Insulin Release Is Glucose Anomeric Specific in the Human

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A BSTRACT The α -glucose anomer produces a greater insulin release than β -glucose in various animal models. These glucose anomers were dissolved rapidly and administered intravenously to human volunteers at a high dose (0.5 g/kg) over a 3-min period or a low dose (3.5 g) over a 20-s period.

Blood samples were obtained at frequent time intervals for measurement of whole blood glucose (ferricyanide), plasma glucose (β -glucose oxidase), and serum immunoreactive insulin.

The high-dose infusion test showed no differences between the anomers of either blood glucose or serum insulin levels. However, at the lower dose, the α -glucose anomer stimulated a significantly greater insulin release than did β -glucose.

It is concluded that the α -glucose anomer stimulates a greater insulin release than the β -glucose anomer in human subjects at low but not at high doses intravenously and that this response is not apparently related to approximations of the degree of mutarotation. These results suggest that a steric specific glucose receptor site exists on the β -cell as a rapid insulin release trigger, although the α -anomer does not exclusively produce this stimulation.

INTRODUCTION

The ability of the β -cell to rapidly release insulin after rapid glucose stimulation has been known for many years, although the mechanism by which this occurs is essentially unknown (1). There are two main theories for the initiation of this glucose-sensitive insulin release process. One suggests that glucose produces insulin secretion by interacting at a membrane receptor (2, 3). The second suggests that glucose metabolism within the β -cell produces insulin secretion (4, 5). Since glucose in solution is present in two anomeric forms, studies investigating the influence of each form upon the β -cell have been pursued using various animal models. For example, the greater ability of the α -glucose vs. the β -glucose anomer in protecting the β -cell against the toxicity of alloxan in rats has previously been demonstrated by this laboratory (6). The capability of the a-anomer to produce a greater stimulation of insulin release than the β -anomer has been observed by our laboratory and others utilizing various animal models and techniques (7-10). However, in the human, the intravenous administration of these glucose anomers (0.5 g/kg)body wt over a 3-min period) has so far been unsuccessful in documenting this differential effect on insulin release (11). The possibility existed that with this large glucose load, mutarotation of the β -anomer would occur sufficiently and rapidly enough to provide a critical concentration of the α -anomer to stimulate the β -cell to a degree which would be undifferentiable upon the insulin release mechanism. It appeared reasonable, therefore, to intravenously administer a small dose (3.5 g) of freshly dissolved glucose anomer over a short period of time (20 s) to evaluate insulin response dynamics in man.

METHODS

Informed consent was obtained from two groups of healthy normal volunteers with no family history of diabetes or lipid disorders, who weighed from 82 to 112% of their ideal weight (Metropolitan Life Insurance Tables, 1959). The subjects were placed on a diet containing 300 g of carbohydrate daily for 3 days before each test; at least 48 h were allowed to elapse between successive tests. All were fasted overnight (12 h) before the test and each subject randomly received either α -, β -, or mutarotated (66% β and 34% α) glucose.

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FIGURE 1 Mean±SEM whole blood glucose and mean± SEM serum insulin levels at various intervals after the administration of α -, β -, and mutarotated glucose at 0.5 g/kg body wt over a 3-min infusion in normal control subjects (n =eight males).

The first group of subjects (eight males) received the anomers at a dose of 0.5 g/kg body wt which was dissolved in 50 ml of 0.9% saline and administered over a 3-min period intravenously as a square wave infusion. The second group of subjects (11 males and 3 females) received the

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glucose anomers as an infusion over 20 s at a final concentration of 17.5% (3.5 g in 20 ml of 0.9% saline). All the subjects were placed in the supine position for approximately 15 min before the test and infusion sets were placed into both antecubital veins (19 gauge butterfly catheter; Deseret Pharmaceuticals, Sandy, Utah; Minicath no. 11 catalogue no. M1912). Saline infusions (0.9%) were used to keep the veins patent (20 ml/h). One side was used for the administration of the glucose solution, while the other was employed for blood sampling. The crystalline anomers were previously cultured for possible bacterial or fungal contamination and assayed for pyrogen (Leberco Laboratory, Rosell Park, N. J.). Further protection of the volunteers was provided by the use of a filter through which all the glucose was infused (Millipore Corp., Bedford, Mass.; Swinnex-25 SX-GSO25, S 0.22 M, catalogue no. 26,396). The crystalline β and a-glucose (Sigma Chemical Co., St. Louis, Mo., G-5250 β -glucose lot no. 44C-1620 analyzed as 98.4% β and 1.6% α , and G-5000, α -glucose lot no. 091-C-1690 analyzed 97.6% α and 2.4% β) were each dissolved by vigorous shaking for 45 s in a sterile 0.9% saline solution just before injection. Standard (mutarotated) stock glucose (Abbott Laboratories, Chicago, Ill.; lot no. 44-448-DK) was diluted with 0.9% saline and the solution was assayed utilizing glucose oxidase (Beckman Instruments, Inc., Fullerton, Calif.) and ferricyanide reduction in an AutoAnalyzer (Technicon Instrument Corp., Tarrytown, N. Y.) to be approximately 66% β and 34% α . In the high dose glucose anomer tests, administered at 0.5 g/kg, blood samples (6 ml) were obtained at 0, 1, 3, 4, 5, 6, 7, 10, 15, 20, 30, 40, 50, and 60 min after the start of the infusion. In the low dose glucose anomer tests, blood samples were obtained 5 min before, just before the infusion, at the end of the infusion, and at 30-s intervals for 5 min, and then every min for an additional 5 min. The blood samples in both tests were assayed for whole blood glucose using the AutoAnalyzer ferricyanide method (12) and for immunoreactive insulin by a twoantibody technique (1). An additional blood sample (2 ml) was obtained in a heparinized pipette at the 0.5-, 1.5-, and 2.5-min time intervals during the low dose test and at the 1-, 3,- 5-, and 7-min time intervals in the high dose test, which was immediately centrifuged and the plasma β -glucose level repeatedly determined over an 80-min period utilizing the glucose oxidase technique (Beckman Glucose Analyzer, Beckman Instruments, Inc., model ERA 2001). Since the glucose oxidase method specifically measures the β -glucose anomer, the change of the plasma glucose level until equilibration is attained can be used to estimate the degree of mutarotation of the injected glucose anomers at the time of obtaining the blood sample.

The results are expressed as the mean \pm SEM and paired t tests were employed comparing the results of the α - to the β -anomer or the α - to the mutarotated anomer (13).

RESULTS

3-min glucose anomeric tolerance test. Fig. 1 shows whole blood glucose and serum insulin levels after the glucose anomeric tolerance tests at 0.5 g/kg i.v. over a 3-min infusion test. There were no significant differences between the tests for either the whole blood glucose or the serum insulin levels.

20-s glucose anomeric tolerance test. Fig. 2 shows whole blood glucose, serum insulin, and insulin levels above base line after the administration of the predominantly pure α -, β -, or the equilibrated (mutarotated 66%)

 β -, 34% α -) glucose. There was essentially no discernible differences among the mean blood glucose concentrations after the administration of each of the glucose anomer solutions. However, at only the 0.5-min time period, the mean glucose level after the injection of the β -glucose anomer was significantly higher than the α -glucose anomer.

Examination of the insulin levels for the three respective glucose tests showed that the α -glucose anomer exhibited a significantly greater insulin level compared to the β -glucose anomer from the 2- to the 5-min interval. Also noteworthy was that the - 5-min, and the end infusion (E) insulin levels were significantly higher in the mutarotated group compared to the α -glucose group. In comparing the insulin levels above base line, the α -glucose anomer produced a significantly greater stimulation of insulin than the β -anomer from the 2nd through the 9th min. Furthermore, there was no statistically significant difference between the α -glucose compared to the mutarotated glucose at any time periods, with the exception of the 10-min time period.



FIGURE 2 Mean blood glucose, mean \pm SEM serum insulin and mean \pm SEM insulin above base-line levels at various time intervals after the intravenous administration of α -, β -, and mutarotated glucose (3.5 g/20 ml) over a 20-s infusion in normal controls (11 male, 3 female). Student's *t* test comparing α -glucose vs. β -glucose or α -glucose vs. mutarotated glucose anomers is shown.



FIGURE 3 The mean \pm SEM insulin areas above base line (μ U/ml per min) at various time intervals after the intravenous administration of α -, β -, or mutarotated glucose (3.5 g/20 ml) over a 20-s infusion in normal volunteer controls (11 male, 3 female). Student's t test comparing the α -glucose vs. β -glucose and α -glucose vs. mutarotated glucose anomers is shown.

To further clarify these differences between α - and β -glucose, certain time intervals were assessed utilizing the insulin area above base line. The time intervals of 0-3, 0-5, 5-10, and 0-10 min are represented in Fig. 3. The α -glucose in the 0-3-min time interval with 52±10 μ U/ml per min compared to the β -anomer which was 41±10 μ U/ml per min (P < 0.01). This difference was also seen at the 0-5-, 5-10-, and 0-10-min intervals; P < 0.001, < 0.01, and < 0.001, respectively. No difference was observed in comparing the α -glucose to the mutarotated glucose at any of these time intervals.

By utilizing the ratio of the insulin area above base line divided by the blood glucose area above base line, an insulin-glucose relationship or an insulinogenic response to glucose can be calculated. In Fig. 4 these ratios for each glucose test for the previously assigned time intervals are shown. The α -glucose produced a significantly greater insulin response per unit of glycemic stimulus during the early part of the test (0-3- and 0-5-min intervals) but no statistically significant difference was observed at the 5-10-min time period. When comparing the α -glucose test to the mutarotated glucose test there was a significant difference (P < 0.05) in insulin release only during the early phase (0-3-min) time interval.

Although not shown, the estimated degree of mutarotation of each glucose anomer from samples obtained at



FIGURE 4 The mean \pm SEM ratio of insulin area above base line (μ U/ml per min) by the blood glucose area above base line (mg/dl per min) at various time intervals after the intravenous administration of α -, β -, or mutarotated glucose (3.5 mg/20 ml) over a 20-s infusion in normal volunteer controls (11 male, 3 female). Student's t test comparing the α -glucose vs. β -glucose and α -glucose vs. mutarotated glucose anomers is shown.

the 0.5-, 1.5-, and 2.5-min time periods was studied. There was great variability between the patients in the estimated degree of mutarotation (ranging from 0 to 100% at the three time intervals). Also notworthy was the lack of a significant correlation between the degree of mutarotation and the insulin release.

DISCUSSION

Various compounds, e.g. glucose, leucine, arginine, glucagon, and tolbutamide have been shown to have a unique capacity to stimulate a rapid insulin release. The mechanisms by which these various compounds produce this effect is unknown but the speed at which insulin release occurs suggests a rapid process initiated at the cell membrane. The plasma membrane, which in various tissues has been shown to be predominantly a lipid, appears to exist in a form of bimolecular lipid leaflet with the hydrocarbon chains toward the center and the polar head groups of the phospholipids facing toward the polar end (14, 15). It has been proposed that for a compound to enter the cell, an interaction between the substance and the predominantly lipid membrane is a prerequisite for penetration of the compound. However, glucose, which is mainly hydrophobic and thus has a strong monosaccharide: water interaction, would penetrate the mem-

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brane to a very low degree. Thus, a hypothesis has been proposed that there is a receptor at the cell membrane which either facilitates transport of the sugar through the membrane or changes the chemical constituent of the sugar by an enzyme and thus translocates the sugar molecule (glucokinase or hexokinase has been proposed as a method of entrance into the cell in contrast to the interaction at the cell membrane). The concept of an enzyme at the β -cell membrane which may be saturable at a low glucose concentration and thus differentiate between the anomers is possible, since at the high glucose infusion there was no evidence of a differential insulin release between the sugars. This could then represent a saturation of the enzymatic system which may then be undifferentiable at the high-dose glucose test, while at the lower dose the enzyme system would provide a specificity at the enzyme receptor site as suggested by Grodsky et al. (9) using glucokinase as an example.

At equilibrium, glucose in solution is present as 66%in the β -anomeric and 34% in the α -anomeric form. In the chair configuration of the α -glucose anomer, the Cl hydroxyl unit is in an axial position to the plane of the ring, but in the β -form the Cl hydroxyl is equatorially positioned. Since the less stable α -glucose anomer has been found to be less hydrophobic than the β -anomer, this might provide a greater carbohydrate: lipid ratio which would allow the α -anomer to penetrate or interact more readily at the cell membrane (15, 16). However, the recent demonstration that both anomers appear to enter the β -cell at the same rate would negate the hypothesis that the α -anomer enters the cell preferentially (17). Another suggestion in the differential effectiveness of the glucose anomers has been that the α -glucose generates more cyclic AMP than the β -glucose anomer (18). These studies suggest that α -glucose stimulation may be initiated at the β -cell membrane receptor which may initiate insulin release through the stimulation of cyclic AMP.

In this report, the *a*-anomer produced a greater stimulation of insulin release than the β -anomer in the human at the lower dose but not at the higher dose. Thus, it may be that if a large concentration of glucose is administered the mutarotation of the β -anomer to a critical concentration of the *a*-anomer may be sufficient to saturate the specific receptor enzyme, and thus stimulating insulin release. This is significant since mutarotation which was estimated in this experiment was almost complete within the first few minutes in the circulation. Thus, the initial stimulation at the β -cell appears to provide an important signal for the perpetuation of insulin release. Although the rate of mutarotation was similar at the two doses studied, the low dose was sufficient to differentiate insulin release by the two glucose anomers.

The mutarotation rate of the anomers is dependent on the pH, temperature, and presence of the enzyme mutarotase (aldose 1-epimerase). In deionized water of pH 6.8 at 24°C, complete mutarotation of the anomers occurs in approximately 2 h (19), while under nearphysiological conditions, complete mutarotation occurs in approximately 7 min (20). In vitro, however, the ubiquitous enzyme mutarotase (which is in high concentration in liver, kidney, and intestine) is able to accelerate further the mutarotation rate (21). In this study, obtaining blood samples from the vein contralateral from the side of the infusion, revealed a great variability of mutarotation among the subjects. Although optimal evaluation of the degree of mutarotation should have been performed directly at the pancreatic effluent, this was considered an unnecessary hazard. In addition, the estimation of the degree of mutarotation in the samples obtained is relatively crude utilizing the Beckman glucose oxidase method (Beckman Instruments, Inc.). Finally, the possibility that some intermediate or secondary phenomenon other than the α -glucose itself may be responsible for these observations cannot be excluded. Similarly, it would be misleading to suggest that the a-glucose anomer exclusively stimulates insulin release.

In summary, this study reveals that in the human

after a small dose intravenous administration of the glucose anomers, the α -anomer provides a significantly greater insulin secretion than the β -anomer. Also, this differential effect is not observed at 10 times this dose and the specificity is not appreciably related to the degree of mutarotation as crudely estimated.

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