Differential Effects of Alpha- and Beta-D-Glucose on Insulin and Glucagon Secretion from the Isolated Perfused Rat Pancreas

Franz M. Matschinsky, M.D., Anthony S. Pagliara, M.D., Barbara A. Hover, Morey W. Haymond, M.D., and Susan N. Stillings, St. Louis

SUMMARY

The efficacy of α and β-D-glucose in causing insulin release and suppressing glucagon release from the isolated perfused rat pancreas was tested. In order to allow simultaneous assessment of the glucose effect on both α and β-cells, the pancreas was continually perfused with a physiological amino acid mixture (10 mM) which provokes glucagon secretion and also stimulates the β-cells, provided glucose is present. Under these conditions the α-anomer of D-glucose at 3 and 6 mM proved significantly more potent than the β-anomer in inducing insulin release and in inhibiting glucagon secretion. These data lend further support to the concept that α-cells and β-cells contain glucoreceptors controlling glucagon and insulin secretion and show that certain physicochemical properties of these receptors are alike in both types of cells. DIABETES *24*:369-72, April, 1975.

Although glucose is the major physiological stimulator and modulator of insulin and glucagon release,[1,2] the mechanisms through which glucose exerts its actions upon pancreatic α and β-cells remains poorly defined. It has been suggested that molecular events during the metabolism of glucose may be the actual cause of insulin release,[3,4] and may control glucagon secretion in an analogous fashion.[5,6] An alternative hypothesis implicates glucoreceptors located on the membrane of α and β-cells[1,7] controlling insulin and glucagon release directly without the necessity of prior metabolism of the glucose molecule.[1]

From the Edward Mallinckrodt Departments of Pediatrics and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110.

Address reprint requests to: Franz M. Matschinsky, M.D., Department of Pharmacology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110; or Anthony S. Pagliara, M.D., Department of Pediatrics, St. Louis Children's Hospital, 500 South Kingshighway, St. Louis, Missouri 63110.

Accepted for publication December 27, 1974.

To submit these two concepts to further experimental testing, studies were performed utilizing the perfused rat pancreas to assess the capacity of α-D and β-D-glucose to stimulate insulin release and to suppress glucagon secretion. The usage of the two anomers offered the possibility of differentiating between the two hypotheses discussed above, since available information indicates that α and β-D-glucose are handled equally well by hexokinases of various organisms and tissues.[8] To allow optimal simultaneous assessment of α and β-cell function the pancreas was continuously perfused with 10 mM of a physiologic amino acid mixture since earlier studies had revealed[1] that glucose (normally present in a mixture of 36 per cent of the α and 64 per cent of the β-anomer[9]) exerted graded effects on the two types of endocrine cells most strongly and reproducibly when amino acids were present at the same time.

METHODS

Male Sprague-Dawley rats weighing 300-400 gm., fed ad libitum with Purina rat chow (Ralston Purina Co., St. Louis, Mo.) were used in all experiments. The pancreas was isolated and perfused by the procedure described by Grodsky[10] et al. with minor modifications.[11] Induction of anesthesia, surgical preparation and composition of the perfusion media have been described in detail.[1] All perfusions were performed with a 10 mM amino acid mixture present throughout. This mixture contained amino acids in proportions found in normal rat serum.[12] Either α-D-glucose or β-D-glucose (Sigma Chemical Co., St. Louis, Mo.) at concentrations of 3 and 6 mM, respectively, was superimposed on this amino acid stimulus.

Because of the relatively rapid spontaneous mutarotation of both anomers at room temperature[13] both

were dissolved ten minutes prior to perfusion in ice cold buffer, and delivered to the pancreas from a flask kept on ice through a separate pump. The ratio of flow between the pump delivering the glucose and that delivering the amino acid mixture ranged between 1:5 and 1:6. During the control periods ice cold glucose free solution was infused at comparable rates to avoid any temperature effects. Due to the dead space of the infusion system the time delay for the glucose pulse was 2.5 minutes.

For both glucose concentrations a minimum of five perfusions were performed. A crossover protocol was used so that each glucose anomer was infused for ten minutes either early or late in the experiment. The detailed protocol was as follows: After preperfusion with the 10 mM amino acid mixture for fifteen minutes (t_{-15}-t_0), either the α or β-anomer was superimposed for ten minutes (t_0-t_{10}); next there was a ten-minute perfusion with the 10 mM amino acid mixture alone (t_{10}-t_{20}); following this the opposite glucose anomer was introduced (t_{20}-t_{30}), again followed by a control period with amino acids alone (t_{30}-t_{40}). Samples were collected at frequent intervals for determination of glucose,[14] insulin and glucagon.[1]

The rate of spontaneous mutarotation of the two anomers was tested in a sham perfusion experiment in which the anomeric glucose solutions were delivered in an identical manner without the pancreas present and aliquots collected and assayed on a Beckman glucose analyzer which is specific for measuring β-D glucose.[15] Spontaneous conversion of the β-anomer was 10 per cent and of the α-anomer 24 per cent before the sugar reached the pancreas.

The rates of insulin and glucagon release were calculated by multiplying the concentrations of the respective samples by the flow rate. Total hormonal release during ten minutes of exposure to the combined amino acid, glucose stimulus was obtained by planimetry and by subtracting the basal secretion rates. For ease in presentation all of the insulin, glucagon and glucose responses obtained with the respective concentrations of α-D glucose are compared to that of the β-D glucose anomer regardless of the sequence of addition (figure 1). Since all of the experiments were performed as crossover studies, the paired *t* test was utilized for determining degree of significance between integrated insulin release and glucagon suppression resulting from perfusion with the two anomers.

RESULTS

At 3 mM both anomers resulted in insulin release and glucagon suppression (figure 1a). However, the α-anomer caused significantly greater stimulation of insulin release (1,628 ± 347 vs 427 ± 119 μU/10 min., $p < 0.001$) and significantly greater inhibition of glucagon secretion (−81 ± 11 vs −57 ± 13 ng/10 min. $p < 0.05$) as compared to the β-D glucose anomer (figure 1a, table 1). Similar results were observed with 6 mM glucose (figure 1b, table 1). Serial determinations of glucose in the perfusate revealed that the total sugar concentrations resulting from the infusion of the two anomeric forms were indistinguishable (figure 1).

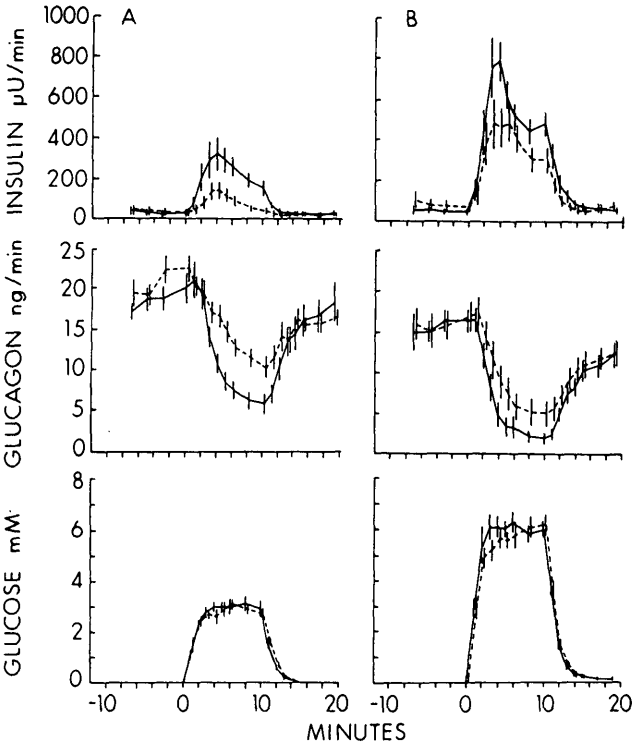

FIG. 1. The actions of α- and β-D-glucose on insulin and glucagon release due to a 10 mM mixture of amino acids. The 10 mM amino acid mixture was present throughout the entire perfusion period. The glucose anomers were infused at different rates as a square wave pulse from t_0 - t_{10} minutes. Panel A depicts perfusion with 3 mM glucose (n = 10) and panel B, 6 mM glucose (n = 12). The means ± S.E.M. are given. α-D-glucose ———; β-D-glucose -----. For statistical analysis see table 1.

DISCUSSION

These studies clearly demonstrate that α-D-glucose is a more powerful permissive agent allowing amino acid induced insulin release to occur and is a more effective inhibitor of amino acid stimulated glucagon secretion than is β-D-glucose. It has recently been reported that in batch incubated isolated rat islets α-D-glucose at 11 mM was a more effective insulin

TABLE 1

Differential action of α- and β-D-glucose on secretion of insulin and glucagon due to a 10 mM mixture of amino acids

Glucose Anomer		Insulin†	Glucagon‡
mM	n	μU/10 min.	ng/10 min.
3.0 α	10	1,628 ± 347	−81 ± 11
3.0 β	10	427 ± 119	−57 ± 13
p*		<0.001	<0.05
6.0 α	12	4,465 ± 624	−99 ± 9
6.0 β	12	2,773 ± 544	−71 ±9
p*		<0.01	<0.02

*Degree of significance between perfusions with α- and β-D-glucose by paired *t* test.
†The values represent the mean ± S.E.M. of the integrated rates of insulin release above baseline (see figure 1).
‡The values represent the mean ± S.E.M. of the integrated rates of glucagon release below baseline (see figure 1).

releasing agent than β-D-glucose.[16] These data together with other information in the literature to be discussed lend further support to the concept that glucose acts through specific receptors on the α and β-cell membrane.

It is well known that D-glucose injected into animals prior to alloxan administration protects against β-cell destruction.[17,18] Recent studies by Rossini et al. have extended that knowledge by showing that the α-D-glucose anomer affords greater protection from the induction of alloxan diabetes in rats than does the β-anomer.[9] Further, 3-0-methyl-D-glucose, a non-metabolizable glucose anologue, is a more effective protective agent than is glucose. Here again, α-3-0-methyl D-glucose is more potent than the equilibrium mixture of its two anomers.[19] From these observations, Cahill, Rossini and Arcangelli suggested that the β-cell membrane had a highly stereospecific site for D-glucose and 3-0-methyl-D-glucose.[19]

The information currently available is insufficient to decide whether this hexose site affording protection against alloxan is a transport site or an independent site. It is also not clear whether the protective site is identical with the insulin releasing site studied in the present investigation and in the independent study cited earlier.[16] However, it is striking that both the protection and releasing sites have in common the ability to differentiate between α and β-anomers of glucose. Since hexose protection against alloxan appears to be unrelated to glucose metabolism[19] and since glycolysis seems to catabolize both anomers equally well,[8,13] the results would seem at least compatible with a unifying hypothesis attributing the protective and releasing actions of glucose to a related site dissociated from its metabolism. It is, however, difficult to reconcile this view with the fact that 3-0-methyl-D-glucose neither stimulates insulin release by itself nor inhibits glucose induced insulin release.[20]

It seems reasonable to suppose that glucose recognition by the α-cells involves mechanisms which are similar to those operative in the β-cells. The present data which demonstrate a greater responsiveness of α-cells to the α-anomer of glucose support this notion. Another observation which would seem to favor the existence of glucoreceptors on the α-cell membrane is the finding that as little as 25 μM iodoacetamide, which has no apparent effect on glycolysis, completely blocked glucose suppression of α-cells, whereas glucose stimulation of the β-cells was greatly enhanced.[21]

In order to more fully assess the significance of the differential effect of α- and β-D-glucose on the insulin and glucagon producing cells of the pancreas, corresponding metabolic studies need to be performed, to see whether the endocrine cells do indeed metabolize the two anomers at comparable rates.

ACKNOWLEDGMENT

We would like to thank Mrs. Veronica Frasier, Mrs. Joy Brothers and Mr. Duane Martin for their technical assistance, and Mrs. Karen Lambarth for typing the manuscript.

Dr. Pagliara is an Investigator of the Howard Hughes Medical Institute. This work was supported by U.S.P.H.S. Research Grants AM-10591, AM-17810 and Clinical Research Centers Grant R-0036.

REFERENCES

[1]Pagliara, A.S., Stillings, S.N., Hover, B., Martin D.M. and Matschinsky, F.M.: Glucose modulation of amino acid-induced glucagon and insulin release in the isolated perfused rat pancreas. J. Clin. Invest. *54*:819-32, 1974.

[2]Gerich, J.E., Charles, M.A. and Grodsky, G.M.: Characterization of effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. J. Clin. Invest. *54*:833-41, 1974.

[3]Coore, H.G., and Randle, P.J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated *in vitro*. Biochem. J. *93*:66-78, 1964.

[4]Ashcroft, S.J.H., Hedescov, C.V., and Randle, P.J.: Glucose metabolism in mouse pancreatic islets. Biochem. J. *118*:143-54, 1970.

[5]Unger, R.H., and Lefebvre, P.J.: Glucagon physiology. *In* Glucagon. Lefebvre, P.J. and Unger, R.H., editors. New York, Pergamon Press Ltd., 1972, 213-44.

[6]Edwards, J.C., and Taylor, K.W.: Fatty acids and the release of glucagon from isolated guinea pig islets of Langerhans incubated *in vitro*. Biochem. Biophys. Acta. *215*:310-15, 1970.

[7]Matschinsky, F.M., Landgraf, R., Ellerman, J., and Kotler-Brajtburg, J.: Glucoreceptor mechanisms in the islets of Langerhans. Diabetes *21*:Suppl. 2, 555-69, 1972.

[8]Salas, J., Salas, M., Eladio, V., and Sols, A.: Glucokinase of rabbit liver: purification and properties. J. Biol. Chem. *240*:1014-18, 1965.

[9]Rossini, A.A., Berger, M., Shadden, J., and Cahill, G.F. Jr.: Beta cell protection to alloxan necrosis by anomers of D-glucose. Science *183*:424, 1973.

[10]Grodsky, G.M., Bates, A.A., Bennett, L.L., Vcella, C., McWilliams, N.B., and Smith, D.F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am. J. Physiol. *205*:638-44, 1963.

[11]Landgraf, R., Kotler-Brajtburg, J., and Matschinsky, F.M.: Kinetics of insulin release from the perfused rat pancreas caused by glucose, glucoamine and galactose. Proc. Natl. Acad. Sci. U.S.A. 68:536-40, 1971.

[12]Scharff, R., and Wool, I.G.: Concentration of amino acids in rat muscle and plasma. Nature (Lond.) *202*:603-04, 1964.

[13]Bailey, J.M., Fishman, P.H., and Pentchev, P.G.: Studies on mutorotases II. Investigations of possible rate-limiting anomerizations in glucose metabolism. J. Biol. Chem. *243*:4827-31, 1968.

[14]Lowry, O.H., Passonneau, J.V., Hasselberger, F.X. and Schulz, D.W.: Effect of ischemia on known substrates and cofactors on the glycolytic pathway in brain. J. Biol. Chem. *239*:18-30, 1964.

[15]The Beckman Glucose Analyzer Operating Manual: Beckman Instruments, Inc. Fullerton, California, July 1970.

[16]Niki, A., Niki, H., Miwi, I., and Okuda, J.: Insulin secretion by anomers of D-glucose. Science *186*:150-51, 1974.

[17]Bhattacharya, G.: Protection against alloxan diabetes by mannose and fructose. Science *117*:270-31, 1953.

[18]Carter, W.J., and Younathan, E.S.: Studies on protection against the diabetogenic effect of alloxan by glucose. Proc. Soc. Exp. Med. *109*:611-12, 1962.

[19]Cahill, G.F., Jr., Rossini, A.A., and Arcangelli, M.A.: Stereospecificity and affinity of β-cell alloxan toxic receptor to 3-0-methyl glucose. Diabetes *23*:Suppl. 1, 339, 1974.

[20]Tomita, T., Lacy, P.E., Matschinsky, F.M., and McDaniel, M.L.: Effect of alloxan on insulin secretion in isolated rat islets perifused in vitro. Diabetes *23*:517-24, 1974.

[21]Pagliara, A.S., Hover, B., and Matschinsky, F.M.: The biochemical basis of the inhibitory and permissive action of glucose in α and β-cells. Fifty-fifth Annual Meeting of the Endocrine Society, A-192, June 1974.

ADDENDUM

Since submission of this manuscript Grodsky et al., (Science *186*:536, 1974) utilizing the isolated perfused rat pancreas, have also demonstrated that the α-D-glucose anomer is a more potent stimulator of insulin release than is the β-anomer.