EFFECTS OF MANNOHEPTULOSE ON THE DYNAMICS OF GLUCOSE OXIDATION IN THE PANCREATIC β-CELLS

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1. Introduction

It has been suggested that glucose stimulates insulin release through its metabolism in the pancreatic β-cells. This hypothesis has partly been based on the observation of striking correlations between insulin release and glucose oxidation in islets incubated for 1 hr or longer [1]. Studies on various types of perfused or superfused pancreas preparations have revealed that the insulin secretory response is multiphasic with time [2–4]. This makes it of interest to measure the oxidation of glucose over periods shorter than 60 min to estimate whether both the initial and the late phases of insulin release are related to glucose metabolism. Such measurements are difficult to perform because of the small size of islets in relation to the sensitivity of the analytical methods available. In this paper a technique is described which makes possible incubation times shorter than 10 min. It will be shown that the 7-carbon sugar mannoheptulose does not significantly affect glucose oxidation during the first 15 min of incubation, although it is known to inhibit insulin release almost immediately [5–6].

2. Materials and methods

[U-^{14}C]D-glucose, sodium [^{14}C]bicarbonate and [1-^{14}C]L-glucose were obtained from the Radiochemical Centre, Amersham, England. All other chemicals were commercially available reagents of analytical grade. Distilled and deionized water was used throughout.

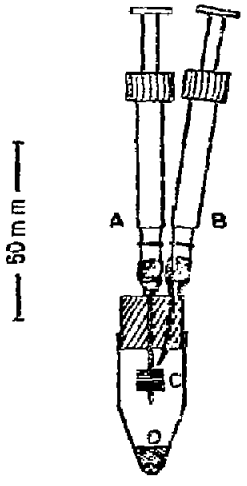

Fig. 1. The incubation equipment consisted of a small glass vessel with a total volume of about 4 cm^3. The vessel was sealed with a rubber stopper through which two needles were run and fitted to syringes, one (A) containing 100 µl 0.1 N HCl and the other (B) containing 100 µl Hyamine. Twelve circular pieces of filter-paper (C) were put on the A-needle. At the end of the incubation the filter-paper was soaked with Hyamine and the $^{14}CO_2$ produced by the incubated islets (D) was absorbed.

Obese-hyperglycemic mice (gene symbol *ob/ob*) from the Umeå colony [7] were used as the source of pancreatic islets. For each experiment about 25 islets were microdissected freehand [8] in Krebs–Ringer bicarbonate buffer (KRB) equilibrated with O_2 and CO_2 (95:5) and supplemented with 3 mM glucose. After dissection the islets were preincubated for 30 min at 37° in the same type of medium as that used during dissection.

KRB media (100 µl) supplemented with 10 mM [U-^{14}C]D-glucose (1.7–2.4 mCi/mmole) and with or without mannoheptulose were pre-warmed in small incubation vessels (see fig. 1) to 37° during continuous gassing. Prior to incubation two needles were run through each stopper and fitted to syringes containing 100 µl 0.1 N HCl and 100 µl Hyamine, respectively. It was checked in control experiments that there was no gas leakage out of the vessels. After the pre-incubation 3–6 islets were transferred to each incubation vessel and the rubber stoppers were inserted. After various periods of incubation the islet metabolism was arrested and $^{14}CO_2$ was liberated by injection of HCl into the incubation medium. Hyamine was then injected onto a small wad of pieces of filter-paper (see fig. 1) and $^{14}CO_2$ trapped into the Hyamine by shaking the vessels (140 strokes per min; 3.5 cm amplitude) for 120 min at room temp. Blanks were obtained by incubating media without islets. The pieces of filter-paper were removed from the needles and transferred to liquid scintillation vials. After the addition of 10 ml scintillation fluid (5 g of 2,5-diphenyloxazole and 50 mg of 1,4-*bis*-2-[5-phenyl-oxazolyl]-benzene in 1 l of toluene), radioactivity counting was performed in a liquid scintillation spectrometer (Packard Model 3375). The observed cpm values were corrected for blanks and then translated to nmoles of glucose equivalents oxidized by comparison with external standards. These consisted of 5 µl incubation medium dissolved in 100 µl Hyamine. After incubation the islets were transferred to aluminium foil, freeze-dried (< 40°; 0.001 mm Hg) overnight and weighed on a quartz fibre balance [9].

To check the recovery of $^{14}CO_2$, vessels were incubated for 30 min with 100 µl KRB supplemented with 0.4 µCi of sodium [^{14}C] bicarbonate. The recovery was 91 ± 2% (n = 7). Diffusion of glucose into the islet extracellular space was analysed with ^{14}C-labelled L-glucose according to a previously described method [10]. The statistical probability that the effect of mannoheptulose was due to chance was estimated from the mean difference between test and control incubations in a series of identical but separate experiments. The expression 'experiment' refers to a set of incubations from a single animal. Within each experiment the factors under study were tested mostly in duplicate or triplicate incubations.

Table 1
Production of $^{14}CO_2$ in islets incubated with ^{14}C-labelled glucose.

Incubation time (min)	Total cpm per vessel	Blank cpm per vessel	µg islet dry weight per vessel
7	510 ± 34 (11)	334 ± 15 (12)	44.1 ± 4.0 (11)
15	620 ± 51 (11)	342 ± 28 (12)	28.4 ± 2.7 (11)
30	1036 ± 101 (14)	317 ± 31 (12)	28.2 ± 3.3 (14)

The data of the controls from fig. 2 are given as radioactivity absorbed in Hyamine after incubation of islets (total cpm), radioactivity in Hyamine from media incubated without islets (blank cpm) and islet dry weight per vessel. The number of incubated vessels is given within parentheses.

3. Results

Table 1 summarizes the mean of the cpm for islets and blanks as well as the islet dry weight for the control incubations presented in fig. 2. At short incubation times the blanks comprise a substantial part of the total cpm values. To obtain sufficient accuracy, two blanks were run in all experiments for each composition of medium. In the 7-min incubations 6 islets were used in each vessel whereas longer incubations were performed with 3 islets. The average dry weight of single islets was about 9 µg.

As shown in fig. 2 the oxidation of 10 mM glucose was a linear function of time except for an initial lag period during the first few minutes. To test whether this lag could be due to slow diffusion of sugar into the extracellular space, isolated islets were incubated with ^{14}C-labelled L-glucose. This sugar represents an ideal extracellular marker [10] for this purpose, since it can be assumed to have the same diffusion constant as D-glucose. Half-maximal uptake was reached at about 45 sec but complete equilibration of L-glucose did not occur until about 15 min of incubation. The lag period of glucose oxidation could therefore probably be explained by the diffusion of glucose into the islet extracellular water.

Whereas mannoheptulose caused a highly significant inhibition ($P < 0.001$) of glucose oxidation at

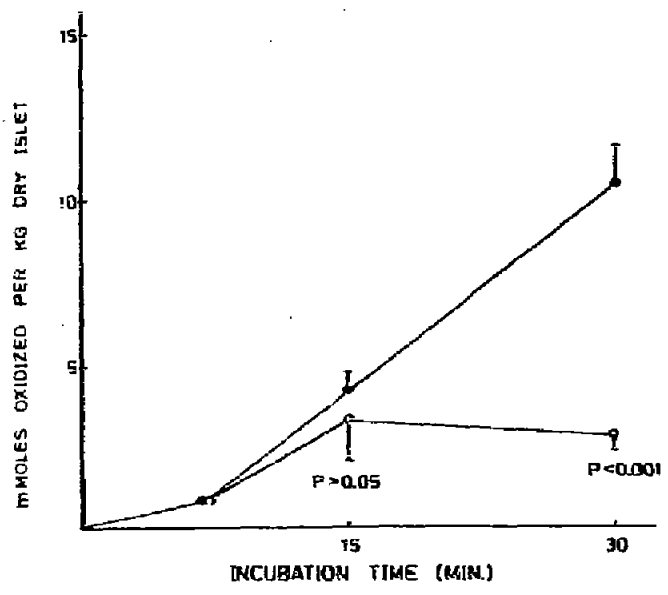

Fig. 2. Islets were incubated for 7, 15 and 30 min in a medium containing 10 mM ^{14}C-labelled D-glucose. In each experiment parallel incubations were performed with (○) and without (●) 5 mM D-mannoheptulose. Rates of $^{14}CO_2$ production are expressed as glucose equivalents oxidized per kg of islet dry weight. Mean values ± S.E.M. are given in the figure except for the 7-min values, where the bars representing S.E.M. were smaller than the circles representing mean values. The number of experiments was 6, 5 and 5, respectively.

30 min of incubation, no significant effect was obtained at 15 min, though there was a slight tendency towards inhibition. During the initial 7 min of incubation mannoheptulose did not affect glucose oxidation at all. The data in fig. 2 further indicate that the islet oxidation of glucose is completely blocked between 15 and 30 min of incubation with mannoheptulose.

4. Discussion

Most studies on the glucose oxidation in pancreatic islets have been performed by means of incubations in small glass wells kept within an outer glass container, preferably liquid scintillation vials [11]. Although useful in incubations for 1 hr or longer, this technique seems to lack enough sensitivity and precision for short-term experiments. This is mainly due to the inadequate temperature equilibration during the first few minutes. In the present procedure this difficulty has been circumvented by keeping the small incubation vials in direct contact with the water bath. Because of this, the media can be appropriately prewarmed to an exact temperature during continuous equilibration with O_2 and CO_2.

Mannoheptulose is a potent inhibitor of glucose-induced insulin release [6, 12–14], whereas it does not inhibit insulin release in response to leucine [15]. Mannoheptulose may therefore be a fairly specific blocker of the β-cell recognition system for glucose as a stimulus of insulin release [1]. It has been observed in long-term experiments that mannoheptulose markedly reduces the oxidation of glucose in the pancreatic islets [6, 16]. This is amply demonstrated with the present experimental set-up. The data also indicate that this inhibition of glucose oxidation is not manifest until 15 min of incubation, after which time glucose oxidation may be totally blocked. In previous 1-hr experiments 5 mM mannoheptulose was found to reduce the oxidation of 12 mM glucose from 36.5 to 7.4 mmoles per kg islet dry weight [6]. The latter value is almost exactly what would be expected for the corresponding control islets after 15 min of incubation if due consideration is paid to the longer lag period with the conventional technique [17].

Inhibition of insulin release starts promptly after the addition of mannoheptulose and is highly significant within the first 5 min of incubation [5–6]. In incubations lasting for 1 hr or longer there is a striking correlation between the apparent rate of oxidation or utilization of glucose and the rate of insulin release at different glucose concentrations [1]. This has been regarded as support for the view that glucose metabolism somehow controls insulin release. The present results do not contradict that this is the case for the late phase of insulin release. The observation of an uninhibited glucose oxidation during the initial 15 min, when insulin release is already retarded, makes it however tempting to assume that a direct receptor mechanism exists in the β-cells that is responsible for at least the early phase of glucose-stimulated insulin release.

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