INDUCTION OF INSULIN BIOSYNTHESIS BY ANOMERS OF D-GLUCOSE

Atsushi NIKI, Hatsumi NIKI
Department of Medicine, Aichigakuin University, Chikusa-ku, Nagoya 464

and

Takayuki OZAWA
Department of Biomedical Chemistry, University of Nagoya, Showa-ku, Nagoya 466, Japan

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1. Introduction

D-glucose is considered to be the most important stimulus for both the releases and the biosynthesis of insulin [1]. We have previously reported that the α-anomer of D-glucose was more effective than the β-anomer in triggering insulin release from isolated rat pancreatic islets [2]. This phenomenon was also observed in other experimental systems in vitro, as well as in vivo including man (for a review, see ref. [1]). Similar anomeric preference was reported in vivo in preventing the diabetogenic effects of alloxan by D-glucose [3,4]. In in-vitro experiments using isolated islets, we have further observed the preference for the α-anomer of D-glucose in protecting pancreatic B cells from the inhibitory effects of alloxan on both glucose-induced insulin release and biosynthesis, and suggested that the α-anomer of D-glucose may also be more effective than the β-anomer in inducing insulin biosynthesis [5]. However, it is difficult to directly compare between the effects of the α- and β-anomers on insulin biosynthesis with a conventional method using batch incubated isolated islets, since the incorporation of the labeled amino acid into (pro)insulin does not occur in a measurable amount within a few minutes, whereas the anomers mutarotate rapidly. In the present study, free cells were prepared from pancreatic islets to expose the B cells directly to glucose, thus avoiding a lag time of its unphysiological diffusion into the islet tissue, and the effects of the two anomers of D-glucose on the biosynthesis of insulin were compared with each other.

2. Materials and methods

Crude collagenase (CLS IV, 140 U/mg) was obtained from Worthington Biochemical Corp.; bovine plasma albumin (fraction V) from Armour Pharmaceutical Co., Chicago; the α- and β-anomers of D-glucose from Sigma Chemical Co., St Louis; L-[4,5-^{3}H]leucine (spec. act. 57.4 Ci/mmol) from New England Nuclear, Boston; glycoletherdiamine-*N, N, N', N'*-tetraacetic acid (GEDTA) and ethylenediamine tetraacetic acid (EDTA) from Wako Pure Chemical Industry Ltd., Osaka; α-mercaptopropionyl glycine (MPG) from Calbiochem, San Diego. Crystalline bovine insulin was kindly gifted by Dr Y. Koga, Shimizu Pharmaceutical Co., Shizuoka, Japan.

Islets of Langerhans were isolated from the pancreata of fed male Wistar rats, weighing 250–350 g, by the collagenase digestion method [6]. The preparation of free cell suspensions from islets was carried out principally by the non-enzymatic method reported by Lernmark [7] with slight modifications. Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2 mg/ml of bovine plasma albumin was used as the basal medium. All incubations were carried out at 37°C under constant gassing 5% CO_2 in oxygen. For disruption of pancreatic islets, 150–250 islets from two pancreata

were preincubated for 30 min in 1 ml Ca^{2+}-omitted basal medium containing 0.5 mg/ml glucose. The incubation medium was replaced by 500 μl Ca^{2+}-free basal medium containing 1 mM GEDTA, 1 mM EDTA, 1 mM MPG and 0.5 mg/ml glucose. After 5 min incubation, islets in the medium were shaken for 10 s with the use of Thermo-mixer (Thermonics Co. Ltd, Tokyo) and a small portion of the suspension was taken to check the degree of disruption of islets tissue under a light microscope. Shaking for 5–10 s was repeated until satisfactory disruption to free cells was completed. Usually, 20–25 s shaking was sufficient. The suspension was washed four times with the Ca^{2+}-containing basal medium supplemented with 0.5 mg/ml glucose at room temperature by repeated centrifugation for 1 min at approximately 80 × *g*. Finally, the sediment of free cells was resuspended in 150–250 μl of the basal medium, depending on the number of islets used, and each 50 μl of the suspension was transferred to small glass tubes having a total capacity of about 1 ml. The approximative number of cells in each tube, determined by simple counting in a haemocytometer, was calculated to be 2×10^4. In the light microscopic examination, the majority of the cells seemed to be intact as was described by Lernmark [7], but several clusters of a few cells were still remained.

After preincubating 50 μl cell suspensions with 5 μCi [^{3}H]leucine for 20 min, the incubation was initiated by adding 50 μl the basal medium, or the media supplemented with either the α- or β-anomer or with equilibrated glucose. The anomers were rapidly dissolved in the prewarmed basal medium just before use. The solution of equilibrated D-glucose was prepared by keeping the solution of the α anomer in 37°C for 5 h prior to the experiment to assure equillibration of the α-anomer capable of undergoing mutarotation. The final concentration of glucose was 1.5 mg/ml. This concentration was chosen since it was sufficient for both anomers to stimulate insulin release, and was suitable to differentiate the effectiveness of the two anomers [8]. The incubation was performed for 7 min, and was terminated by the addition of 300 μl acid alcohol, containing 0.5 mg of non-radioactive leucine and 0.5 mg of crystalline bovine insulin. The methods of extraction, purification and gel-filtration of (pro)insulin were previously described [5]. Total radioactivity in the (pro)insulin fractions, measured in a liquid scintillation system (Beckman LS-355), was used to indicate the biosynthesis of insulin.

Statistical analyses were performed by means of the unpaired *t*-test.

3. Results

The effects of the α- and β-anomers of D-glucose on the incorporation of [^{3}H]leucine into the (pro)-insulin fractions during 7 min incubation of free islet cells in suspension is shown in fig.1. In the basal medium, the radioactivity (cpm) incorporated into the (pro)insulin fractions was 1195.0 ± 91.6 (mean value ± SE for five experiments) and the radioactivities in the media with the α- and the β-anomer of D-glucose were 3128.6 ± 225.2 and 2202.6 ± 173.5, respectively. Thus, the α-anomer of D-glucose is significantly more effective than the β-anomer ($p < 0.01$) in inducing insulin biosynthesis.

Figure 2 shows the result of another series of

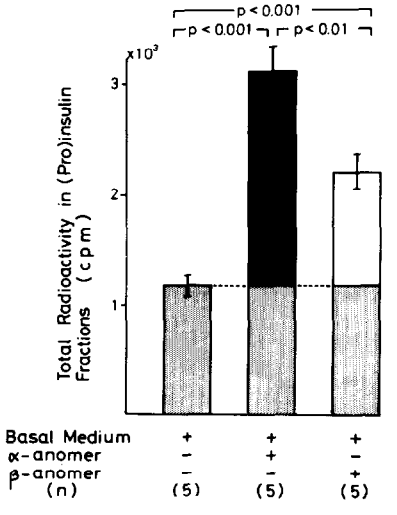

Fig.1. Effects of the α- and β-anomers of D-glucose on insulin biosynthesis. Free cells from rat pancreatic islets in suspension (about 2×10^4 cells/tube) were preincubated with [^{3}H]leucine for 20 min, and then incubated for 7 min after adding the basal medium or the media supplemented with either the α- or β-anomer at the final concentration of 1.5 mg/ml. Each bar represents the mean value of the total radioactivities of [^{3}H]leucine incorporated into (pro)insulin fraction, SEM being shown by the vertical line. The values in parentheses (*n*) indicate the number of experiments.

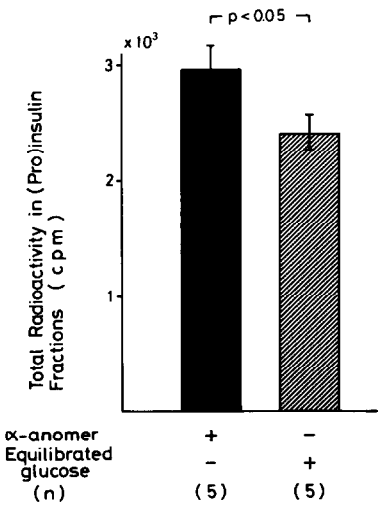

Fig.2. The effects of the α-anomer of D-glucose and equilibrated glucose on insulin biosynthesis. The solution of equilibrated glucose was prepared by keeping the α-anomer solution in 37°C for 5 h before use. The final concentration of D-glucose was 1.5 mg/ml. (See caption for fig.1.)

experiments in which we compared the effect of the α-anomer of D-glucose on insulin biosynthesis to that of equilibrated glucose. The amount of radioactivity incorporated into the (pro)insulin fractions in the medium with equilibrated glucose (2405.0 ± 141.6) was smaller than that in the medium with the α-anomer (2973.2 ± 199.7) ($p < 0.05$).

4. Discussion

The isolated pancreatic islet is surrounded with connective tissue capsule and has lost its capillary system, so that the glucose molecule in the medium is supposed to come into contact with the B cells in the islet tissue through unphysiological diffusion. The free cell system offers certain advantages in the study of insulin biosynthesis during a short incubation period. Namely, in this system, the glucose solution directly contracts with the B cells without being diluted with intercellular fluid. It is especially important for comparing the effects of the α- and β-anomers of D-glucose on insulin biosynthesis, since each anomer mutarotates very rapidly under our experimental condition [2], while insulin biosynthesis occurs rather slowly comparing to insulin release, which promptly occurs after an adequate stimulation. Moreover, in the free cell system, a great number of B cells can be equally suspended in a limited amount of the medium, thus saving the amount of [^{3}H]leucine used and, nevertheless, resulting in an increase of radioactive incorporation into the newly synthesized insulin. Fragility of the free cells, which may greatly affect the measurements of insulin release by a contamination with insulin from damaged B cells, is considered a relatively minor disadvantage in the study of insulin biosynthesis.

Our present data indicated that the α-anomer of D-glucose was more effective in inducing insulin biosynthesis than the β-anomer or the equilibrium at the concentration of 1.5 mg/ml, as in triggering insulin release [8]. Similar results were obtained by Zucker and Lin (personal communication), who preincubuted the isolated islets with either the α- or β-anomer for four periods of 5 min each, the last period together with [^{3}H]leucine, and determined the incorporation of [^{3}H]leucine into proinsulin by the immunoprecipitation method [9].

Although there are discrepancies between the effects of individual substances on insulin release and biosynthesis, a close parallelism seems to exist between the two processes so far as the regulator is D-glucose [1]. The present data provide further evidence that the anomeric preference in the B cell recognition of D-glucose is also the same for both release and the biosynthesis of insulin. However, the mechanism by which the pancreatic B cell distinguishes the α-anomeric configuration from the β-anomeric one is still remained to be elucidated.

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