The Dual Function of Glucose in Islets of Langerhans*

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SUMMARY

It is widely accepted that metabolites of glucose rather than glucose itself trigger insulin release from β-cells. If so, level changes of such metabolites ought to occur in β-cells within seconds of a glucose pulse. To examine this hypothesis, glucose, glucose-6-P, fructose-diP plus triose-P, 6-P-glucosone, ATP, and P-creatine were measured in individual rat islets after intravenous infusion for 1 to 60 min of glucose, mannoheptulose, and xylitol, or combinations of these compounds. Insulin levels in serum were determined concomitantly. Some of the animals were fasted for 5 or 6 days prior to glucose infusion.

Individual islets were dissected from freeze-dried sections sampled with quick freezing methods. The sensitivity and specificity needed for analysis of metabolites and cofactors were gained by using enzymatic fluorometric procedures combined with an oil well method and enzymatic cycling of pyridine nucleotides. Serum insulin was determined immunochemically.

Insulin levels in peripheral blood rose within 1 min of injection of glucose. Mannose-6-P as well as fasting blocked this glucose effect. Penetration of glucose, although very rapid, was carrier mediated, since under special conditions mannoheptulose and also xylitol caused exit countertransport of glucose from the β-cells. This shows that mannoheptulose has effects on glucose metabolism of islets that are independent of its ability to inhibit phosphorylation. Most of the metabolites and cofactors measured were unchanged during the first 5 min of glucose infusion. Fructose-diP plus triose-P rose within 1 min in fed animals, but also increased when insulin release was blocked in various ways.

After 1 hour of hyperglycemia there was a marked rise in all metabolites and cofactors save ATP. The metabolic changes induced by glucose infusion in severely fasted animals, indicate that islet cell metabolism was stimulated, although insulin release was blocked.

These results and reports by other investigators suggest that glucose itself rather than its metabolites triggers insulin secretion.

Glucose is the principal stimulus that touches off insulin release from the β-cells. The cellular constituents and biochemical events subserving this release are still largely unknown, in spite of intensive research efforts. There are at least three hypothetical mechanisms that, a priori, might explain the phenomenon. The pancreatic islets might be responsive: (a) to glucose directly, (b) to changes in intermediates, cofactors, or both, resulting from metabolism of glucose, or (c) to glucose directly subject to modulation by levels of intermediates, cofactors, or both, of glucose metabolism.

Most investigators active in this field have promoted the second hypothesis in one form or another (e.g., References 3-6). We submitted this metabolism theory to further testing in this study. If the theory were true, the crucial alterations of intermediate and cofactor levels in islets should occur prior to release of the hormone. Also, if such level changes were inductive, they should not appear independent of enhanced secretion. The levels of glucose, glucose-6-P, the sum of fructose-diP, and the triose-P, 6-P-glucosone, ATP, and P-creatine were measured in individual rat islets at intervals between 1 and 60 min of intravenous infusion of glucose. The effects of mannoheptulose, xylitol, and of prolonged fasting on above parameters of metabolism were also explored. The insulin levels in serum were determined concomitantly in all experiments.

METHODS

Animal Experiments and Tissue Handling—Seventy-seven Holtzman rats of both sexes, weighing about 400 g, were used. The animals were either fed, or fasted for 12 to 120 hours. They were anesthetized with pentobarbital (30 to 45 mg per kg of weight, intraperitoneally). In preparation for the anesthesia, a small dose of atropine (0.2 mg per kg intraperitoneally) was injected. This cholinergic blockade prevented pulmonary edema and minimized the danger of hypoxia during the experiment. The trachea, the right jugular vein, and the left carotid artery were cannulated with polyethylene tubing (Clay Adams, Parsippany, New Jersey: PE 200, 50, and 60, respectively). The pancreas was exposed and mobilized. Concentrated solution (1 to 2 ml) of glucose and mannoheptulose, alone or combined, or of xylitol, were infused into the vein at constant rates. An infusion pump (Harvard Apparatus model 600-910/020) was used for this purpose.

Blood samples of 0.2 to 0.4 ml were collected from the artery, with use of the blood pressure as a convenient force to accelerate sampling (~0.5 ml every 10 sec). After clotting (~30 min at 25°) the serum was separated from the cells by centrifugation and was stored at −20°. At the end of the experiment, immediately...
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6-P-GLUCONATE, moles x 10^-15

FIG. 1. Yield of product in the assays for 6-P-gluconate (A) and for fructose diphosphate (B). The ordinates record the yield of cycling product 6-P-gluconate in A and glutamate in B from 6-P-gluconate (A) and fructose diphosphate (B) shown on the abscissas. Amplifications at the cycling steps were approximately 20,000- (A) and 10,000-fold (B).

after taking the last blood probe, the pancreas was excised and frozen instantly in Freon 12 (CCl2F2) chilled to its freezing point (-150°) with liquid N2. Sections of the pancreas were cut in the cryostat 20 μm thick and were submitted to freeze-drying for 40 hours at -40° (7). Islets were dissected at low magnification and without prior staining in a room that provided low humidity (50%) and constant temperature (18°). The weights of the specimens were obtained with the quartz fiber fish pole balance (7).

Chemical Analytical Procedures—Serum glucose was measured in duplicate by an enzymatic fluorometric method (8); serum insulin was measured, also in duplicate, by the Hales and Randle version of the double antibody technique (9). Porcine insulin served as the standard. Intermediates in the islets were determined by enzymatic fluorometric methods (1, 10). The necessary sensitivity was gained by combined usage of enzymatic cycling and an oil well method (10, 11, 12).

RESULTS

Evaluation of Micromethods for 6-P-Gluconate and for Sum of Fructose Diphosphate and Triose Phosphates (Fig. 1)—High accuracy and reproducibility were obtained with authentic 6-P-gluconate and fructose-diphosphate. The amounts of the standards ranged from 0.5 to 2.5 x 10^-14 and from 2 to 10 x 10^-14 moles of 6-P-gluconate and fructose-diphosphate, respectively. The over-all blanks of the two assays were kept as low as 1.5 x 10^-14 and 2 x 10^-14 moles, respectively. The assays were for practical purposes linear and the standard deviations less than 5%. No losses occurred in 6-P-gluconate and fructose-diphosphate, when they were added to freeze-dried tissue samples at the first analytical step (a heat treatment in acid to destroy enzymes and endogenous TPNH or DPNH (10)). When analyzing individual islet samples from the same pancreas the variability was greater than with standards (S.E.M. usually less than ±10%). The other methods used for analysis of metabolites and cofactors were of comparable precision (1, 10).

Metabolic Changes in Pancreatic Islets during Insulin Release Provoked by Glucose (Fig. 2)—The insulin levels in arterial blood

2 In the group marked with the letter a, levels of glucose were calculated assuming a water content of 75% and equal distribution between intra- and extracellular space (1, 13).
had tripled as early as 1 to 1½ min after intravenous injection of glucose. This indicates that β-cells must have responded within seconds. Insulin levels in the serum did not increase further during continued glucose infusion. The apparent peak of the relative levels in the 5-min group is caused by the atypically low serum concentrations of the hormone in the controls. Toward the end of 1 hour the insulin levels showed a tendency to fall. The concentration of intracellular glucose rose to the serum level within the first time interval after the pulse dose was given. The sum of fructose-diphosphate and triose-P had almost doubled by that time. The levels of all other intermediates and cofactors measured were unchanged; 5 min after glucose infusion, fructose-diphosphate plus triose-P had increased even more, to 250% of control levels. In addition, P-creatine stores were strikingly elevated. After 60 min of glucose infusion, the concentration of all substances measured except ATP had increased 2- to 3-fold. It is worth emphasizing that the ATP content of the β-cells remained extremely constant under greatly differing experimental conditions.

The intermediate and cofactor patterns of islet tissue, measured 1 to 1½ min after glucose dosage, do not necessarily reflect the situation at the moment when insulin release commenced. Extrapolations from the data obtained at 1 min to the critical first few seconds are therefore tentative. With this reservation, since the islet content of fructose-diphosphate plus triose-P rose substantially within the first time interval, one of these intermediates might qualify as the signal for release. Analogous studies with the isolated perfused pancreas, which allows accurate sampling to be performed within 15 sec of a glucose pulse, should give a better account of the initial kinetics. The decrease of the ratio of glucose-6-phosphate to fructose-diphosphate plus triose-P (from 3.5 to 2) following glucose treatment suggests that P-fructokinase was activated, or else that blockade occurred below the triose-P step. The first explanation seems more plausible on the basis of general knowledge about regulation of glycolysis (8, 14) (since glucose-6-phosphate did not fall, hexokinase was presumably also activated but to a lesser extent). The mechanism of P-fructokinase activation is obscure, particularly since the energy potential, which usually governs glycolytic rate, was apparently unaltered. In the search for an explanation one should not discount the possibility that intracellular alterations of the levels of free Ca++, K+, or both, which presumably occur during glucose-stimulated insulin release (15-17) may also affect the glycolytic flux.

The lack of early change of glucose-6-phosphate in rat islets is surprising in view of the rapid rise of this metabolite in islets of obese mice after glucose administration. Glucose-6-phosphate rose 400% or more within 1 to 1½ min (18). The species difference in the behavior of the metabolite may possibly be explained by a difference in the islet’s hexokinase or hexokinases. Kinetic studies in vitro with mouse islet tissue gave evidence for a "hexokinase" (low KM for glucose) and a "glucomokinase" (high KM). One hexokinase activity was found in rat islets (1, 13). The species difference in glucose-6-phosphate response emphasizes the importance of comparative biochemical studies, when studying such basic mechanisms as glucose-provoked insulin release.

The changes of intermediary metabolism that became apparent after 1 hour of glucose infusion are in agreement with the results obtained in other comparable long term studies in vivo and in vitro (13, 19).

Effect of Long Term Fasting on Metabolism and Insulin Release

It is well documented (20) and was confirmed in this study (Fig. 3) that fasting or prolonged fasting paralyzes insulin release provoked by glucose. Metabolite levels in islets in general were found to be lower than in the fed state, but P-creatine was twice as high as in fed controls. When glucose was infused into fasted animals several puzzling alterations of intermediary metabolites were observed. Five minutes after glucose injection the levels of glucose-6-phosphate and 6-phosphogluconate were about 2 times higher than in the 0.9% NaCl solution controls, but concentrations did not surpass those found in fed control animals. Fructose-diphosphate and triose-P were also increased 2-fold at this time and were maintained at this elevated level for an hour. The levels of 6-phosphogluconate were comparably high in controls and experiments when glucose infusion lasted for 1 hour, quite in contrast to the findings in fed animals. At this time, glucose-6-phosphate levels were equally high but increased in the control and the glucose-treated group. Prolonged anesthesia in addition to the state of severe fasting might explain this result, which is also quite different from the data obtained with fed animals.

Regardless of some uncertainty about the meaning of the elevated glucose-6-phosphate content in fasted animals after prolonged anesthesia results show that fasting has blunted a component or components of the glucose-sensitive release mechanism, that seem to be unrelated to glucose penetration or to the first two glycolytic phosphorylation steps. Power failure within the islets as a possible explanation of fasting diabetes can also be ruled out, since the energy potential of the cells (ATP plus P-creatine) actually rose during fasting. If metabolism is in fact linked with the re-
lease mechanism, fasting must have uncoupled it. From the data gathered in fed and fasted animals it seems likely that the rise of 6-P-glucuronate is an essential feature of the late phase of glucose stimulated insulin release. Montague and Taylor (18) arrived at a similar viewpoint based on results gathered with isolated islets. Since glucose-6-P and 6-P-glucuronate rose concomitantly, it might be inferred that the flux through the pentose-P pathway was increased in the late phase. But 6-P-glucuronate can hardly qualify as a signal for early release, since the tissue concentration rose only after a considerable delay (>5 min).

Similar conclusions were drawn from the results of analogous studies with obese hyperglycemic mice (21).

Effects of Mannoheptulose and Xylitol on Metabolite Patterns (Fig. 4)—Mannoheptulose, injected with equimolar amounts of glucose, blocked insulin release but barely influenced levels of ATP, P-creatine, glucose-6-P, and 6-P-glucuronate. Even the rise of fructose-di-P and triose-P seen after injection of glucose alone, was not entirely prevented. In fact, the ratio of glucose-6-P to fructose-di-P plus triose-P fell to half the control ratio as was seen with glucose alone. However, when mannoheptulose was given alone it caused drastic perturbations of the metabolite pattern. The levels of islet glucose and of its phosphorylated products dropped far below control values. Fructose-di-P plus triose-P became almost undetectable. ATP and P-creatine concentrations remained unaltered despite the depletion of glycolytic intermediates.

The action of xylitol was studied in two groups of three and four animals each. Measurements were made 75 to 105 sec after starting the injection, at which time the estimated xylitol concentration in serum was about 40 mmol per liter. In the smaller group serum insulin levels rose from 81 ± 15 to 133 ± 14 μunits per ml. In the second group serum insulin levels were unchanged at 105 sec after the injection started (48 ± 5 versus 51 ± 9). Reports from various laboratories also suggest that insulin release caused by xylitol varies greatly (18, 22). Nevertheless, the metabolic response of the islets of these two groups of animals was the same, no matter what happened functionally. The islet glucose levels fell from 35 ± 4 (6) to 17 ± 2 (7) mmol per kg of tissue, dry weight; glucose-6-P dropped from 232 ± 30 (6) to 136 ± 15 (5) μmol per kg of tissue, dry weight; 6-P-glucuronate was hardly altered, 31 ± 4 (6) versus 25 ± 0.5 (7) μmol per kg of tissue, dry weight, and fructose-di-P plus triose-P rose from 68 ± 6 (6) to 102 ± 10 (7) μmol per kg of tissue, dry weight. As a result the ratio of glucose-6-P to fructose-P plus triose-P fell from 3.5 to 1.3.

Hexokinase levels of rat islets are too low (0.1 mole per kg of tissue, dry weight, per hour at 38° (1)) to explain the drop of intracellular glucose that was observed after mannoheptulose and xylitol injection. The results can be explained if the two substances compete with glucose for the same membrane carrier. In this case the high external concentrations of the competitors would result in exit counterflow of glucose. These data serve as additional support for the opinion expressed earlier, that glucose penetration into pancreatic islets is carrier mediated (1, 3, 23).

DISCUSSION

Glucose might play a double role in the islet cells. It might act as a purely chemical stimulant in addition to its universal role as substrate of metabolism. A number of reports from the literature and the results published in this paper, concur with such a dual function.

The stimulation of insulin release by mono- or disaccharides (3–5, 24), polyols (18, 22), and N-acetylglucosamine (24) can be best explained if the mechanism of action of all of these compounds involves a glucoreceptor of very broad specificity. This receptor is presumably located in the cell membrane, either part of a carrier system or an independent site. It is accessible from the outside or from the inside of the cell. The order of potency of these stimulants would be determined by the order of affinity for the receptor. The inhibition by mannoheptulose (4), and 2-deoxyglucose (5, 25) of glucose provoked insulin release can be equally well understood if one assumes that these antimitabolites compete with glucose for the receptor site and have little or no releasing potency. The two actions of glucosamine, which can stimulate release by itself (4, 24) and can also block glucose-induced insulin release (4, 5) are also consistent with the receptor hypothesis. The action of inhibitory sugars is complicated, since they probably also interfere with the metabolism of glucose, when they reach sufficiently high intracellular concentration (26–28).

Another argument for the existence of a glucoreceptor can be derived from the studies of Renold et al. (29) showing that 2-deoxyglucose and mannoheptulose enhanced insulin release by tolbutamide. A possible explanation is that the drug receptor and a glucoreceptor are located close to each other in the membrane.

The evidence coming from the present study favors the glucoreceptor hypothesis. Among the intermediates and cofactors of glucose metabolism studied only fructose-di-P plus triose-P have exhibited sufficiently rapid changes in concentration to qualify as triggers for release. But since these compounds also rose in various conditions in the absence of insulin release, it seems probable that the changes merely indicate activation of glycolysis. Furthermore, since glucose-6-P was initially unchanged, the elevation of fructose-di-P and triose-P was probably a secondary event conceivably caused by changes in the ionic environment or by a rise in cyclic 3',5'-AMP (see References 29–35).

The fact that the early intermediates and several cofactors of glucose metabolism, which might serve as signals for release, behave in a fashion that apparently disqualifies them for such a role, does not rule out the possibility that other less prominent metabolites might function as triggers. A definite answer to the question must be delayed until other metabolites, which are more re
note from the step of glucose activation (e.g., P-glycerates) and which are less abundant (e.g., acetyl-CoA), have also been studied.

Prolonged hyperglycaemia influences islet cell metabolism as indicated by the present study and by the findings of other investigators. Montague and Taylor (19) found that 6-P-glucose levels of islets were elevated 30 min after stimulation in vitro with glucose in the presence or absence of thymidine. They concluded from this that the pentose-P pathway was an essential part of the release mechanism. Similarly, Ashcroft, Hedeskov, and Randle (30) observed that glucose-6-P concentrations of islets were increased 30 min after stimulation in vitro with glucose and that fructose-6-P levels were elevated after the same time interval, when glucose was replaced by mannose. They hypothesized that glucose-6-P might be the trigger, when glucose is the stimulus and that fructose-6-P might serve as the signal, when mannose is present. The question is whether the changes in metabolite levels occur rapidly enough to explain the endocrine response to glucose which occurs within seconds.

It is conceivable that the late effects of glucose on islet cell metabolism might modify the responsiveness of the secretory complex. This is suggested, because after prolonged superfusion of pancreas tissue with glucose the β-cells were sensitized to renewed stimulation by glucose (37). Curry, Bennett, and Grosky (38) proposed that metabolic feedback processes could explain the multiphasic secretory response of the pancreas to glucose. Our results are not inconsistent with this attractive idea.

REFERENCES


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