SHORT COMMUNICATION

The Effect of Glucose Anomers upon Insulin and Glucagon Secretion*

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Summary. The precise mechanism of insulin release is unknown, though the presence of a beta cell surface receptor has been postulated. As D-glucose exists in at least two anomeric forms, α and β , these were employed to study insulin release in three animal models. After rapid dissolution, each anomer was rapidly injected into

intact rats, dogs, and a rat pancreatic perfusion system. There was a trend toward greater insulin secretion and greater suppression of glucagon by the α anomer.

Key words: glucose anomers, insulin release, glucagon, mutarotation.

D-glucose is known to exist in the crystalline state in either the α or β anomeric form. After placing either of the anomers in aqueous solution there is a transformation into an equilibrium state in which 64% is in the β form and 36% is in the α form. Glucose has been shown to be a major stimulant to insulin release, although the exact mechanism is unknown. Current theories include either a direct interaction of glucose upon a receptor site on the beta cell, or a glucose metabolite which causes insulin secretion, or both. To study the effect of these two D-glucose anomers on insulin release, three different animal models were used. This preliminary survey suggests that there may be some difference between the effect of the two forms upon insulin and glucagon secretion.

Materials and Methods

1. Acute α or β Glucose Administration to Rats

Male Charles River Strain albino rats weighing 180–200 g were fasted for 24 h prior to experimentation. They were anesthetized with intraperitoneal sodium pentobarbital (0.1 mg/100 g body weight). A midline abdominal incision was made. Surrounding tissues were displaced laterally and the aorta and vena cava were separated. A small 3" tubing (Minicath PRN-Deseret Cat #5084) was placed into the vena cava 3 cm below the renal vein. A stock glucose solution was prepared in sterile normal saline twelve hours prior to use to assure complete mutarotation (64% β , 36% α). Alpha and Beta glucose (Sigma Chemical G-5250, Beta, lot #052e-0810 analyzed as 99.2% β and 0.8% α , and Sigma Chemical G-5000, Alpha, lot #091C-1690 analyzed as 97.6% α and 2.4% β) were each dissolved by vigorous shaking for 10 sec in sterile

saline just prior to injection. These glucose solutions were infused over 5 sec in a volume of 1 ml at a concentration of 200 mg/ml and blood was withdrawn and flushed through the tubing three times to assure completeness of the infusion. A volume of 1 ml blood was removed at time 0, and after the infusion at 1, 3, 5 and 10 min for glucose and insulin determinations [1, 2].

2. Acute α and β Glucose Administration to Dogs

Mongrel dogs weighing between 22 and 26 kg were employed. Twenty-four hours prior to the studies the animals were anesthetized with sodium pentobarbital (10 mg/kg) and catheters were placed into both jugular veins. The tubings were kept patent with a heparin solution (3 units/1 ml of saline). One catheter was used for infusion and the other for blood sampling. A 24 h recovery period from the surgery was allowed. The animals were fasted 12 h prior to each experiment. All the dogs received α , β and mutarotated glucose infusions in random order with a 48–72 h period between each experiment. The α or β glucose (0.5 g/kg of body weight) was dissolved by vigorous shaking in 50 ml of sterile de-ionized H₂O about 10–20 sec prior to use. The time from the addition of the water to the commencement of infusion ranged from 36 to 47 sec. The solution was infused as a square wave pulse over 2 min. Blood samples (8 ml) were taken at 0 time, the glucose infusion was started and additional blood samples obtained every 30 sec for 3 min, then every 2 min for 10 min, and then every 10 min for 40 min. The samples were assayed for glucose and insulin.

3. Rat Pancreas Perfusion Study

The rat pancreas perfusion technique was a modification of the method of Penhos *et al.* [3]. The pancreas was left *in situ* in the animal, and at no time during the operation was the pancreas without oxygen. The perfusate was a modified Krebs-Ringer-bicarbonate buffer

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with 4% dextran T70 and 0.2% bovine serum albumin (fraction V), and was constantly bubbled with 95% O₂ and 5% CO₂. A nonpulsatile flow four-channel roller bearing pump (Buchler Polystatic) was used and the entire apparatus was housed in a plexiglass box at 38°C. Pancreatic flow rate was 2–3 ml/min, and the perfusate was not recirculated. Perfusate samples were

Statistical Analysis

Comparisons of mean glucose and insulin levels following α , β , or mutarotated glucose infusion were made by analysis of variance, and Scheffes' tests of differences between means [6]. Insulin-glucose relationship were represented by the regression coefficient (b)

Table 1. Rat IVGTT

Time (mins)	Glucose infused		α (n = 8)		β (n = 7)	
	Mutarotated (n = 6)		BG	IRI	BG	IRI
	BG (mg/dl)	IRI (uU/ml)	(mg/dl)	(uU/ml)	(mg/100 ml)	(uU/ml)
0	138 ± 6	12 ± 2	141 ± 3	14 ± 3	139 ± 4	11 ± 2
1	428 ± 12	30 ± 11	465 ± 11	47 ± 8	430 ± 8	38 ± 10
3	402 ± 23	22 ± 5	398 ± 17	35 ± 6	364 ± 3	15 ± 3
5	383 ± 28	23 ± 4	357 ± 11	27 ± 4	340 ± 9	18 ± 3
10	348 ± 21	23 ± 7	326 ± 11	23 ± 4	309 ± 4	12 ± 5

Results are expressed as Mean ± SEM
n = number of rat experiments in each group

Table 2. IVGTT in dogs (n = 5). Values listed are the slopes (b) of linear regression of serum insulin upon blood glucose

Time (mins)	Glucose infused:		α		β	
	Mutarotated		b	p	b	p
	b	p				
0–63	0.4660	< 0.001	0.0097	n.s.	0.0367	< 0.05
0–3	0.5051	< 0.005	0.0025	n.s.	0.0181	n.s.
4–63	0.4145	< 0.001	0.3852	< 0.001	0.2045	< 0.001

p = significance of the correlation coefficient

collected from the cannulated portal vein into chilled glass tubes containing EDTA for glucagon [4, 5] and without EDTA for insulin and both AutoAnalyzer glucose and Beckman (Model ERA-2001) glucose oxidase glucose determinations.

During the time periods when the pancreas was perfused with 80 mg/100 ml glucose, the oxygenated perfusate was pumped from a reservoir kept at 38°C. When α , β , or mutarotated glucose was infused, a second perfusate with a high concentration (3000 mg/100 ml) of the particular glucose form was introduced with a Harvard pump into the channel containing the oxygenated, warm perfusate with no glucose. The final glucose concentration during this 5 min perfusion was 300 mg/100 ml. Either α , or β glucose was dissolved in solution approximately 15 sec before being infused. The switch from the perfusate with 80 mg/100 ml mutarotated glucose to the perfusate with the test sugar was accomplished almost instantaneously, and after the latter was infused for five minutes there was a rapid switch back to the reservoir with 80 mg/100 ml mutarotated glucose. There was a 15 min "rest period" between test infusions, and three test infusions in random order were done in each of the five animals perfused.

pooling all the glucose-insulin pairs for each sugar infusion study group. Differences of group b-values were tested for significance by t-test [7]. Paired t-tests were utilized to determine the first significant change in the glucose, insulin and glucagon levels and to test the significance between glucose disappearance rates kg.

Results

1. Acute α and β Glucose Administration to Rats

The α or β or mutarotated glucose was infused in random order into the rats. The mean glucose and insulin levels from each study group are shown in Table 1. The basal blood glucose and insulin values were essentially the same in each group. Though no statistically significant differences between the groups infused with different glucoses was observed, it is noteworthy that the α glucose group of animals showed a greater increase of insulin at one minute than was observed with the other two glucose preparations.

2. Acute α and β Glucose Administration to Dogs

Each dog served as his own control. The sequences of the infusion of the glucose form were randomized in

each dog, and the time interval elapsing between each infusion was constant in all experiments. The mean ($\pm$ SEM) fasting insulin levels were α - 6 ± 7 μ U/ml, β - 9 ± 1 μ U/ml, mutarotated- 8 ± 2 μ U/ml. The higher mean basal insulin level in the α group was due to the one dog with a level of 40 μ U/ml. This animal had a more normal insulin level when used in the β or mutarotated experiments, and a possible contamination of the sample or inadvertent feeding of the animal could not be excluded. Excluding the animal from the study produced no significant change in the results.

Fig. 1. Mean blood glucose and insulin levels in 5 dogs during and following the infusion of either mutarotated, α or β glucose

Figure 1 shows the means of the glucose and insulin following glucose administration. There were no statistically significant differences in mean glucose or insulin levels among the groups. In Table 2 the b-values, which represent the linear regression of serum insulin upon blood glucose, are shown for the total test period (0–63 min) as well as the 0–3 min and the 4–63 min intervals. In the mutarotated glucose experiments there was a significant correlation between serum insulin and blood glucose for all periods analyzed. However, the α and β glucose groups did not show this significant relationship in the 0–3 min period of

the experiment nor did a significant correlation appear in the α group for the 0–63 min period. During the 4–63 min periods, a significant correlation was observed in all glucose groups. The insulin released per unit glucose (b-value) during the 4–63 min time period is significantly less in the β group when compared to the mutarotated glucose group ($p < 0.05$), but not significantly different from the α group.

The mean, ($\pm$ SEM) rates of glucose utilization were 4.06 ± 0.50 , 3.70 ± 0.63 and 2.76 ± 0.42 for the α , β and mutarotated glucose groups, respectively. These values are similar to other studies employing dogs [8]. The mean k_G was significantly higher in the α group compared to the mutarotated group.

3. Rat Pancreas Perfusion Study

A 15 min "rest period" between administration of the subsequent glucose solutions was used to assure re-equilibration and re-establishment of a basal status. Samples were taken at 30 sec intervals, i.e. the 30 sec sample represents the effusate from 0–30 sec, the 60 sec from 30–60 sec, etc. The samples were then divided for insulin, glucagon, and determination of total glucose by the AutoAnalyzer as well as for glucose levels using the glucose oxidase technique. The basal glucose level was 80 mg/100 ml and the basal insulin was 2 μ U/ml.

In Fig. 2 the means of the glucose and insulin levels for the three glucose perfusions are shown at various time intervals. No significant differences between the groups were observed. The first statistically significant increase over baseline for the insulin and the glucose levels in all three groups occurred at approximately 1.5 min of the perfusion. The glucagon levels are presented in Table 3. There was a statistically significant

Table 3. Glucagon levels during perfusion of rat pancreas

Time (mins)	Glucagon (pg/ml)		
	Mutarotated	α	β
0.5	156 $\pm$ 71	126 $\pm$ 29	114 $\pm$ 36
1.5	98 $\pm$ 29	118 $\pm$ 27	132 $\pm$ 26
3.0	110 $\pm$ 24	116 $\pm$ 32	122 $\pm$ 25
5.0	70 $\pm$ 19	70 $\pm$ 18*	82 $\pm$ 14
8.5	74 $\pm$ 14	74 $\pm$ 12*	46 $\pm$ 11*
10.5	72 $\pm$ 22	80 $\pm$ 18*	52 $\pm$ 18*
14.5	112 $\pm$ 36	88 $\pm$ 19*	96 $\pm$ 29

* $p =$ or < 0.05 (paired comparison with 0.5 min sample).

fall of glucagon in the α group which commenced at the 5 min interval and continued throughout the study period. Though a reduction in glucagon was observed in the mutarotated group, it was not statistically significant. The β glucose group showed a significant glucagon decrease only at the 8.5 and 10.5 min interval.

To ascertain that the α or β glucose which was infused was similar to that in the effusate, selected samples obtained at various times during the glucose perfusion were immediately analyzed using the glucose

oxidase method. Since the glucose oxidase method specifically measures only the β glucose, the degree of rotation of either sugar can be analyzed throughout the experiment by repetitive measurement of a sample until no further change of the glucose level is observed. The α and β glucose employed was dissolved into the buffer solution used in the experiments on three separate occasions and the percent β glucose as a function of time was established. Mutarotation to equilibrium of 64% β and 36% α occurred in approximately one hour in the buffer solution. Samples were obtained from the pancreatic effusate at 1, 3, and 5 min for the α , β , and mutarotated glucose groups and repetitive glu-

vious reports have shown that various other tissues have preference of one glucose anomeric form above the other when evaluating metabolic or tissue membrane penetration studies [9, 10, 11, 12].

The possible roles of the two glucose anomers were compared to the mutarotated equilibrated glucose with respect to insulin and glucagon secretion in three different animal models. In the intravenous glucose infusion into rats, though no statistical significance was observed, there is a suggestion that the α glucose group at one minute showed a higher insulin release than either the β or mutarotated groups with the same glucose levels.

In the perfused rat experiments the glucose anomers were only slightly altered during the passage through the system over the five minute infusion. Although the islets represent approximately 1% of the tissue mass there would be no way of evaluating the exposure of this tissue to either glucose anomer. There was no statistical difference in the insulin release, although the highest levels were obtained with α glucose. The glucagon levels were significantly reduced at the 5 to 14.5 min period when the α glucose anomer was infused but only at 8.5 and 10.5 min during β administration and none were significantly reduced during mutarotated glucose infusion.

The intravenous glucose infusion tests in the dogs revealed higher blood glucose levels in the α group when compared to the other groups during the infusion period. The glucose concentrations after the end of the infusions were comparable. The possibility of a difference in the distribution of the anomers is the most plausible explanation for this observation. In the first few minutes of the glucose infusion there is a definite disparity between the levels in the α and β group when compared to the mutarotated group. The K_G value of the mutarotated group is similar to the findings of previous experiments, as mentioned earlier, but the α and β groups have somewhat higher K_G values. Only the α group, however, was significantly higher than the mutarotated group. The insulin release in the α group, though not statistically significant, is slightly higher than in the other two groups.

Though no marked statistical differences were observed in the three models used in this study, trends appear in both insulin and glucagon levels. In a recent report, the α glucose anomer was observed to produce greater protection from alloxan diabetes than the β anomer as evidenced by both plasma glucose levels and B-cell morphology [13]. Thus, there is still much question as to the possible role the α and β glucose anomers play in insulin secretion.

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Fig. 2. Mean blood glucose and insulin levels in the perfused pancreas ($N = 5$) during and following the infusion of either mutarotated, α or β glucose

cose oxidase determinations were made until complete mutarotation had occurred. By comparing these glucose levels against the changes observed in the buffer solution alone a percentage of the degree of mutarotation in the perfused pancreas preparation could be made. It was noted that the effusate was only minimally altered when perfused through the pancreas.

Discussion

Although glucose is able to stimulate insulin release, the mechanism of this action is unknown. Pre-

References

1. Hoffman, W.S.: A rapid photoelectric method for the determination of glucose in blood and urine. *J. biol. Chem.* **120**, 51—55 (1937)
2. Soeldner, J.S., Slone, D.: Critical variable in the radioimmunoassay of serum insulin using the double antibody technic. *Diabetes* **14**, 771—779 (1965)
3. Penhos, J.C., Wu, C.H., Basabe, J.C., Lopez, N., Wolff, F.W.: A rat pancreas-small gut preparation for the study of intestinal factor(s) and insulin release. *Diabetes* **18**, 733—738 (1969)
4. Unger, R.H., Aguilar-Parados, E., Miller, W.A., Eisentraut, A.M.: Studies of pancreatic alpha-cell function in normal and diabetic subjects. *J. Clin. Invest.* **49**, 837—848 (1970)
5. Unger, R.H.: Personal communication
6. Steel, R.G.D., Torrie, J.H.: *Principles and Procedures of Statistics*. New York: McGraw-Hill Book Inc., p. 173 (1960)
7. Armitage, P.: *Statistical Methods in Medical Research*. Oxford: Blackwell Scientific Publications, p. 207 (1971)
8. Rasio, E.A., Hill, G.J., Soeldner, J.S., Herrera, M.G.: Effect of pancreatectomy on glucose tolerance and extracellular fluid insulin in the dog. *Diabetes* **16**, 551—556 (1967)
9. Bailey, J.M., Fishman, P.H., Pentchev, P.G.: Studies on mutarotases: Investigation of possible rate-limiting anomerizations in glucose metabolism. *J. biol. Chem.* **243**, 4827—4831 (1968)
10. Faust, R.G.: Monosaccharide penetration into human red blood cells by an alter diffusion mechanism. *J. cell. comp. Physiol.* **56**, 103—121 (1960)
11. Sachs, W., Sachs, S.: Cerebral metabolism of glucose anomers in human subjects *in vivo*, in Paoletti, R., Fumagelli, R., Galli, G., (eds): *Second International Meeting of the International Society for Neurochemistry*. Milan, Tamburini Editore, p. 349—350 (1969)
12. Bailey, J.M., Fishman, P.H., Pentchev, P.G.: Studies on mutarotases: Purification and properties of a mutarotase from higher plants. *J. biol. Chem.* **242**, 4263—4269 (1967)
13. Rossini, A.A., Berger, M., Shadden, J., Cahill, G.F., Jr.: Beta cell protection to alloxan necrosis by anomers of D-glucose. *Science* **183**, 424 (1974)

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