations the potentiating effect declined, and with 20 mM *N*-bromoacetyl-β-D-galactosylamine

there was significant inhibition of the D-glucose-induced insulin release.

The dynamics of the effects of *N*-bromoacetylglycosylamines on D-glucose-induced insulin release are shown in Fig. 4. The results agree well with the net effects over 1 hr described in Table 2 and Fig. 3. Thus, when the *N*-bromoacetylglycosylamines at 10 mM were suddenly introduced into a perifusion medium containing 17 mM D-glucose, the L-glucose derivative caused a rapid, marked potentiation of insulin release with a maximum after about 100 min. No inhibition of the D-glucose-induced insulin release was observed during perfusion with the L-glucose derivative. The corresponding D-glucose derivative also caused a rapid potentiation of the D-glucose-induced insulin release. However, the effect was less than that of the L-glucose derivative and seemed to decline after the first 15 min of stimulation. With the D-galactose derivative there was no evidence of even a transient potentiation of insulin release, whereas after about 30 min the D-glucose-induced insulin release was inhibited.

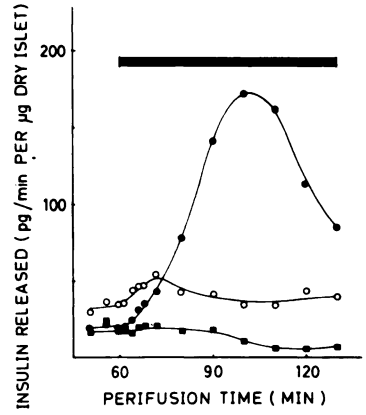

FIG. 4. *Dynamics of insulin release in response to N-bromoacetylglycosylamines*

Islets were perifused with basal medium containing 10 mM D-glucose throughout the experiments. After 60 min of perifusion, 10 mM *N*-bromoacetyl-β-D-glucosylamine (○), 10 mM *N*-bromoacetyl-β-L-glucosylamine (●), or 10 mM *N*-bromoacetyl-β-D-galactosylamine (■) was quickly added to the perifusion medium. Perifusion with *N*-bromoacetylglycosylamine lasted for 75 min (bar). The points denote the average rate of insulin release during each sampling period.

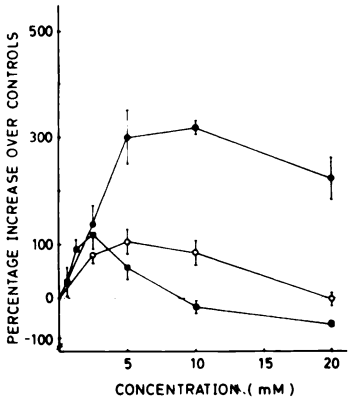

FIG. 3. *Dose-response curves for effects of N-bromoacetylglycosylamines on insulin release*

After preliminary incubation for 40 min in glucose-free basal medium, islets were incubated for 60 min in basal medium containing 10 mM D-glucose as well as *N*-bromoacetyl-β-D-glucosylamine (○), *N*-bromoacetyl-β-L-glucosylamine (●), or *N*-bromoacetyl-β-D-galactosylamine (■) at the concentrations indicated on the abscissa. Rates of insulin release are expressed as a percentage of the control rate with 10 mM D-glucose alone. Values are means ± standard errors of eight (○), 10 (●), or seven (■) different experiments.

Table 3 summarizes experiments designed to test for any difference in latency between the effects of *N*-bromoacetyl-β-D-glucosylamine on insulin release in the absence and presence of 10 mM D-glucose. The effect of *N*-bromoacetyl-β-D-glucosylamine alone was found to be manifest during the first 5 min of incubation, while the potentiating effect was not statistically significant during this early phase. Mannoheptulose, which is known to inhibit D-glucose-induced insulin release (2, 3), significantly decreased insulin release in response to a combination of D-glucose and *N*-bromoacetyl-β-D-glucosylamine (Table 4). The secretory response to *N*-bromoacetyl-β-D-glucosylamine alone was not significantly inhibited by mannoheptulose, but there was a 20–30% reduction of the mean values (Table 4).

Table 5 shows that 10 mM *N*-iodoacetyl-2-amino-2-deoxy-D-glucose inhibited the D-glucose-induced secretory response, while a 0.1 mM concentration of the same com-

TABLE 3

Effect of N-bromoacetyl-β-D-glucosylamine on early and late phases of insulin release

After preliminary incubation for 40 min in glucose-free basal medium, islets were incubated for 5 or 60 min in basal medium containing D-glucose and *N*-bromoacetyl-β-D-glucosylamine as indicated. Amounts of insulin released during the 5- and 60-min periods are presented as mean values ± standard errors of seven different experiments. In addition, mean values ± standard errors of differences between incubations with and without *N*-bromoacetyl-β-D-glucosylamine are given. *p* values refer to *t*-tests on paired observations.

Phase	*N*-Bromoacetyl-β-D-glucosylamine	D-Glucose	Insulin release		*p*
			Primary data	Effect of *N*-bromoacetyl-β-D-glucosylamine	
min	*mM*	*mM*	*ng/hr/μg dry islets*		
5	0	0	0.05 ± 0.01		
	10	0	0.14 ± 0.03	0.09 ± 0.03	<0.02
	0	10	0.82 ± 0.16		
	10	10	0.93 ± 0.16	0.11 ± 0.11	>0.05
60	0	0	0.23 ± 0.05		
	10	0	2.02 ± 0.58	1.79 ± 0.58	<0.025
	0	10	3.83 ± 0.79		
	10	10	9.90 ± 1.57	6.07 ± 0.97	<0.001

TABLE 4

Effects of mannoheptulose on insulin release in response to N-bromoacetyl-β-D-glucosylamine and D-glucose

After preliminary incubation for 40 min in glucose-free basal medium, islets were incubated for 60 min in basal medium containing *N*-bromoacetyl-β-D-glucosylamine, D-glucose, and mannoheptulose as indicated. Amounts of insulin released during the 60-min period are presented as mean values ± standard errors of seven (no D-glucose) or eight (10 mM D-glucose) different experiments. In addition, the mean values ± standard errors of differences between parallel incubations with and without mannoheptulose are given. Statistical significances were judged by *t*-tests on paired observations.

D-Glucose	*N*-Bromoacetyl-β-D-glucosylamine	Mannoheptulose	Insulin release		*p*
			Primary data	Effect of mannoheptulose	
mM	*mM*	*mM*	*ng/hr/μg dry islets*		
0	0	0	0.40 ± 0.09		
	10	0	1.05 ± 0.28		
	10	10	0.73 ± 0.33	−0.32 ± 0.24	>0.05
	20	0	1.33 ± 0.45		
	20	10	1.07 ± 0.40	−0.26 ± 0.40	>0.05
10	0	0	6.71 ± 1.23		
	10	0	12.37 ± 1.51		
	10	10	0.98 ± 0.39	−11.39 ± 1.37	<0.001
	20	0	6.66 ± 0.87		
	20	10	1.10 ± 0.46	−5.56 ± 0.87	<0.001

pound seemed to have no effect. Basal insulin release appeared to be unaffected by *N*-iodoacetyl-2-amino-2-deoxy-D-glucose at all concentrations tested.

DISCUSSION

Bromoacetylated analogues of substrates for enzymes (25) and transport carriers (19) have been used to achieve covalent bonding to the specific catalytic regions. Because of the stability of the ensuing complex, such "affinity labeling" markedly inhibits the interaction of the scalar or vectorial catalyst with other substrates. For example, *N*-bromoacetyllactosylamine and *N*-bromoacetylgalactosyl-

TABLE 5

Effects of N-iodoacetyl-2-amino-2-deoxy-D-glucose on insulin release

After preliminary incubation for 40 min in basal medium containing 3 mM D-glucose, islets were incubated for 60 min in medium containing D-glucose and *N*-iodoacetyl-2-amino-2-deoxy-D-glucose (IAA-glucose) as indicated. Amounts of insulin released during the 60-min period are presented as mean values ± standard errors for the numbers of experiments shown in parentheses. Statistical significances were judged by *t*-testing the differences between parallel incubations with and without *N*-iodoacetyl-2-amino-2-deoxy-D-glucose.

D-Glucose	IAA-glucose	Insulin release
mM	*mM*	*ng/hr/μg dry islets*
3	0	0.55 ± 0.14 (20)
3	0.1	0.43 ± 0.13 (11)
3	1	0.56 ± 0.21 (8)
3	10	0.80 ± 0.19 (8)
20	0	3.61 ± 0.51 (20)
20	0.1	3.82 ± 0.65 (11)
20	1	2.51 ± 0.46 (8)
20	10	0.58 ± 0.08[a] (8)

[a] $p < 0.001$.

amine are irreversible inhibitors of the lactose transport system in *Escherichia coli*, while *N*-bromoacetylglucosylamine has no effect (19).

As reported here, *N*-bromoacetyl-β-D-glucosylamine or the corresponding analogue of L-glucose did not inhibit the β-cell secretory response to D-glucose; an inhibitory effect of *N*-bromoacetyl-β-D-galactosylamine was observed only at 10 and 20 mM concentrations of the D-galactose derivative. This resistance to inhibition may indicate that the β-cells do not contain secretagogic glucose receptors with a chemical configuration suitable for the covalent bonding of *N*-bromoacetyl-β-D-glucosylamine. Such a result should perhaps be expected, in view of the finding that *N*-acetyl-β-D-glucosylamine lacked insulin-releasing ability and thus may not fit the direct glucose receptor if it exists at all. From this point of view, it is more striking that insulin release was markedly inhibited by *N*-iodoacetyl-2-amino-2-deoxy-D-glucose. *N*-Acetyl-2-amino-2-deoxy-D-glucose has been found to potentiate insulin release in response to D-glucose or D-glyceraldehyde, possibly by interacting with a regulatory glucose receptor site (3). If, again, such a receptor exists, the present results suggest that *N*-iodoacetyl-2-amino-2-deoxy-D-glucose might be a useful tool in attempts to label and isolate it. However, our results do not prove the existence of such a site. Iodoacetamide at equimolar concentrations is also a potent inhibitor of glucose-stimulated insulin release (15). The inhibitory action of iodoacetamide is probably due to blockade of the 3-phosphoglyceraldehyde dehydrogenase reaction in glycolysis (15). So far, we do not know whether *N*-iodoacetyl-2-amino-2-deoxy-D-glucose can enter the β-cells to produce the same effect.

The interpretation of the present results is also complicated by the fact that a certain alkylation of the β-cell plasma membrane sensitizes the β-cells to stimulation with D-glucose. Such sensitization was previously evident from the marked potentiating effects of low concentrations of iodoacetate or iodoacetamide on glucose-stimulated insulin release (15). As shown here, all the *N*-bromoacetylglycosylamines reacted with L-cysteine and potentiated the insulin secretory responses to D-glucose, presumably by a mechanism similar to or identical with that responsible for potentiation by low concentrations of iodoacetate or iodoacetamide.

Because iodoacetamide does not cause significant insulin release in the absence of glucose but potentiates the glucose-induced secretory response, it may be asked whether the initiating function of glucose and the potentiating function of alkylating thiol reagents can be incorporated at all into one molecule. Unlike *N*-iodoacetyl-2-amino-2-deoxy-D-glucose, *N*-bromoacetyl-β-D-glucosylamine exerted a small but significant insulin-releasing action in the absence of a stimulatory glucose concentration. In contrast to the potentiating action of *N*-bromoacetyl-β-D-glucosylamine, the insulin-releasing action of this compound alone resembled that of D-glucose in being manifest during the first 5 min of incubation in closed vials. Neither *N*-bromoacetyl-β-L-glucosylamine nor *N*-bromoacetyl-β-D-galactosylamine exhibited the same

capacity to initiate insulin release. Other studies have shown that L-glucose[1] and D-galactose (4) do not initiate insulin release from the present type of islets. The analogous behaviour of the *N*-bromoacetylglycosylamines may therefore suggest that the β-cell can somehow distinguish between them by means of its system for stimulus recognition. However, this interpretation suffers from the objection that mannoheptulose did not significantly inhibit insulin release in response to *N*-bromoacetyl-β-D-glucosylamine alone. Mannoheptulose is a potent inhibitor of the D-glucose-induced insulin release in the absence (2, 3, 26) and, as shown here, in the presence of *N*-bromoacetylglycosylamines. Moreover, *N*-bromoacetyl-β-D-glucosylamine was not a strikingly more efficient potentiator of the D-glucose-induced insulin release than the other derivatives under study. This lack of specificity may suggest that the target groups, are not preferentially located in the vicinity of a specific D-glucose receptor.

The dose-response curves for the effects of *N*-bromoacetyl-β-D-glucosylamine and *N*-bromoacetyl-β-D-galactosylamine on D-glucose-induced insulin release were biphasic. With increasing concentrations of these compounds a maximum was reached that was followed by a decline of the potentiating effect at still high concentrations. In the case of the D-galactose derivative, significant inhibition of the D-glucose-induced insulin release was even observed at the highest concentrations of the derivative tested. The same phenomenon of a biphasic dose-response curve was previously noted in studies on the effects of iodoacetamide on insulin release (15). It is conceivable, therefore, that when *N*-bromoacetyl-β-D-glucosylamine and *N*-bromoacetyl-β-D-galactosylamine are present at high concentrations in the medium, they may enter the β-cells in amounts sufficient to inhibit glycolysis and insulin release. It is not known whether and how these compounds are transported across the β-cell plasma membrane. However, the β-cells possess a carrier system for D-glucose (27), and this system may perhaps accept the present D-glucose and D-galactose derivatives as substrates. The transport system has a strict stereospecificity for the D isomer of glucose, resulting in the apparent exclusion of L-glucose from the cell interior (27). If, therefore, the L-glucose derivative does not enter the β-cells to inhibit glycolysis, its potentiating action on the plasma membrane would also predominate at high concentrations of the compound.

[1] Unpublished observations.

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