BBA 75680

EVIDENCE FOR MEDIATED TRANSPORT OF GLUCOSE IN MAMMALIAN PANCREATIC β-CELLS

BO HELLMAN, JANOVE SEHLIN AND INGE-BERT TÄLJEDAL
Department of Histology, University of Umeå, Umeå (Sweden)
(Received February 15th, 1971)

SUMMARY

Uptake of glucose by microdissected pancreatic islets of obese–hyperglycemic mice was studied at 8°. The use of a double-label procedure permitted correction for label in the extracellular space. The following observations and interpretations were made:

1. L-Glucose was restricted to the sucrose space, whereas D-glucose was uniformly equilibrated over the β-cell membrane.

2. L-Glucose (5–40 mM) had no effect on the uptake of D-glucose (1 mM).

3. The uptake of D-glucose was saturable with a v_{max} of about 400 mmoles/h per kg dry islet, and with a K_m around 50 mM.

4. At a medium concentration of 5 mM D-glucose, the uptake of this sugar was almost completely blocked by 10 mM phlorizin. Under similar conditions, 20 mM mannoheptulose had no significant effect on D-glucose uptake.

The results contradict the previous hypothesis that the β-cell membrane is freely permeable to D-glucose. It is suggested that the uptake of glucose by these cells is mediated by a membrane-located transport molecule with stereospecificity for D-glucose. Renewed attention should therefore be given to the β-cell membrane as a possible locus for the triggering of insulin release by D-glucose.

INTRODUCTION

The mechanism by which glucose stimulates insulin release is obscure. It is not even known whether it is the glucose molecule as such or some metabolite that serves to trigger insulin release. Failure of phlorizin to inhibit insulin release has been suggested to indicate that the β-cell is freely permeable to glucose and that triggering of insulin release is distal to glucose entry[1,2]. In a preliminary report, however, we have suggested that glucose uptake by the mammalian β-cell depends on the stereochemical configuration of the molecule, indicating an interaction between D-glucose and the β-cell membrane[3]. The present paper gives a more precise and extended demonstration of the difference between D-glucose and L-glucose uptake by mammalian β-cells. In addition, two new arguments are added to the discussion as it is shown that the uptake of D-glucose by the β-cell is a saturable and, indeed, a phlorizin-sensitive process.

MATERIALS AND METHODS

Radioactive chemicals were obtained from The Radiochemical Centre, Amersham, England. The phlorizin used was a chromatographically pure (purity > 99.5 %) preparation from Fluka AG, Buchs, Switzerland. All other reagents were of analytical grade. Distilled and deionized water was used throughout.

Adult obese–hyperglycemic mice (gene symbol: obob) were starved overnight. For each experiment about 25 fresh pancreatic islets were isolated by free hand microdissection at 2° (ref. 4) in gassed (O_2 + CO_2, 95:5) Krebs–Ringer bicarbonate buffer supplemented with 0.3 % (w/v) human serum albumin. Gassed buffer containing 0.3 % (w/v) human serum albumin was also used in all subsequent incubations.

After pre-incubation at 37°, batches of three islets each were incubated at 8° in 200 μl medium supplemented with labelled sugar. The concentrations and specific activities of these sugars, the medium concentrations of other additives, as well as the incubation times varied between experiments, as indicated in the legends to figures and tables. All incubations were performed in duplicate with shaking (140 strokes/min, 3.5 cm amplitude). After incubation, the islets were placed on aluminium foils and gently freed of contaminating fluid with the aid of a micropipette. The islets were then freeze-dried at −40° and 0.001 mm Hg overnight, weighed on a quartz-fibre balance and dissolved by incubation for 45 min in 100 μl of hyamine at room temperature. 10 ml of scintillation fluid (5 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-[5-phenyloxazolyl]-benzene in 1 l of toluene) was then added, and counting was carried out in a liquid scintillation spectrometer (Packard model 3375). The setting of the discriminators was such that less than 0.5 % of the counts in the ^{3}H channel were also counted in the ^{14}C channel. Spill-over to the ^{3}H channel was 25 % of the counts in the ^{14}C channel. The specific radioactivities were chosen to give approximately the same counts/min in either channel. The observed counts/min values were translated to mmoles of sugar by comparison with external standards counted in parallel with the islets. These standards consisted of 5 μl incubation medium dissolved in 100 μl of hyamine.

RESULTS

Distribution of sucrose, D-*glucose and* L-*glucose*

The distribution of D-glucose in relation to that of sucrose was studied in three different series of experiments. In order to use each islet as its own control, these incubations were performed in media containing both [^{14}C]glucose and [^{3}H]sucrose. The first series of experiments was designed to check whether the presence of sucrose in such double-label experiments would disturb the uptake of D-glucose. As shown in Table I, the islet uptake of [6,6′-^{3}H]sucrose was linearly related to sucrose concentration within the range 0.1–20 mM. Sucrose at these concentrations did not affect the islet content of uniformly ^{14}C-labelled D-glucose after 15 min of incubation (Table I).

Islet uptake of D-glucose and L-glucose with time was studied in the next two series of experiments. Fig. 1 shows that the uptake of L-glucose was more rapid than that of sucrose, which resulted in an initial excess of the former sugar. However, this excess declined with time and at equilibrium (45 min) the islet content of L-glucose was not different from that of sucrose. In contrast to L-glucose, D-glucose was not restricted

TABLE I

UPTAKE OF D-GLUCOSE IN THE PRESENCE OF SUCROSE

Five experiments were performed. In each of these, islets from a single animal were pre-incubated for 70 min in glucose-free Krebs–Ringer buffer. Parallel incubations were then performed for 15 min at 8° in media containing [6,6′-^{3}H]sucrose (1.3–250 mC/mmole) at the concentrations listed. All of these media also contained 5.0 mM uniformly ^{14}C-labelled D-glucose (2.0 mC/mmole). The amount of D-glucose taken up by islet cells was calculated by correcting for label in the sucrose space. Results are given as mean values ± S.E.

Medium concn. of [^{3}H]sucrose (mM)	*Sugar content of islet (mmoles/kg dry weight)*	
	Total [^{3}H]sucrose	*Intracellular D-[^{14}C]glucose*
0.1	0.28 ± 0.02	5.03 ± 0.29
1.0	2.56 ± 0.13	5.08 ± 0.18
5.0	14.1 ± 1.6	5.08 ± 0.70
20.0	59.3 ± 2.0	4.57 ± 0.34

to the sucrose space (Fig. 1). If it is assumed that sucrose was distributed within the extracellular space, it can be concluded that D-glucose but not L-glucose enters the β-cells. In a previous study[5], the distribution of urea and sucrose in the islets of obese–hyperglycemic mice indicated that the intracellular space is 1.2 times the islet dry weight. On these premises, it can be calculated from the present results that the intracellular concentration of uniformly ^{14}C-labelled D-glucose was 4.7 ± 0.3 mM (mean values ± S.E. for 5 experiments) after equilibration with 5 mM uniformly ^{14}C-labelled D-glucose in the medium.

Since L-glucose was restricted to the sucrose space, the difference between the uptake curves for D-glucose and L-glucose should be a measure of D-glucose uptake by

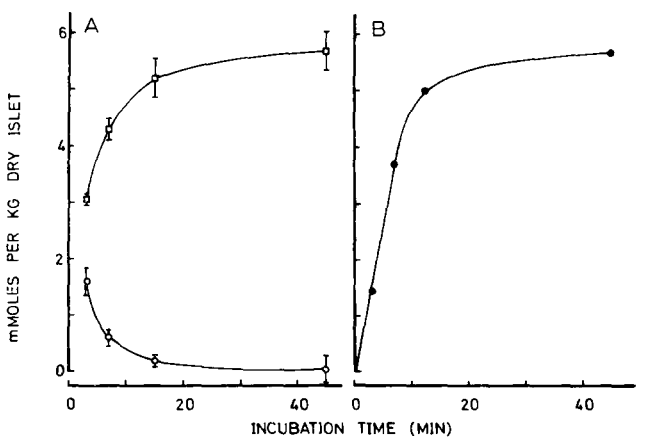


Fig. 1. Effect of time on the sugar uptake by islets. After pre-incubation for 70 min in glucose-free Krebs–Ringer buffer, islets were incubated for different periods of time in media supplemented with 5.0 mM [6,6′-^{3}H]sucrose (5.0 mC/mmole) as well as with 5.0 mM uniformly ^{14}C-labelled D-glucose (2.0 mC/mmole) or 5.0 mM L-[1-^{14}C]glucose (3.0 mC/mmole). A, the islet content of D-glucose (□—□) and L-glucose (○—○) from which the islet content of sucrose was subtracted. B, the islet content of D-glucose from which the islet content of L-glucose was subtracted. Mean values ± S.E. for five (D-glucose) or six (L-glucose) different experiments.

TABLE II

EFFECT OF L-GLUCOSE ON THE UPTAKE OF D-GLUCOSE

Six experiments were performed. In each of these, islets from a single animal were preincubated for 30 min in glucose-free Krebs–Ringer buffer. Parallel incubations were then performed for 3 min at 8° in media containing non-radioactive L-glucose at the concentrations listed. All of these media also contained 1.0 mM uniformly ^{14}C-labelled D-glucose (10.0 mC/mmole) and 0.1 mM [6,6′-^{3}H]-sucrose (250 mC/mmole). The amount of D-glucose taken up by islet cells was calculated by correcting for label in the sucrose space. Results are given as mean values ± S.E. for each medium as well as for the differences between test and control media.

Medium concn. of L-*glucose (mM)*	*Intracellular content of uniformly* ^{14}*C-labelled* D-*glucose (mmoles/kg dry weight)*	*Test minus control*	*% change of uptake*
0 (control)	0.58 ± 0.11	—	—
5	0.65 ± 0.11	0.07 ± 0.04	+ 12
20	0.52 ± 0.11	−0.06 ± 0.06	− 10
40	0.60 ± 0.11	0.02 ± 0.07	+ 3

the β-cells. As suggested by the data of Fig. 1, the rate of net uptake of D-glucose was approximately linear for more than 5 min. This linear net uptake at 5 mM D-glucose amounted to about 30 mmoles/h per kg dry islet.

Since D-glucose and L-glucose are almost identical in size, solubility and other physical properties, the above results point to L-glucose as the ideal extracellular marker in studies of D-glucose uptake by the β-cells. Before being used as such, however, L-glucose had to be tested for possible effects on the uptake of D-glucose. It can be seen in Table II that 5–40 mM L-glucose did not interfere with the uptake of D-glucose by β-cells at a medium concentration as low as 1 mM.

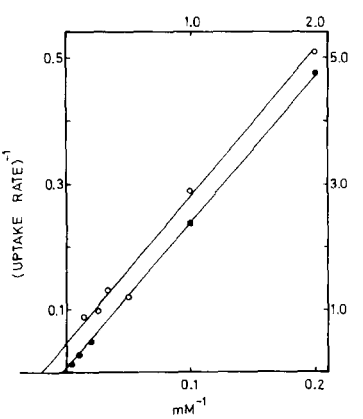


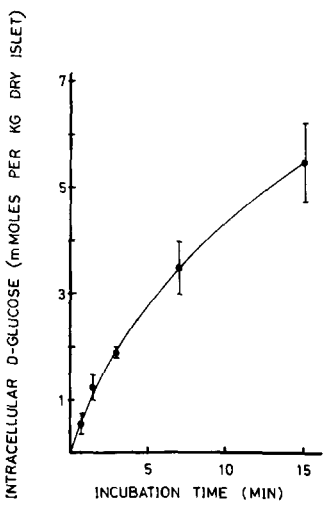
Fig. 2. Double-reciprocal plot of D-glucose uptake by β-cells in relation to the D-glucose concentration of the medium. After pre-incubation for 30 min in glucose-free Krebs–Ringer buffer, islets were incubated for 3 min in media containing 0.5–70 mM D-[6-^{3}H]glucose (0.3–40 mC/mmole) and 2.5 mM L-[1-^{14}C]glucose (3.0 mC/mmole). The amounts of D-glucose taken up by β-cells were calculated by correction for label in the L-glucose space. Uptake rates are expressed as mmoles/3 min per kg dry islet. Note that two different scales are used on the ordinate as well as on the abscissa. ●—●, results obtained with 0.5–20 mM D-glucose (right and top scales.) ○—○, results obtained with 7–70 mM D-glucose (left and bottom scales). Each point is the mean of 3–8 different experiments.

Concentration dependence of net uptake of D-*glucose*

Islets were incubated for 3 min in media containing different concentrations of D-[6-^{3}H]glucose. The simultaneous presence in the media of 2.5 mM L-[1-^{14}C]glucose permitted direct correction for extracellular label in each batch of islets. The net uptake of D-glucose by islet cells was clearly saturable. A double-reciprocal plot of the data (Fig. 2) resulted in an estimation of v_{max} as about 400 mmoles/h per kg dry islet, and of K_m as about 50 mM.

Equilibrium exchange of D-*glucose*

Fig. 3 shows the time course of D-[6-^{3}H]glucose uptake by islets equilibrated with nonradioactive D-glucose. The initial rate of uptake was maintained for at least 1 min. It can therefore be assumed that the uptake by preloaded β-cells at 45 sec is predominantly a measure of initial unidirectional D-glucose flux. The concentration dependence of D-glucose entry, measured by the equilibrium-exchange method, is shown in Fig. 4. Although these data are attended by comparatively great random errors, they are clearly compatible with a similarly low affinity for the transport system as suggested by Fig. 2.



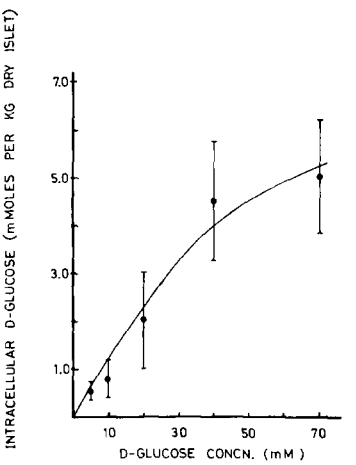


Fig. 3. Effect of time on D-glucose uptake by pre-loaded β-cells. The β-cells were equilibrated with 5.0 mM nonradioactive D-glucose by pre-incubation for 30 min. Islets were then incubated for different periods of time in media containing 5.0 mM D-[6-^{3}H]glucose (4.0 mC/mmole) and 2.5 mM L-[1-^{14}C]glucose (3.0 mC/mmole). The amounts of D-glucose taken up by the β-cells were calculated by correction for label in the L-glucose space. Each point represents the mean $\pm$ S.E. for different experiments. Listed in order of increasing incubation time, the numbers of experiments were 6, 5, 6, 4, and 3.

Fig. 4. Concentration dependence of the rate of exchange of D-glucose at equilibrium. The β-cells were equilibrated with 5.0–70 mM nonradioactive D-glucose by pre-incubation for 30 min. Islets were then incubated for 45 sec in media in which the nonradioactive D-glucose had been replaced by equimolar amounts of D-[6-^{3}H]glucose (0.3–4.0 mC/mmole). These incubation media also contained 2.5 mM L-[1-^{14}C]glucose (3.0 mC/mmole). The amounts of D-glucose taken up by the β-cells were calculated by correction for label in the L-glucose space. Each point represents the mean $\pm$ S.E. for six different experiments.

TABLE III

EFFECT OF PHLORIZIN ON THE UPTAKE OF D-GLUCOSE

Six experiments were performed. In each of these, islets from a single animal were pre-incubated for 30 min in Krebs–Ringer buffer supplemented with 5.0 mM nonradioactive D-glucose and with phlorizin at the concentrations listed. Parallel incubations were then performed for 3 min at 8° in fresh media containing the same concentrations of phlorizin as used during pre-incubation. During the 3-min incubations the media also contained 5.0 mM D-[6-^{3}H]glucose (4.0 mC/mmole) and 2.5 mM L-[1-^{14}C]glucose (3.0 mC/mmole). The amount of D-glucose taken up by the islet cells was calculated by correcting for label in the L-glucose space. Results are given as mean values ± S.E. for each medium as well as for the differences between test and control media.

Medium concn. of phlorizin (mM)	*Intracellular content of* D-[6-^{3}H]*glucose (mmoles/kg dry weight)*	*Test minus control*	*% change of uptake*
0 (control)	1.23 ± 0.20	—	—
1	0.65 ± 0.11	−0.58 ± 0.16*	−47
5	0.25 ± 0.11	−0.98 ± 0.21**	−80
10	0.08 ± 0.13	−1.15 ± 0.27**	−94

*$P < 0.02$.
**$P < 0.01$.

Effect of phlorizin or mannoheptulose on uptake of D-*glucose*

The effect of phlorizin on uptake of D-glucose by pancreatic islets is shown in Table III. At a medium concentration of 5 mM D-glucose, the uptake was progressively inhibited by increasing concentrations of phlorizin. A considerable and significant effect was obtained with as little as 1 mM phlorizin. At a phlorizin concentration of 10 mM there was an almost complete block of D-glucose uptake under the present experimental conditions.

Nine experiments were performed to test the effect of D-mannoheptulose on D-glucose uptake. Islets from a single animal were pre-incubated for 30 min in the presence or absence of 20 mM mannoheptulose. Parallel incubations were then performed for 3 min in a medium containing 20 mM mannoheptulose, 5 mM D-[6-^{3}H]-glucose (4.0 mC/mmole) plus 2.5 mM L-[1-^{14}C]glucose (3.0 mC/mmole), and in a mannoheptulose-free control medium. After correction for label in the L-glucose space, the islet cells incubated with mannoheptulose contained 0.89 ± 0.12 mmoles of D-[6-^{3}H]glucose per kg dry islet (mean value ± S.E.) as compared to 1.25 ± 0.17 mmoles/kg dry islet for the controls. The difference between test and control incubations, 0.36 ± 0.16 mmoles/kg dry islet, was not statistically significant ($P \approx 0.05$).

DISCUSSION

A difference between the uptake of D-glucose and that of L-glucose by islet tissue was first reported in the fish *Opsanus tau*[6]. The β-cells of such islets are relatively few in number and may not be directly comparable to those of mammals. In a preliminary communication, however, we have published data in support of a stereospecific glucose uptake also in the islets of obese–hyperglycemic mice[3]. In that preliminary study, contaminating and extracellular label was removed by washing the islets after incubation. Although there is good evidence that such washing did not greatly affect the intracellular content of glucose, a more accurate method was needed

in studies of transport kinetics. In the present investigation, washing of the islets was therefore omitted. Instead a double-label procedure was used to determine the amount of extracellular label in each batch of incubated islets.

The results obtained with the present method strongly suggest that the uptake of D-glucose by the β-cell is brought about by a stereospecific transport system similar to those postulated for a number of other cells[7,8]. This conclusion is based on the following line of evidence. First, the islets used contain more than 90 % β-cells. It should be emphasized that these islets exhibit an adequate release of insulin in response to a variety of secretagogues including glucose[9] and that their hyperplasia is probably due to peripheral insulin resistance in the obese–hyperglycemic mouse[10,11]. Second, L-glucose was restricted to the sucrose space, whereas D-glucose was uniformly equilibrated over the islet cell membrane. Third, D-glucose uptake was saturable and, fourth, was inhibited by phlorizin.

The mechanism by which glucose stimulates insulin release is obscure. Alleged failure of phlorizin to inhibit insulin release has been suggested to indicate that the β-cell membrane is freely permeable to glucose and that glucose triggers insulin release by virtue of its metabolism[1,2]. Consequently, most interest has so far been focussed on glucose metabolism in the β-cell. An important implication of the present results is, however, that D-glucose does interact with the β-cell membrane. The possibility that insulin release is triggered by the D-glucose molecule should therefore be reconsidered. This idea has recently also been expressed in a discussion of electrophysiological islet data[12].

Mannoheptulose inhibits insulin release and glucose oxidation in the β-cell[1,2,13]. It has also been reported that the β-cell content of glucose *in vivo* can be influenced by injecting mannoheptulose into rats, which might suggest that mannoheptulose inhibits insulin release by interfering with glucose entry into the β-cell[14]. In the present study, mannoheptulose had no significant effect on D-glucose uptake, although the mean rate for islets incubated with mannoheptulose was lower than that of controls. Since some effect was seen with washed islets in our preliminary report[3] the possibility cannot be excluded that mannoheptulose utilizes the same transport system as D-glucose. It seems clear, however, that any effect of mannoheptulose on glucose transport is small in comparison to its drastic effects on insulin release. This suggests that mannoheptulose does not inhibit insulin release by limiting the flow of D-glucose into the β-cell.

In discussing the kinetics of D-glucose uptake by the β-cell, it must be remembered that the present results were obtained at 8°. This low temperature was chosen in order to reduce metabolism to a minimum. Furthermore, there are great technical difficulties in measuring linear rates in islets incubated at 37°. The maximum velocity for D-glucose uptake at 8° was found to be as great as 400 mmoles/h per kg dry islet. This value is 6 times greater than the maximum rate of glucose oxidation at 37° (ref. 15), which indicates that glucose transport is normally not rate-limiting for glucose utilization in the β-cells. The observed concentration dependence of the uptake of D-glucose suggests that the transport molecule has at least one binding site of low affinity similar to that suggested for erythrocytes[16]. In a hypothesis considering insulin release as triggered at the β-cell membrane, this low-affinity binding site could, perhaps, provide the necessary sensitivity to changes in the extracellular glucose concentration. Such a hypothesis might furthermore be related to the internal transfer model of LIEB AND STEIN[16], who suggested that glucose transport involves cyclic con-

formational changes of a membrane-bound protein. A test of the applicability of the internal transfer model to the β-cell also requires data on the initial rate of net loss of D-glucose from preloaded β-cells. For technical reasons such measurements have not yet been possible with sufficient accuracy.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (12X-562; 12X-2288), the Swedish Diabetes Association, and the U.S. Public Health Service (AM-12535).

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