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INHIBITION OF PANCREATIC B-CELL FUNCTION BY D-MANNOHEPTULOSE*)

By

Fukashi ISHIBASHI, Toshie SATO, Motoki TSUBOTA and Ryoso KAWATE

The Second Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan

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ABSTRACT

We have reported that alloxan inhibits glucose metabolism and glucose-induced ^{45}Ca uptake in the pancreatic B-cells, while non-B-islet cells are not inhibited by alloxan pretreatment. Therefore, it is possible to assess glucose metabolism and Ca uptake in the B-cells by measuring these parameters in alloxan treated and non-alloxan treated islets and determining the difference. In our previous paper, we found that the glucose dose-response relationships of glucose oxidation, glucose-induced ^{45}Ca uptake and insulin release were closely interrelated.

In this paper we have investigated the effect of D-mannoheptulose on glucose oxidation, glucose-induced ^{45}Ca uptake and insulin release in the B-cells by incubating isolated rat islets with and without alloxan pretreatment (20 mg/d*l*, for 6 minutes) in order to investigate the role of hexokinase in the regulation of glucose-induced insulin release in the pancreatic B-cells.

In the absence of D-mannoheptulose, the threshold of the three parameters ($^{14}CO_2$ formation from ^{14}C-U-D-glucose, glucose-induced ^{45}Ca uptake and insulin release) in the B-cells was approximately 5.5 mM glucose. The half-maximal responses for each of the three parameters occurred with 11.5 mM, 13.5 mM and 11.7 mM glucose, respectively. The maximal response for these parameters occurred with 20 mM glucose. When 1.0 mM D-mannoheptulose was added to glucose, the threshold for the three parameters increased to 11.0 mM with half maximal responses at 15.5 mM, 15.8 mM and 17.0 mM glucose, respectively. The maximal responses of the three parameters were obtained at 27.8 mM glucose. The addition of 2.5 mM D-mannoheptulose to glucose required more glucose to achieve half-maximal responses of the three parameters (27.4 mM, 27.5 mM and 25.5 mM, respectively) with maximal responses at 38.8 mM glucose.

The inhibitions by D-mannoheptulose of glucose oxidation, glucose-induced ^{45}Ca uptake and insulin release respectively were closely related to each other. These data indicate that hexokinase may play a regulatory role in glucose metabolism in the pancreatic B-cells resulting in the tight control of glucose-induced Ca mobilization and insulin release.

*) 石橋不可止，佐藤敏枝，坪田元記，川手亮三：D-mannoheptulose による膵B細胞機能の阻害

INTRODUCTION

The seven carbon sugar, D-mannoheptulose, is known to inhibit glucose-induced insulin release in islets[1,2] by inhibiting glucose metabolism at the phosphorylating steps[3,4]. Of the various metabolic inhibitors available to study the influence of glucose metabolism on glucose-induced insulin release, D-mannoheptulose is particularly appropriate[5], because at concentrations sufficient to block the effects of hexoses on islet tissue, it is without effect on metabolism and insulin release by D-glyceraldehyde[3] and other metabolizable substances[3].

Although collagenase-digested rat pancreatic islets are composed of at least 4 different cell populations[6], it has been assumed that function of isolated islet is representative of B-cell.

We and other investigators have reported that the B-cytotoxic agent, alloxan inhibits glucose metabolism[7–9] in pancreatic B-cells and induces disturbances of ionic fluxes[10,11] essential to the initiation of glucose-induced insulin release. Therefore, it is possible to examine the relationships among glucose metabolism, glucose-induced ^{45}Ca uptake and insulin release in the pancreatic B-cell by subtracting glucose metabolism and Ca uptake in alloxan-treated islets from those in untreated islets. Using this method, we found a close interrelationship among these three parameters in the B-cell[12].

The present investigation is devoted to a comparison of the effects of D-mannoheptulose on glucose oxidation, glucose-induced ^{45}Ca uptake and insulin release from the B-cell. We have analyzed the effects of D-mannoheptulose on the glucose dose-responses of glucose oxidation, glucose-induced ^{45}Ca uptake and insulin release.

MATERIALS AND METHODS

Isolation of rat pancreatic islets and alloxan treatment

Pancreatic islets were isolated by collagenase digestion[13] from ad libitum fed male Wistar rats weighing 350–400 g. Islets were preincubated for 30 minutes with a modified Krebs-Ringer bicarbonate (KRB) medium containing 115 mM NaCl, 1 mM KH_2PO_4, 20 mM $NaHCO_3$, 5 mM KCl, 1 mM $MgCl_2$ and 0.5% (W/V) bovine serum albumin. Islets were then exposed to alloxan (20 mg/d*l*) for 6 minutes. Alloxan was dissolved in the glucose-free KRB medium immediately before use in order to prevent decomposition. The alloxan treated islets were washed twice with KRB medium before use in subsequent experiments.

Effect of D-mannoheptulose on glucose-induced insylin release

Batches of 5 islets were placed in petri dishes (30 mm I. D. and 10 mm high) and incubated for 60 minutes at 37°C in KRB medium containing glucose (2.7, 5.5, 11.0, 16.7, 20.0, 27.8, 38.8 and 55.5 mM) without or with D-mannoheptulose (1.0 mM or 2.5 mM). The pH of the medium was kept at 7.4 by continuous gassinh with a mixture of 95%O_2/5%CO_2. At the end of incubation, the medium was removed and frozen at −20°C for insulin assay. Insulin content in medium was determined by the double antibody radioimmunoassay method of Morgan and Lazarow[14].

Effect of D-mannoheptulose on glucose oxidation in pancreatic B-cell

The following experiments were performed with untreated and alloxan-treated islets. Batches of 10 islets were placed in siall incubation vials (10 mm I. D. and 20 mm high) containing 200 μl of glucose free KRB medeum supplimented with ^{14}C-U-D-glucose (2.7–55.5 mM) without or with D-mannoheptulose (1.0 mM or 2.5 mM). Incubation vials containing islets were placed in scintillation vials which were sealed and kept at 37°C for 60 minutes. Other technical aspects of the method have been described elsewhere[9]. Glucose oxidation in the B-cell was calculated by subtracting $^{14}CO_2$ formed in the alloxan treated islets from values obtained in the untreated islets.

Effect of D-mannoheptulose on glucose-induced ^{45}Ca uptake in the pancreatic B-cells

Batches of 10 alloxan-treated or untreated islets were preincubated for 30 minutes in 2 m*l* of calcium free KRB medium. This was followed by incubation for 60 minutes in 2 m*l* of the same medium containing $^{45}CaCl^2$ (10 μCi/m*l*) and glucose (2.7–55.5 mM) without or with D-mannoheptulose (1.0 mM or 2.5 mM). Islets were separated from washing medum by Millipore filtration (pore size 0.45

μ). The islets and filter were placed in a mixture of toluene-PPO-POPOP and ^{45}Ca radioactivity was determined by a liquid scintillation spectrometer. The glucose-induced ^{45}Ca uptake in the B-cell was calculated by subtracting ^{45}Ca uptake in the alloxan treated islets from that of untreated islets.

The materials used and their sources were as follows; Collegenase type IV 160 U/mg (Worthington Biochem. Co.,), alloxan monohydrate, bovine serum albumin, D-glucose and D-mannoheptulose (Sigma Chemical Co., ^{125}I-insulin and ^{14}C-U-D-glucose (3 mCi/mM) (Radiochemical Center, Amersham, Engl.), and $^{45}CaCl_2$ (4. 8mCi/mg Ca) (New Engl. Nucl. Co.).

All results were expressed as mean or mean $\pm$ SEM.

RESULTS

In Fig. 1, the suppression of glucose-induced insulin release by 1. 0 mM or 2. 5 mM D-mannoheptulose was plotted as a function of glucose concentration. When islets were incubated with glucose alone, 5. 5 mM glucose was the lowest concentration to elicit insulin release and half-maximal release ocurred at 11. 7 mM.

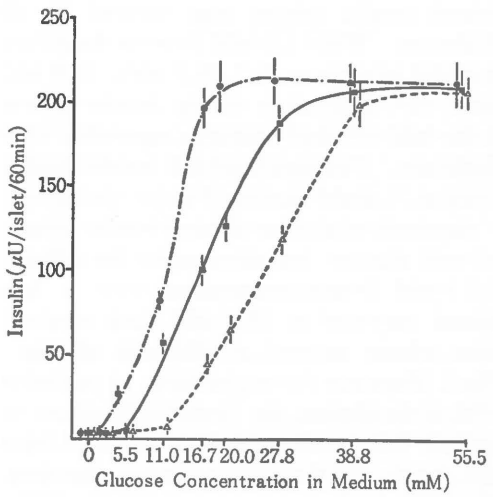

Fig. 1. Effect of D-mannoheptulose on glucose-induced insulin release was plotted as a function of glucose concentration in medium. After 30 minute preincubation with glucose free KRB medium, 5 islets per dish were incubated for 60 minutes at 37°C in KRB medium containing glucose (2. 7-55. 5 mM) without (●- - -●) or with 1. 0 mM (■——■) or 2. 5 mM (△- - -△) of D-mannoheptulose. At the end of incubation, medium was removed and frozen at −20°C for insulin radioimmunoassay.

Values are the mean $\pm$ SEM of 11-15 incubations.

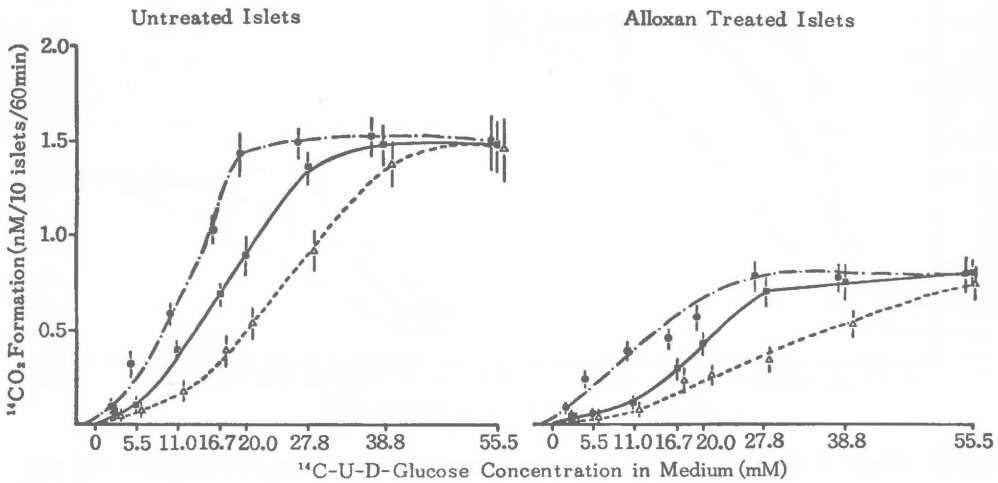

Fig. 2. Effects of D-mannoheptulose on glucose oxidation in alloxan treated (right panel) and non-treated (left panel) islets were plotted as a function of ^{14}C-U-D-glucose concentration in medium. After 30 minute preincubation with glucose free KRB medium, 10 islets per vial were incubated for 60 minutes at 37°C with 200 μl of glucose free KRB medium supplemented with ^{14}C-U-D-glucose (2. 7-55. 5 mM) without (●- - -●) or with 1. 0 mM (■——■) or 2. 5 mM (△- - -△) of D-mannoheptulose in the sealed scintillation vial. Blank value was obtained by incubating the medium without islets.

Values are the mean $\pm$ SEM of 9-12 incubations.

Maximal insulin release was induced by 20 mM glucose. When 1.0 mM D-mannoheptulose was added to glucose (2.7–55.5 mM), 11.0 mM glucose was required to induce insulin release and the half maximal response ocurred at 17.0 mM glucose. The near maximal insulin release ocurred at 27.8mM glucose. Further elevation of the threshold of glucose-induced insulin release (16.7 mM glucose) was observed by the addition of 2.5 mM D-mammoheptulose with a half maximal response at 25.5 mM, and maximal insulin release ocurred at 38.8 mM glucose.

Fig. 2 illustrates the suppression of oxidation of ^{14}C-U-D-glucose by D-mannoheptulose in untreated (left panel) and alloxan-treated islets (right panel). In the untreated islets, the threshold ^{14}C-U-D-glucose concentration for $^{14}CO_2$ formation without, with 1.0mM, or with 2.5mM D-mannoheptulose was 5.5 mM, 11.0 mM and 11.0 mM, respectively. Half-maximal responses occurred at 13.0 mM, 17.4 mM and 25.3 mM, respectively. As shown in the right pajel, alloxan inhibited $^{14}CO_2$ formation at concentrations greater than 5.5 mM ^{14}C-U-D-glucose, but did not influence the ^{14}C-U-D-glucose concentration required for half-maximal responses, while markedly reducing maximal responses. As with untreated islet, in alloxan treated islets the addition of 1.0 mM or 2.5 mM D-mannoheptulose resulted in the elevation of ^{14}C-U-D-glucose concentrations for threshold and half maximal response.

Fig. 3 shows the effects of D-mannoheptulose on the glucose-dose response curve of glucose-induced ^{45}Ca uptage in the untreated and alloxan treated islets. The inhibition of ^{45}Ca uptake by D-mannoheptulose ocurred at low glucose concentrations in the untreated as well as alloxan-treated islets and higher concentrations of glucose overcame the inhibition by D-mannoheptulose.

Fig. 4 demonstrates the close interrelationships among $^{14}CO_2$ formation, glucose-induced ^{45}Ca uptake and insulin release in the B-cell as a function of glucose concentration. Value of each parameter produced by 16.7 mM glucose without D-mannoheptulose was regarded as 100%. The glucose oxidation and ^{45}Ca uptake in the B-cell were obtained by subtracting glucose oxidation and ^{45}Ca uptake in the

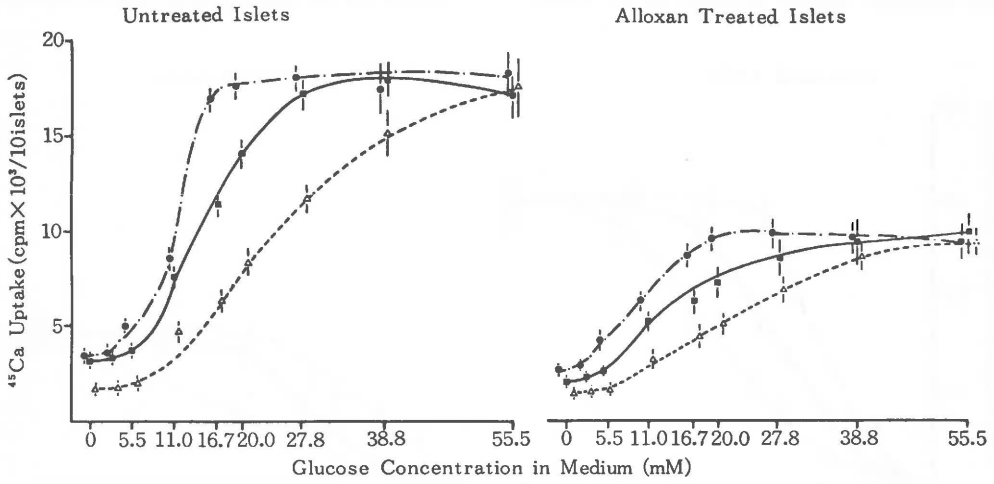

Fig. 3. Effects of D-mannoheptulose on glucose-induced ^{45}Ca uptake in alloxan treated and non-alloxan treated islets were plotted as a function of glucose concentration in medium. After 30 minute preincubation with calcium and glucose free KRB medium, batches of 10 islets (alloxan treated; right panel, or non-treated; left panel) were incubated for 60 minutes in 2 m*l* of calcium free KRB medium containing $^{45}CaCl_2$ (10 μCi/m*l*) and glucose (2.7–55.5 mM) without (●–·–●) or with 1.0 mM (■——■) or 2.5 mM (△– – –△) of D-mannoheptulose. After 10 times washings with cold glucose and calcium free KRB medium, islets were separated from washing medium by Millipore filtration (pore size 0.45 μ). ^{45}Ca radioactivity in 10 islets was counted in a scintillation spectrometer.

Values are the mean ± SEM of 10–14 experiments.

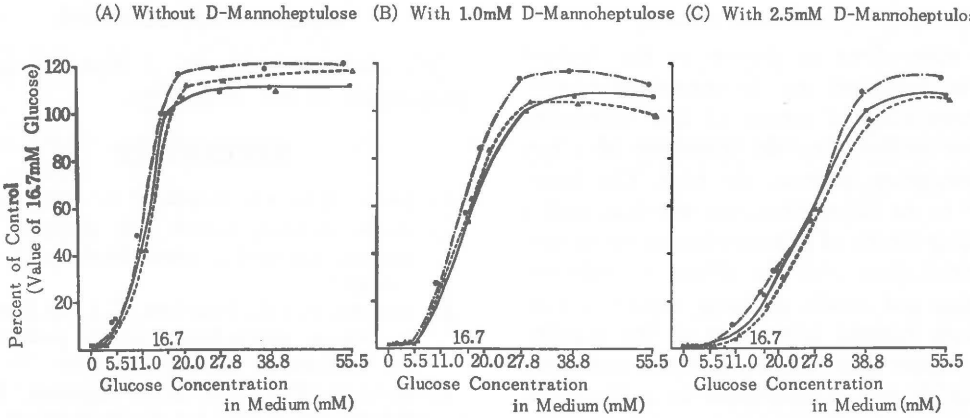

Fig. 4. The correlation between B-cell functions (glucose oxidation ●—•—●, glucose-induced ^{45}Ca uptake △- - -△, and insulin release ■——■) and glucose concentration in medium without (left panel) or with 1.0 mM (middle panel) or 2.5 mM (right panel) of D-mannoheptulose. Glucose oxidation and ^{45}Ca uptake in B-cell were obtained by substracting those of the alloxan treated islets from those of the non-treated islets found in Fig. 2 and Fig. 3. Value of each parameter produced by 16.7 mM glucose without D-mannoheptulose was regarded as 100%.

Values are the mean of 9-15 experiments.

alloxan-treated islets from those of untreated islets. Without D-mannoheptulose (Fig. 4-A), the threshold of $^{14}CO_2$ formation, glucose-induced ^{45}Ca uptake and insulin release was 5.5 mM glucose with half-maximal responses at 11.5 mM, 13.5 mM and 11.7 mM, respectivily. Apparent maximal responses ocurred by about 20 mM glucose.

When 1.0 mM D-mannoheptulose was added to glucose (Fig. 4-B), glucose concentrations greater than 5.5 mM were required to elicit responces of the three parameters, with half-maximal responses at 15.5 mM, 15.8 mM and 17.0 mM, respectivily. The maximal responses of the three parameters ocurred at 27.8 mM glucose.

With 2.5 mM D-mannoheptulose present in medium (Fig. 4-C), threshold glucose concentration was greater than 11.0 mM with half-maximal responses at 27.4 mM, 27.5 mM and 25.5 mM, respectively. Maximal responses were encountered at 38.8 mM glucose.

The glucose dose-response curves for the three parameters in the B-cell appeared sigmoidal regardless of the existence of D-mannoheptulose in medium.

DISCUSSION

How the pancreatic B-cell recognizes glucose as an insulin secretagogue is still unknown. For several years, two contrasting hypotheses were advocated concerning the mechanisms of glucose recognition by B-cells. According to the glucose receptor hypothesis, the glucose molecule itself serves as a signal for insulin release by interacting with the α-stereospecific glucose receptor probably located on the B-cell plasma membrane. According to the glucose metabolism hypothesis, a signal for insulin release is generated by the metabolism of glucose in the pancreatic B-cells in the form of a metabolite or a cofactor.

Tomita et al.[15] found that brief ehposure of isolated islets to alloxan inhibited glucose-induced insulin release while retaining the capability to respond to other insulin secretagogues than glucose[16]. McDaniel et al.[17] reported that alloxan inhibited neither hexose transport nor metabolism in the islets. However, recent investigations including our own revealed that alloxan inhibits glucose oxidation[7-9] and utilization[8,11] in the islets, which results in the inhibition of ionic fluxes[10,11] necessary for the initiation of insulin release. When smaller amount of alloxan was used, disturbance of islet function appeared to be limited to the B-cells[10]. Therefore, it was possible to relate changes in glucose metabolism to glucose-induced calcium accumlation and insulin release in the B-cell by substracting values in alloxan treated is-

lets from those in untreated islets[12].

The metabolism of glucose in the isolated islets is inhibited by D-mannoheptulose[3,4]. The mechanism of action of this compound has been attributed to the inhibition of a key phosphorylating enzyme, the high Km hexokinase[5] in the islets. However, previous studies correlating effects of D-mannoheptulose on glucose metabolism with its effects on calcium-mobilizing and insulin releasing capacities were done with isolated islets (not in the B-cells), and a detailed dose-response analysis over wide range of glucose concentration in medium has never been investihated.

In the present study, the suppression of glucose oxidation by D-mannoheptulose in the B-cell was found to be associated with superimposable sigmoidal inhibition of glucose-induced ^{45}Ca uptake and insulin release. Higher concentrations of glucose overcame the inhibition of these parameters by D-mannoheptulose. These data indicate that hexokinase activity[18] in the B-cell had an extremely flexible capacity and D-mannoheptulose inhibited the hexokinase with relatively higher Km as reported by Zawalich[5]. Since broad changes in glucose oxidation induced by D-mannoheptulose were closely associated with changes in glucose-induced ^{45}Ca uptake and insulin release hexokinase activity and subsequent changes in glucose metabolism might play a regulatory role in controlling glucose-induced calcium uptake and insulin release.

The present study does not elucidate the specific metabolite or cofactor responses to glu cose metabolism which might trigger insulin release. Malaisse et al.[19] found that inhibition of glucose-induced insulin release by D-mannoheptulose was due to the inhibition of reduced pyridine nucleotide generation in the B-cell.

Several limitations of the present study should also be kept in mind. First, glucose oxidation in the islets or B-cell is not necessarily representative of glucose metabolism[20]. Second, the possicility of alloxan affecting other cell populations in the islet besides the B-cell has never been investigated. And last, we canot exclude the possible action of D-mannoheptulose on the hypothetical glucose receptor of the B-cell plasma membrane[21,22].

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