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The Pancreatic β-Cell Recognition of Insulin Secretagogues

I. Transport of Mannoheptulose and the Dynamics of Insulin Release

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SUMMARY

Microdissected pancreatic islets of obese-hyperglycemic mice were used to study the uptake of mannoheptulose by β-cells in relation to the dynamics of mannoheptulose-inhibited insulin release. Mannoheptulose uptake was faster at 37° than at 8° and was inhibited by glucose, 3-*O*-methylglucose, or phlorizin. The transport rate for mannoheptulose was much lower than that previously observed for glucose. Nevertheless, a few minutes of exposure to 5 mM mannoheptulose were enough to establish intracellular concentrations of the same magnitude as the concentrations inhibiting insulin release and glucose oxidation when added to the medium. This observation makes it possible to explain the prompt inhibition of insulin release noted in microperfusion experiments as due to mannoheptulose interference with the β-cell metabolism of glucose. The mediated and glucose-sensitive transport of mannoheptulose, however, is also compatible with the idea that insulin release is governed by the binding of sugar to a receptor at the β-cell plasma membrane.

INTRODUCTION

Mannoheptulose is a 7-carbon sugar with pronounced ability to inhibit glucose-stimulated insulin release (1). In support of the hypothesis that the signal for insulin release is derived from glucose metabolism, it has been shown that mannoheptulose inhibits glucose phosphorylation in extracts of pancreatic islets (2–4). To our knowledge, however, the ability of mannoheptulose to enter intact β-cells has not been demonstrated. It thus seems possible that interference of mannoheptulose with β-cell function must occur at the plasma membrane. This possibility was recently stressed after measuring the levels of glucose *in vivo* in pancreatic islets of rats after the injection of mannoheptulose (5). Although experiments *in vitro* revealed that mannoheptulose has little effect on the transport of glucose across the β-cell membrane (6), it may be able to block membrane receptors that are not transport sites or that are responsible for only a fraction of the total glucose uptake. It was felt that the existence of such membrane-located receptors would seem more plausible if the effect of mannoheptulose on insulin release could be shown to be independent of its entry into the β-cells. We have therefore investigated the time course of mannoheptulose uptake by isolated islets in relation to the dynamics of insulin release. The monitoring of insulin

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release from a few islets composed almost exclusively of β-cells was made possible by using a specially designed microperfusion system.

MATERIALS AND METHODS

^{3}H-Labeled D-mannoheptulose was prepared by New England Nuclear Corporation. Before use it was purified by chromatography on Whatman No. 3 paper, using butanol–ethanol–borate buffer (40:11:19 by volume; pH 9) as eluent. Uniformly ^{14}C-labeled sucrose, D-glucose, [^{125}I] insulin, and insulin antibodies were obtained from the Radiochemical Centre, Amersham, England. Nonradioactive D-mannoheptulose and 3-*O*-methyl-D-glucose were purchased from Sigma Chemical Company. Reagents for insulin assay were kindly donated by Novo A/S, Copenhagen. Nonradioactive D-glucose and sucrose were products of British Drug Houses, Ltd., and phlorizin was obtained from Pfaltz and Bauer, New York.

Adult, obese-hyperglycemic mice (gene symbol, *obob*) of either sex were taken from a local colony (7). Unless otherwise stated, the animals were fasted overnight before being killed. The excised pancreas was suspended in Krebs-Ringer-bicarbonate medium supplemented with 0.3–0.5% human serum albumin and equilibrated with O_2–CO_2 (95:5). For each experiment up to 25 fresh islets were isolated by freehand microdissection (8). This kind of islet preparation contains more than 90% β-cells (7, 8). All experiments, except the perfusion experiments, were started by preliminary incubation of the islets at 37° for 30–70 min in basal medium of the same composition as was used during microdissection. This type of medium was also employed as the basal medium in all further incubations.

For studies of mannoheptulose uptake, batches of three islets were incubated in 200 μl of basal medium supplemented with 5 mM ^{3}H-labeled mannoheptulose (at least 2.2 mCi/mmole) and 0.1 mM ^{14}C-labeled sucrose (100 mCi/mmole). The incubation times, temperature, and additions to the medium are specified under RESULTS. After incubation, the islets were placed on pieces of aluminum foil and were freed of as much incubation fluid as possible, using a micropipette. The islets were then freeze-dried, weighed, and analyzed for ^{3}H and ^{14}C as previously described (6).

To study the effects of various concentrations of mannoheptulose on insulin release, batches of two islets were incubated for 60 min at 37° in 315 μl of basal medium supplemented with 20 mM glucose and mannoheptulose as required. To study the dynamics of mannoheptulose action, batches of two islets were placed in a small polyethene-nylon chamber (4.5 μl volume) and were perfused at a rate of 37 μl/min, using basal medium at 37° supplemented with mannoheptulose and/or glucose as specified under RESULTS. A screen oxygenator and the design of the medium inlet ensured optimal equilibration of the medium with O_2–CO_2 (95:5). The temperature used in perfusion of islets was 37°. A detailed description of this microperfusion apparatus will be given elsewhere.[1]

Oxidation of uniformly ^{14}C-labeled glucose was measured as previously described (9). Insulin was assayed radioimmunologically. Separation of free and bound insulin was achieved by precipitation with ethanol (10). Rates of insulin release and glucose oxidation were expressed per unit of dry weight after freeze-drying (−40°; 0.001 mm Hg) overnight and weighing on a quartz fiber balance.

RESULTS

Uptake of mannoheptulose and sucrose with time. As shown in Fig. 1, the islet uptake of mannoheptulose was greater than that of sucrose, which suggests that mannoheptulose entered the β-cells. Uptake was much faster at 37° than at 8°, as would be expected if entry into the β-cells was facilitated by some transport system in the plasma membrane.

At both 8° and 37° the differences between the islet contents of mannoheptulose and sucrose were represented by time curves of complex kinetics. This was most obvious at 8°, at which temperature a clear peak was obtained after 7 min of incubation. The same phenomenon has previously been observed for the islet uptake of L-glucose (6). Although the latter sugar was restricted to the sucrose space at equilibrium, there was an

[1] L.-Å. Idahl, manuscript in preparation.

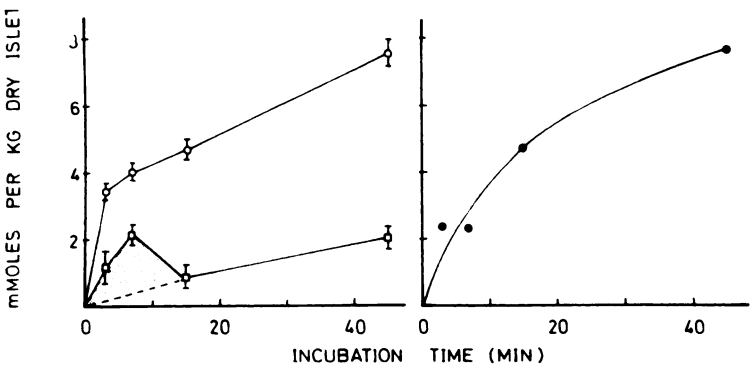

FIG. 1. *Islet uptake of mannoheptulose with time*

Islets were incubated at 8° (□——□) or 37° (○——○) in a medium containing 5 mM ^{3}H-labeled mannoheptulose as well as ^{14}C-labeled sucrose. The left-hand diagram shows the islet content of mannoheptulose corrected for label in the sucrose space. Each point represents the mean of eight (8°) or five (37°) experiments. Bars denote standard errors of the mean. The stippled area represents the excess of mannoheptulose over sucrose due to slow equilibration of sucrose in the extracellular space (see the text). To obtain an estimate of the β-cell uptake of mannoheptulose at 37°, the stippled area was subtracted from the upper curve of the left-hand diagram. The resulting curve is shown at right.

initial overshoot of L-glucose in relation to sucrose. Therefore the present differences between mannoheptulose and sucrose after brief incubation times are probably not truly representative of the β-cell uptake of mannoheptulose. The previous results with L-glucose lead us to conclude rather that mannoheptulose was equilibrated more rapidly within the extracellular space than did sucrose.

Since equilibration of sucrose in the islets is complete after about 15 min (6), it should be possible to estimate the difference in extracellular equilibration rates between mannoheptulose and sucrose by extrapolating the late parts of the uptake curves back to zero time. This is most conveniently done with the curve obtained at 8°, at which temperature the cellular uptake of mannoheptulose was slow. Since diffusion in the extracellular water should not be greatly affected by temperature changes between 8° and 37°, we have used this extrapolation to correct the data obtained at 37°. The resulting curve (Fig. 1) indicates that the uptake of mannoheptulose by β-cells is much slower than that of D-glucose. With the latter sugar, equilibrium was reached within 15 min even at 8° (6). In the present study, β-cells incubated at 8° for 15 min contained only 0.9 mmole of mannoheptulose per kilogram of islet, dry weight. If the intracellular water is taken to be 1.2 times the islet dry weight (11), this figure corresponds to only 15% of the medium concentration of mannoheptulose. Uptake was also fairly slow at 37°, since a cellular concentration of mannoheptulose equal to that in the medium was not reached until after 20 min of incubation.

Effects of glucose, 3-O-methylglucose, and phlorizin on mannoheptulose uptake. The transport of mannoheptulose was significantly inhibited by both glucose and 3-*O*-methylglucose (Table 1). These data are consistent with an inhibitory effect of mannoheptulose on glucose uptake *in vitro* (6), and suggest that mannoheptulose and glucose have at least one transport route in common. The same conclusion has been drawn after measuring the levels of glucose in β-cells of mannoheptulose-treated rats *in vivo* (5). It is further supported by the fact that phlorizin strongly inhibits the transport of glucose (6, 12) as well as that of mannoheptulose (Table 1) in the islet cells.

Effect of mannoheptulose on insulin release. Figure 2 shows the time course of mannoheptulose-induced inhibition of insulin release. Islets perfused with 12 mM glucose for 45 min exhibited a rapid, steady decline in insulin release when the perfusion medium was switched to one containing both 12 mM

TABLE 1

Effects of glucose, 3-O-methylglucose, and phlorizin on islet cell uptake of mannoheptulose

Islets were incubated for 10 min at 37° in a medium containing 1 or 10 mM ^{3}H-labeled mannoheptulose and 0.1 mM ^{14}C-labeled sucrose. In each experiment parallel incubations were performed without further additions to the medium (controls) and in medium that also contained glucose, 3-*O*-methylglucose, or phlorizin as indicated. Test substances were also present during preliminary incubation for 30–40 min in the nonradioactive medium. The contents of mannoheptulose in islet cells were calculated by correction for label in the sucrose space. Mean values ± standard errors are given for each medium as well as for the differences between test and control media over a series of repeated experiments. The numbers of experiments were as tabulated. Statistical significances were estimated from the differences between paired test and control data.

Test substance	No. of experiments	Islet cell content of mannoheptulose			*p* value for inhibition
		Control	Experimental	Experimental minus control	
		mmoles/kg, dry wt			
Incubations with 1 mM ^{3}H-labeled mannoheptulose					
20 mM glucose	12	0.99±0.06	0.84±0.05	−0.16±0.06	<0.025
20 mM 3-*O*-methylglucose	6	1.00±0.07	0.74±0.04	−0.26±0.05	<0.005
10 mM phlorizin	6	1.00±0.07	0.44±0.06	−0.56±0.11	<0.005
Incubations with 10 mM ^{3}H-labeled mannoheptulose					
1 mM phlorizin	8	10.69±1.17	5.21±0.52	−5.48±0.96	<0.001
5 mM phlorizin	8	10.69±1.17	4.64±0.34	−6.05±1.12	<0.001
10 mM phlorizin	8	10.69±1.17	4.14±0.49	−6.55±0.49	<0.001

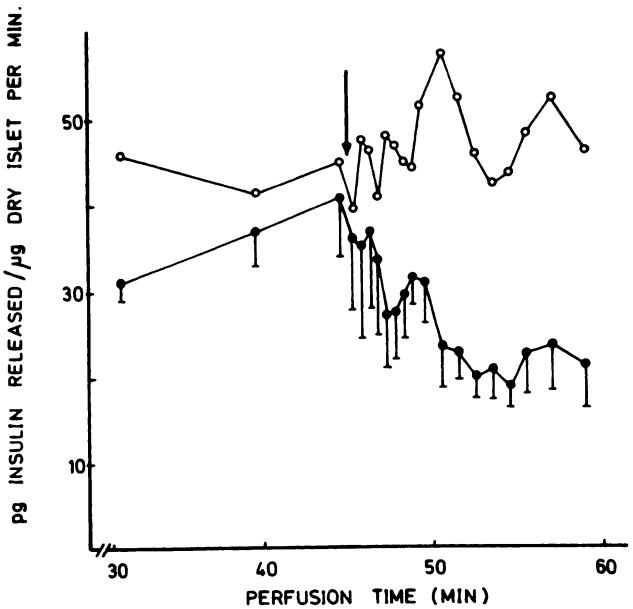

FIG. 2. *Dynamics of mannoheptulose-induced inhibition of insulin release*

Islets were perfused with a medium containing 12 mM glucose. After 45 min (arrow) the perfusion medium was abruptly changed to one containing 12 mM glucose plus 5 mM mannoheptulose (●——●) or 12 mM glucose (○——○). Before the medium was changed, 1-min samples of effluent were taken for insulin assay. Samples were then removed at 30-sec intervals during the first 4 min, and later at 1–2-min intervals. Each point represents the mean of two (○——○) or three (●——●) experiments. Bars denote standard errors of the mean. The basal insulin release in this system is 5–8 pg/µg of islet, dry weight, per minute.

glucose and 5 mM mannoheptulose. The random variations between experiments obliterated the statistical significance of inhibition immediately after mannoheptulose had reached the perfusion chamber. However, an immediate effect of mannoheptulose on insulin release is suggested by the general appearance of the curve.

The possibility that the technical procedure of altering the perfusion medium could in itself inhibit insulin release was tested in two control experiments. In these, the flow to the perfusion chamber was shifted from one reservoir containing 12 mM glucose to another containing the same kind of medium. This change did not inhibit insulin release (Fig. 2). However, it made the recordings fluctuate somewhat, which explains the minor irregularities in the curve obtained during perfusion with mannoheptulose.

Although the volume of the perfusion chamber was as small as 4.5 μl, it cannot be assumed that newly released insulin emerged from the chamber instantaneously. There was also a certain lag period before the mannoheptulose-containing medium reached the perfusion chamber. These technical aspects are illustrated in Fig. 3, which shows the results of a control experiment performed without islets in the chamber. When an insulin-containing medium was abruptly changed to a medium free of insulin, there was a time lag of about 1 min before the insulin concentration of the effluent decreased more than 50%. Therefore the mannoheptulose-induced retardation of insulin release must be assumed to have been even faster than is indicated by Fig. 2.

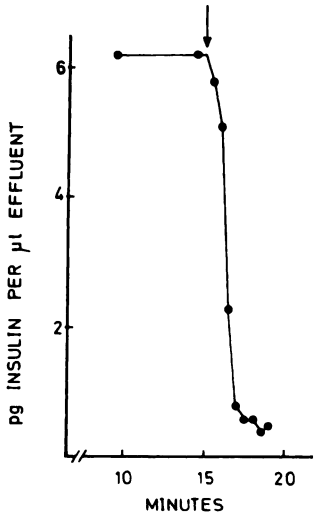

FIG. 3. *Insulin washout from microperfusion chamber.*

The microperfusion chamber was fed basal medium supplemented with ox insulin at a concentration of 6 ng/ml. After 15 min (arrow) the medium was rapidly switched to insulin-free basal medium. It can be seen that the insulin concentration of the effluent was reduced by 50% about 1 min after the change of medium. The flow rate was the same as in Fig. 2.

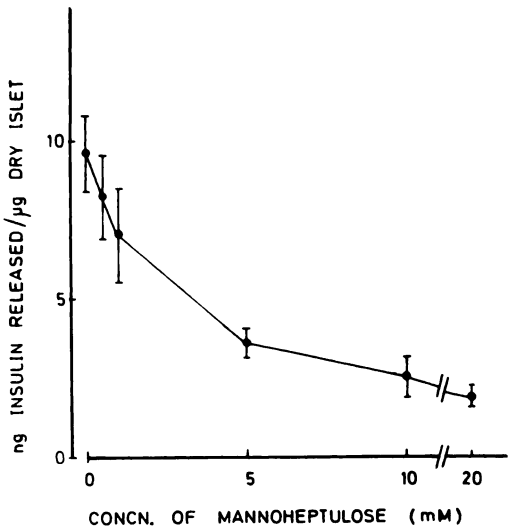

FIG. 4. *Effect of mannoheptulose on insulin release.*

Islets were incubated in medium containing 20 mM glucose plus mannoheptulose at various concentrations. The amounts of insulin released during 60 min are given as mean values ± standard errors for five or six experiments.

Inhibition of insulin release by low concentrations of mannoheptulose is suggested by Fig. 4, which shows a concentration-dependent reduction of the amounts of insulin released over a 60-min incubation.

Effect of mannoheptulose on glucose oxidation. Oxidation of glucose was measured in an attempt to determine whether the apparently immediate inhibition of insulin release could be explained by a similarly rapid inhibition of glucose metabolism. As in previous studies (9), the time course of glucose oxidation was characterized by an initial lag period, which precluded the demonstration of a prompt effect of mannoheptulose on

TABLE 2

Effects of mannoheptulose on glucose oxidation in pancreatic islets

Islets were incubated for 60 min at 37° in medium containing 12 mM uniformly ^{14}C-labeled glucose. In each experiment parallel incubations were performed without further additions to the medium (controls) and in medium containing 0.5, 1.0, or 5.0 mM mannoheptulose. The latter compound was also present during preliminary incubation for 30–60 min in glucose-free medium. Rates of $^{14}CO_2$ production are expressed as glucose equivalents oxidized per kilogram of islet (dry weight) per hour. Mean values ± standard errors are given for each medium as well as for the differences between test and control media over a series of five experiments. Statistical significance was estimated from the differences between paired test and control data.

Mannoheptulose	Rate of glucose oxidation	
	Experimental	Experimental minus control
mM	*mmoles/kg (dry wt)/hr*	
0[a]	36.5 ± 8.4	
0.5	18.4 ± 4.2	−18.0 ± 4.8[b]
1.0	15.0 ± 3.3	−21.5 ± 5.2[b]
5.0	7.4 ± 2.5	−29.1 ± 8.5[c]

[a] Control.
[b] $p < 0.02$.
[c] $p < 0.05$.

glucose metabolism. Incubations conducted for 60 min, however, revealed that glucose oxidation was inhibited by about 50% in the presence of as little as 0.5 mM mannoheptulose, using a glucose concentration of 12 mM (Table 2). A similarly substantial inhibition of glucose oxidation by mannoheptulose has previously been reported for collagenase-isolated islets of normal mice (13).

DISCUSSION

Our present observations suggest the following conclusions with respect to the mechanism of glucose-stimulated insulin release. Mannoheptulose is taken up by a glucose-sensitive transport site in the β-cells and has a prompt effect on insulin release. This is compatible with the hypothesis that insulin release is triggered by the binding of glucose to a membrane-located receptor (5, 14, 15), which may be a transport site. Such a hypothesis resembles the one previously proposed as an explanation of insulin release in response to amino acids (16–19). It should be pointed out that the membrane-receptor hypothesis is complicated by a considerable discrepancy between the extent to which glucose transport is inhibited by phlorizin or mannoheptulose, on the one hand, and the effects on insulin release, on the other. Whereas phlorizin has a much stronger effect on glucose transport than on insulin release (12), the reverse is true for mannoheptulose (6). From this we conclude that insulin release is not governed by total glucose transport. If insulin release is triggered by the binding of glucose to a membrane-located receptor, this receptor either is not a transport site or is responsible for only a small fraction of the total glucose uptake. The question whether there exist multiple transport systems for glucose in the β-cells is likely to be answered by detailed studies of the transport kinetics with particular reference to the mutually inhibiting actions of mannoheptulose and glucose. At present we are unable to report such data because of the technical problems involved in measuring the true rate of mannoheptulose uptake by β-cells after very short incubation times.

Besides demonstrating an interaction between mannoheptulose and the β-cell membrane, the present data remove one reason for uncertainty as to whether mannoheptulose inhibits insulin release by intracellular interference with glucose metabolism (2–4). Although transport of mannoheptulose was slow, a few minutes of incubation with 5 mM mannoheptulose resulted in intracellular concentrations of the same magnitude as the concentrations added to the medium inhibiting insulin release and glucose oxidation. If insulin release depends on glucose metabolism, inhibition of glucose phosphorylation is probably a functionally significant event that could at least contribute to the effect caused by blocking of the hypothetical membrane-located receptor.

No categorical statement as to the site at which glucose triggers insulin release is presently warranted. Perhaps the membrane-receptor alternative should be favored, because insulin release does not always parallel

changes in the glucose 6-phosphate level in β-cells (5, 12, 20) and because compounds such as phlorizin (12), galactose (14, 21), and glucosamine (14, 21, 22), as well as mannoheptulose (23), can stimulate insulin release under appropriate conditions. If such a membrane-located receptor exists, the scrutiny of sugars for structure-activity relationships should help to establish the stereochemical requirements for stimulation and inhibition of insulin release. From this viewpoint it may prove informative that, in addition to mannoheptulose, D-glucoheptulose elicits a hyperglycemic response when administered to rats (24).

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