Regulatory Role of Fructose-2,6-Bisphosphate in Pancreatic Islet Glucose Metabolism Remains Unsettled

PAMELA TRUEHEART BURCH, DONNA K. BERNER, HABIBA NAJAFI, MARTIN D. MEGLASSON, AND FRANZ M. MATSCHINSKY

SUMMARY

Fructose-2,6-P_2 was measured in perifused, isolated rat pancreatic islets. Fructose-2,6-P_2 was present in pancreatic islets at low levels approximately equal to fructose-2,6-P_2 content of liver from fasted rats. In islets perifused with glucose at physiologic concentrations, fructose-2,6-P_2 was increased from 0.8 μM in the presence of 5.5 mM glucose to 1.0 μM at 10 mM glucose and 1.3 μM at 16.7 mM glucose, but did not increase further at higher glucose concentration. Therefore, only modest increases in the phosphofructokinase-1 activator, fructose-2,6-P_2, occur at glucose concentrations stimulating insulin secretion. DIABETES 1985; 34:1014–18.

Glucokinase controls glucose use by pancreatic islet β-cells, and recently it has been proposed to be the sensor for glucose stimulation of insulin release.[1] There are many arguments in favor of this concept: the measured phosphorylation capacity of glucokinase in islet homogenates is equal to the actual glycolytic rate of intact islets in vitro,[2,3] and the biophysical characteristics of the enzyme glucokinase are such that they explain the concentration dependency, substrate specificity,[2,4,5] sensitivity to inhibition by mannoheptulose[4,6] and alloxan,[7–9] and the anomeric discrimination of hexose use and hexose-induced insulin release.[10–16] The rate of other regulatory glycolytic enzymes is adjusted to the pace-setting rate of glucokinase through complex level changes of substrates and known allosteric modifiers. For example, at the phosphofructokinase (fructose-6-P, 1-kinase; PFK-1) step, flux is generally believed to be gated by altered levels of glucose-6-P, fructose-6-P, glucose-1,6-P_2, fructose-2,6-P_2,[17] fructose-1,6-P_2, P-glycerate, P-enolpyruvate, citrate, adenine nucleotides, P-creatine, and P_i (reviewed in refs. 18 and 19). The pH and the levels of K^+ and NH_4^+ also help govern PFK-1 activity.[18] Considering the high complexity of the known glucose-induced changes of the intermediates and co-factor constellation in islet tissue and realizing our ignorance about the kinetic characteristics of islet PFK-1, it is at present impossible for the biochemist to quantitatively assess the regulatory role of any single one of these modifiers. Previously reported studies proposing a pivotal role of fructose-2,6-P_2[20,21] in controlling glycolysis in glucose-stimulated islets have to be viewed in light of the above comments.

Fructose-2,6-P_2 content in pancreatic islets has been reported with widely varying results. Glucose-deprived islets have been reported to contain relatively high levels of fructose-2,6-P_2 (i.e., ≈15 μM*) and exposure to a high glucose concentration is claimed to raise fructose-2,6-P_2 further to about 50 μM.[20] In a later study by the same group, fructose-2,6-P_2 content of islets was found to be about one-tenth of those levels published previously.[21] Levels of fructose-2,6-P_2 in islets even lower than these values and increasing little with glucose have also been observed, i.e., 0.6 μM in glucose-deprived islets and 1.3 μM in islets exposed to 16.7 mM glucose.[22] Since the fructose-2,6-P_2 increment due to physiologic changes of glucose concentration in the medium was at most 50%, this led us to doubt that fructose-2,6-P_2 could play a unique role as activator of PFK-1 in β-cells. The present article provides a detailed report of this study.

MATERIALS AND METHODS

Laboratory animals. Male Wistar rats from Hilltop Laboratory Animals (Scottdale, Pennsylvania) were used. The animals were 3–4 mo old. They were fed ad libitum with Purina rat chow and water. In case of fasting, food was withheld for 48 h with free access to water.

This study was presented in part at the International Symposium on Effects of Hormones on Cellular Membrane Systems, Woudschoten Conference Center, Zeist, The Netherlands, September 18–22, 1983.

From the Diabetes Research Center and Departments of Biochemistry and Biophysics and Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania.

Address reprint requests to Dr. Franz M. Matschinsky, Diabetes Research Center, University of Pennsylvania, 414 Anatomy-Chemistry Building G3, 36th and Hamilton Walk, Philadelphia, Pennsylvania 19104.

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*The comparison of data from different studies is based on the assumption of 3 nl water space/μg dry wt or protein.

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Islet isolation, perifusion, and quick-freezing of islets. Islets were isolated from the pancreas of Wistar rats as described previously.[4] Islets in batches of about 500 were loaded onto Nitex filters (Tetko, Elmsford, New York) in perifusion chambers using a system described previously[23] and perifused using Krebs bicarbonate buffer supplemented with 0.25% bovine serum albumin (Reheis, Kankakee, Illinois) and continuously gassed with 95% O_2/5% CO_2 to maintain a pH of 7.35–7.45. Islets were first perifused for 30 min with 5.5 mM glucose to obtain stable baselines. This control period was followed by 15-min perifusion in the absence of glucose, or with 5.5, 10, or 16.7 mM glucose; in two experiments, islets were perifused with 27.5 mM glucose. Perifusate samples were collected at intervals and stored at −20°C for later analysis of insulin by radioimmunoassay.[24] To harvest the tissue, the perifusion was stopped and the filters containing the islets were quickly removed from the plastic chamber and frozen on a slab of dry ice. The frozen islets were stored at −80°C.

Freeze-drying and extraction of islet tissue. The filters carrying islets were placed into plastic beakers capped with perforated lids and were freeze-dried for 60 h at −40°C and were then stored under vacuum at −20°C. Batches of about 3000 islets each were pooled (usually the harvest from 5–7 perifusion experiments) and processed as described below. Considering the multiple perifusion conditions listed above, we processed altogether 26 batches of 3000 islets each. Each batch of 3000 islets was treated as a single experiment.

Freeze-dried powder prepared from crushed, quick-frozen liver of 48-h-fasted rats served as biologic standard. Aliquots of 5 mg liver powder were processed as described for freeze-dried islets.

Before extraction, the freeze-dried islets were warmed to room temperature still under vacuum. Four to six filters with freeze-dried islets or 5 mg of freeze-dried liver powder were placed in the bottom of 1.5-ml plastic centrifuge tubes fitting the Beckman microfuge (Beckman Instruments, Fullerton, California). Then, 600 μl of extraction buffer (1 mM Na-Hepes, pH 8.5) preheated to 75°C was quickly added; the tubes were briefly vortexed and then incubated for 10 min in a waterbath of 75°C. Vortexing was repeated and the tubes placed on ice to terminate extraction. The filters were removed and the tubes were briefly centrifuged to separate a precipitate that had formed during the heat treatment. Two aliquots were prepared in a manner that allowed the measurement of acid-stable and acid-labile PFK-1 activators. A 225-μl aliquot of supernatant was removed and 75.5 μl of buffer C was added followed by mixing (buffer C was prepared as follows: 1.9 ml of 400 mM Na-Hepes, pH 7.4, containing 0.8 mM EDTA, 20 mM $MgCl_2$, and 2 mM NH_4Cl was mixed with 100 μl of 3 N HCl and 30.6 μl of 10 N NaOH). To another aliquot of 300-μl supernatant was added 5.15 μl of 3 N HCl followed by mixing. After 25 min at room temperature, 100 μl of buffer D was added (buffer D was prepared as follows: 1 ml of 400 mM Na-Hepes, pH 7.4, containing 0.8 mM EDTA, 20 mM $MgCl_2$, and 2 mM NH_4Cl was combined with 15.3 μl of 10 N NaOH). These samples were centrifuged for 30 min at 2000× *g* on Centrifree filters (Amicon, Danvers, Massachusetts). Each filtrate was divided into three aliquots that were stored at −80°C. Fructose-2,6-P_2 standards were treated in the same manner. In recovery studies, fructose-2,6-P_2 standards were simultaneously admixed to the extraction buffer alone or to extraction buffer containing freeze-dried islets or liver tissue.

Assay of fructose-2,6-P_2. Fructose-2,6-P_2 was assayed by measuring activation of PFK-1 from rabbit muscle inhibited by ATP. The assay was carried out in 10 × 75-mm fluorometer tubes in a volume of 100 μl. Each tube contained 15–30 μl of islet or liver extract equivalent to 75–150 islets or 120–250 μg of liver tissue. The final concentration of the assay reagent was as follows: 100 mM Na-Hepes, pH 7.4, 0.2 mM EDTA, 5.0 mM $MgCl_2$, 0.5 mM NH_4Cl, 0.02% bovine serum albumin, 2.5 mM dithiothreitol, 1.0 mM fructose-6-P, 0.15 mM NADH, 2 mM ATP, 0.45 U/ml aldolase, 0.7 U/ml glycero-P dehydrogenase, 5 U/ml triose-P isomerase, and 0.015 U/ml muscle PFK-1. These enzymes were obtained from Boehringer (Indianapolis, Indiana). Aldolase, glycero-P dehydrogenase, and triose-P isomerase were dialyzed against 100 mM Na-Hepes, pH 7.4, 0.2 mM EDTA, 5 mM $MgCl_2$, and 0.5 mM NH_4Cl and stored at 4°C. PFK-1 was dialyzed against 50 mM Na-Hepes (pH 7.4), 4 mM EDTA, 150 mM KCl, 4 mM $MgCl_2$, 2.5 mM dithiothreitol, 0.2% bovine plasma serum albumin, 1 mM 5′-AMP, 3 mM K_3PO_4, 1 mM K_2SO_4, and stored in aliquots at −80°C. All assay tubes were kept on ice until the reaction was started with 2 mM ATP. Incubation was for precisely 1 h at 21°C. The reaction was stopped by adding 1 ml of 0.05 N NaOH containing 1 mM cysteine HCl. Fluorometry was used to quantitate the oxidation of NADH. Each routine assay included four islet samples, one liver sample (each treated with acid or weak base), and a set of 10 fructose-2,6-P_2 standards ranging from 25 to 1130 fmol. Samples and standards were measured in triplicate. Including several blanks, this resulted in 80–90 tubes per assay. To assess interassay reproducibility each islet sample was measured in triplicate in two different assays.

Standardization of fructose-2,6-P_2. As standards for the present study, we have employed fructose-2,6-P_2 purchased from Sigma and a sample from Dr. K. Uyeda (University of Texas Health Science Center, Dallas, Texas). Fructose-2,6-

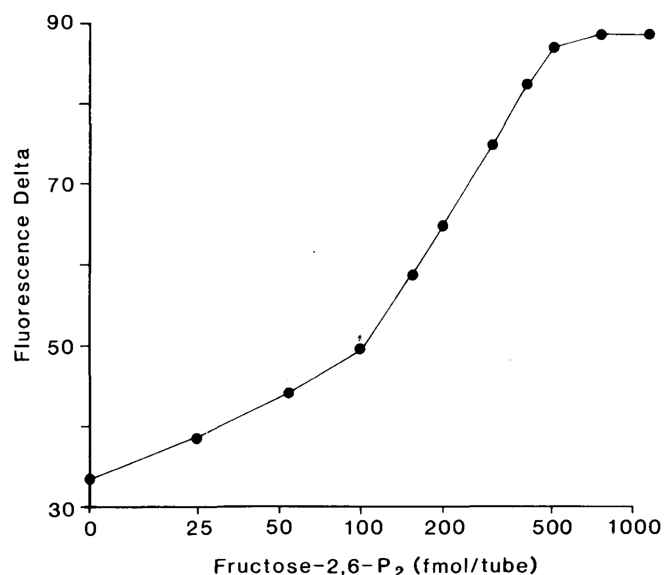

FIGURE 1. Standard curve for activation of muscle phosphofructokinase by fructose-2,6-P_2. Data are shown as decrease in NADH fluorescence. Note that fructose-2,6-P_2 concentration is plotted on a logarithmic scale.

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TABLE 1
Fructose-2,6-P_2 content of perifused rat islets as a function of glucose concentration

(Glucose, mM)	0			5.5			10			16.7			27.5		
(Exp.)	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	2.13	1.30	0.83	1.79	1.21	0.58	2.50	1.25	1.25	2.55	1.06	1.50	3.28	1.34	1.94
2	1.64	1.13	0.51	1.75	1.08	0.67	2.38	1.22	1.16	2.56	1.14	1.42	3.02	1.64	1.38
3	1.72	1.11	0.61	2.60	1.29	1.31	2.78	1.18	1.60	1.98	0.96	1.02			
4	2.51	0.95	1.56	3.27	1.30	1.97	3.42	1.53	1.89	3.48	1.55	1.92			
5	2.53	1.34	1.18	2.85	1.74	1.11	3.04	1.44	1.60	5.12	1.13	3.99			
6	2.26	1.41	0.85	3.09	1.22	1.87	3.00	1.30	1.70	3.18	1.13	2.05			
Mean	2.13	1.21	0.92	2.56	1.30	1.25	2.85	1.32	1.53	3.15	1.16	1.98	3.15	1.49	1.66
SEM	0.16	0.07	0.16	0.28	0.09	0.24	0.16	0.06	0.11	0.46	0.09	0.43			

Data are shown as individual values and means ± SEM for total (column A), acid-stable (column B), and acid-labile (fructose-2,6-P_2; column C) PFK-1 activator. PFK-1 activation is expressed relative to fructose-2,6-P_2 standard and shown as femtomol/islet. Statistical analysis of the effect of glucose on islet fructose-2,6-P_2 content (P-values shown are for one-tail tests): F-test: 0 vs 5.5 mM, $P = 0.38$; 0 vs 10 mM, $P = 0.11$; 0 vs 16.7 mM, $P < 0.01$; and 0 vs 27.5 mM, $P = 0.17$. SS-STP test: 5.5 vs 10 mM, $P > 0.50$; 5.5 vs 16.7 mM, $0.25 > P > 0.50$; 5.5 vs 27.5 mM, $P > 0.50$; and 16.7 vs 27.5 mM, $P > 0.50$.

P_2 was dissolved in 0.1 N NaOH to provide stock solutions of 0.25 to 7.7 mM. The stock solutions were standardized fluorometrically by measuring fructose-6-P before and after acid treatment of the stock solution. Different standard solutions gave similar results when compared with the above fluorometric PKF-1 activation assay.

Statistical methods. Statistical analysis was performed by one-way analysis of variance. Subsequent comparison of sample means for a priori hypotheses was by the F-test and for a posteriori hypotheses by the sums of squares simultaneous test procedure (SS-STP, ref. 25).

RESULTS

Methodologic studies. The standard curve presented in Figure 1 shows that the assay is able to detect as little as 25 fmol fructose-2,6-P_2, has a 40-fold range, and is most reliable between 50 and 500 fmol. Acid treatment totally destroyed authentic fructose-2,6-P_2. Using liver tissue from fed rats, we found 7.5 μM fructose-2,6-P_2.* In liver tissue from 48-h-fasted rats, we observed 0.6 μM fructose-2,6-P_2. In liver tissue from fed animals the contamination of acid-resistant PFK-1 activator was <10% of fructose-2,6-P_2. However, in livers from fasted animals, the content of acid-resistant activator was about equal to acid-labile fructose-2,6-P_2. Fructose-2,6-P_2 added to liver or islet tissue at the heat-extraction step was fully recovered. Interassay differences of fructose-2,6-P_2 content in islets or liver tissue were <10%.

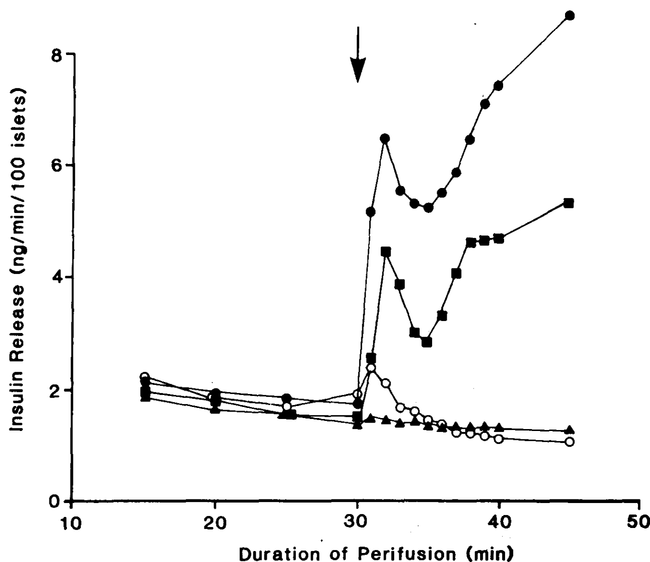

FIGURE 2. Insulin secretion by pancreatic islets perifused with 5.5 mM glucose (0–30 min), then 0 (○), 5.5 (▲), 10 (■), or 16.7 (●) mM glucose for an additional 15 min before rapidly freezing the islets for determination of the fructose-2,6-P_2 content. Data are shown as mean values for insulin secretion at each glucose concentration. The apparent stimulation of insulin secretion by islets switched to glucose-free medium does not represent a significant increase.

Results with islet tissue. The shape of insulin release profiles attests to the functional preservation of the islet tissue studied herein (Figure 2). We were unable to obtain DNA measurements in the present study. An estimate indicates, however, that the DNA content is about 10 ng/islet. This estimate is based on comparison of insulin release rates for the present study with the results of a similar previous study in which DNA data are available[26] and is consistent with DNA values obtained in another study from our laboratory in which islets were prepared using Ficoll gradients as employed in this study.[4] From this, we derive an average islet size of about 2 nl. In calculating fructose-2,6-P_2 concentrations, a water space of 1.5 nl/islet is assumed.

The fructose-2,6-P_2 content of islet tissue sampled during the second phase of glucose-stimulated insulin secretion is very low, about 10% of that reported in liver tissue from fed rats (compare Table 1 with refs. 27–29). In fact, fructose-2,6-P_2 levels in freshly isolated islet tissue from fed rats were comparable to levels reported in livers from fasted rats (≤1 μM) and observed herein in studies of liver tissue. This was the reason for choosing liver tissue from fasted animals as biologic standards in the present study. The analysis of fructose-2,6-P_2 with muscle phosphofructokinase is complicated by the presence in islet tissue of an acid-resistant PFK-1 activator. In the glucose-deprived state, the acid-stable activator contributes 50% to the total activator level. High glucose causes some elevation of islet fructose-2,6-P_2, whereas the acid-stable component is not influenced by glucose (Figure 3). In the physiologically relevant range of glucose concentrations, 5.5–16.7 mM, the fructose-2,6-P_2 level rises 58%, yet this rise fails to achieve statistical significance

*Please see footnote, page 1014.

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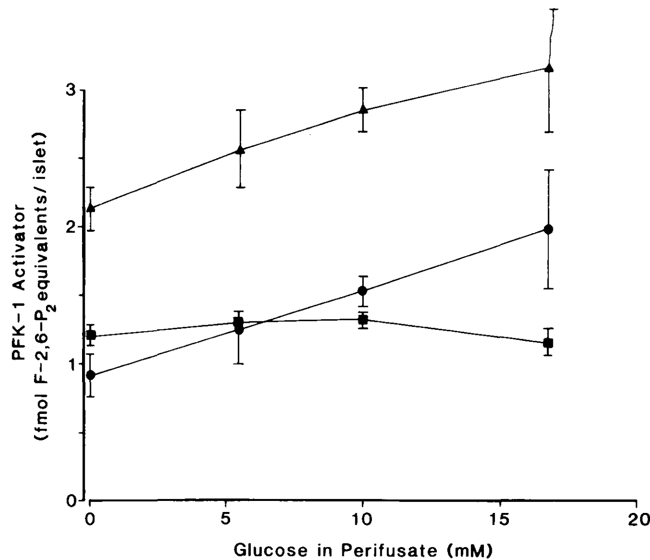

FIGURE 3. Phosphofructokinase-1 activator in pancreatic islets as a function of perifusate glucose concentration. Data are shown as means ± SEM for N = 6 determinations of total (▲), acid-stable (■), and acid-labile fructose-2,6-P₂ (●) PFK-1 activator.

($0.25 > P_{1\text{-tail}} > 0.50$). Increasing the glucose concentration to 27.5 mM did not cause any further increase in fructose-2,6-P_2 (Table 1).

DISCUSSION

In previous studies of rat pancreatic islets, widely disparate fructose-2,6-P_2 contents have been reported.[20,21] Variation in fructose-2,6-P_2 measured in islets has been attributed to differences in the assay methods used in these studies.[21] Previous reports of fructose-2,6-P_2 content of liver indicate similar values, however, whether assays are performed by measuring fructose-6-P formed by acid hydrolysis of fructose-2,6-P_2,[30] by activation of muscle PFK-1 inhibited by ATP,[28,31] or by activation of PP_i:fructose 6-phosphate 1-phosphotransferase.[29] In the present study, fructose-2,6-P_2 was measured by its ability to relieve inhibition by ATP of PFK-1 using a sensitive fluorometric microassay. This method provides results for fructose-2,6-P_2 content in liver samples from rats fed ad libitum or fasted similar to those reported in other studies of liver.[27-29] These data show that the fluorometric microassay used herein is reliable when applied to samples using tissue concentrations selected to contain fructose-2,6-P_2 at 50–500 fmol and when appropriate tissue blanks are included to correct for acid-stable PFK-1 activators.

The results of the present study demonstrate that islet tissue contains only 10% of the hepatic level of fructose-2,6-P_2. Physiologic alterations of glucose levels influence the fructose-2,6-P_2 content of perifused islet tissue very little, i.e., fructose-2,6-P_2 is increased about 20% when glucose rises from 5.5 to 10 mM and about 50% when glucose rises from 5.5 to 16.7 or 27.5 mM. However, these increases in fructose-2,6-P_2 content are not statistically significant; a statistically significant increase in islet fructose-2,6-P_2 content occurs only when glucose concentration in perifusate medium is increased from 0 to 16.7 mM. The concentration-dependency curve differs clearly from the sigmoidal glucose dependency curves of glucose use[2,3] and of insulin release.[32]

The present results are corroborated by an earlier report,[21] but are also contradicted by another study from the same group,[20] which suggests that islet and liver tissue have similarly high levels of fructose-2,6-P_2. Another important distinction of fructose-2,6-P_2 metabolism in liver as compared with islet tissue has also been shown; in contrast to liver tissue,[33,34] glucagon is unable to influence islet fructose-2,6-P_2 levels no matter what the glucose level in the media.[21] This is consistent with the lack of effect of glucagon on glucose oxidation by islet tissue.[35]

It remains to be established what controls the basal fructose-2,6-P_2 levels and how, in molecular terms, glucose brings about the small rise in fructose-2,6-P_2. Many factors govern the level of fructose-2,6-P_2 in liver. Presently available information suggest that in islets the level of fructose-6-P is the major determinant. The significance of other determinants is not clear: 5′-AMP and P_i, which activate fructose-6-P, 2-kinase (PFK-2, ref. 36) in the case of the liver enzyme, are lowered in islets by glucose;[37,38] P-enolpyruvate and citrate, which inhibit PFK-2, rise in islets in the presence of glucose;[39,40] also, α-glycero-P, which enhances fructose-2,6-bisphosphatase, may be elevated in islets by glucose.

It appears then that fructose-2,6-P_2 is one among about a dozen factors that adjust the PFK-1 activity to the pace-setting phosphorylation of glucose by glucokinase. It remains a major challenge for future studies to provide a quantitative analysis of this important but highly complex issue.

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REFERENCES

[1] Meglasson, M. D., and Matschinsky, F. M.: New perspectives on pancreatic islet glucokinase. Am. J. Physiol. 1984; 246:E1–13.

[2] Trus, M. D., Zawalich, W. S., Burch, P. T., Berner, D. K., Weill, V. A., and Matschinsky, F. M.: Regulation of glucose metabolism in pancreatic islets. Diabetes 1981; 30:911–22.

[3] Garfinkel, D., Garfinkel, L., Meglasson, M. D., and Matschinsky, F. M.: Computer modeling identifies glucokinase as glucose sensor of pancreatic β-cells. Am. J. Physiol. 1984; 247:R527–36.

[4] Meglasson, M. D., Burch, P. T., Berner, D. K., Najafi, H., Vogin, A. P., and Matschinsky, F. M.: Chromatographic resolution and kinetic characterization of glucokinase from islets of Langerhans. Proc. Natl. Acad. Sci. USA 1983; 80:85–89.

[5] Zawalich, W. S., Rognstad, R., Pagliara, A. S., and Matschinsky, F. M.: A comparison of the utilization rates and hormone-releasing actions of glucose, mannose, and fructose in isolated pancreatic islets. J. Biol. Chem. 1977; 252:8519–23.

[6] Zawalich, W. S., Pagliara, A. S., and Matschinsky, F. M.: Effects of iodoacetate, mannoheptulose and 3-0-methylglucose on the secretory function and metabolism of isolated pancreatic islets. Endocrinology 1977; 100:1276–83.

[7] 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 1974; 23:517–24.

[8] Henquin, J. C., Malvaux, P., and Lambert, A. E.: Alloxan-induced alteration of insulin release, rubidium efflux and glucose metabolism in rat islets stimulated by various secretagogues. Diabetologia 1979; 16:253–60.

[9] Meglasson, M. D., Burch, P. T., Berner, D. K., Schinco, M. A., and Matschinsky, F. M.: Identification of glucokinase as an alloxan-sensitive glucose sensor of the pancreatic β-cell. Submitted for publication, 1985.

[10] Grodsky, G. M., Fanska, R., and Lundquist, I.: Interrelationships between α and β anomers of glucose affecting both insulin and glucagon secretion in the perfused rat pancreas. Endocrinology 1975; 97:573–80.

[11] Matschinsky, F. M., Pagliara, A. S., Hover, B. A., Haymond, M. W., and Stillings, S. N.: Differential effects of alpha- and beta-D-glucose on insulin and glucagon secretion from the isolated perfused rat pancreas. Diabetes 1975; 24:369–72.

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[12] Malaisse, W. J., Sener, A., Koser, M., and Herchuelz, A.: Stimulus-secretion coupling of glucose-induced insulin release. Metabolism of α- and β-D-glucose in isolated islets. J. Biol. Chem. 1976; 251:5936–43.

[13] Niki, A., Niki, H., and Miwa, I.: Effect of anomers of D-mannose on insulin release from perfused rat pancreas. Endocrinology 1979; 105:1051–54.

[14] Sener, A., Malaisse-Lagae, F., Lebrun, P., Herchuelz, A., Leclercq-Meyer, V., and Malaisse, W. J.: Anomeric specificity of D-mannose metabolism in pancreatic islets. Biochem. Biophys. Res. Commun. 1982; 108:1567–73.

[15] Meglasson, M. D., and Matschinsky, F. M.: Discrimination of glucose anomers by glucokinase from liver and transplantable insulinoma. J. Biol. Chem. 1983; 258:6705–708.

[16] Meglasson, M. D., Schinco, M. A., and Matschinsky, F. M.: Mannose phosphorylation by glucokinase from liver and transplantable insulinoma: cooperativity and discrimination of anomers. Diabetes 1983; 32:1146–51.

[17] Furuya, E., and Uyeda, K.: An activation factor of liver phosphofructokinase. Proc. Natl. Acad. Sci. USA 1980; 77:5861–64.

[18] Passonneau, J. V., and Lowry, O. H.: The role of phosphofructokinase in metabolic regulation. Adv. Enzyme Res. 1964; 2:265–74.

[19] Uyeda, K.: Phosphofructokinase. Adv. Enzymol. 1979; 48:193–244.

[20] Malaisse, W. J., Malaisse-Lagae, F., and Sener, A.: Glucose-induced accumulation of fructose-2,6-bisphosphate in pancreatic islets. Diabetes 1982; 31:90–93.

[21] Sener, A., Van Schaftingen, E., Van De Winkel, M., Pipeleers, D. G., Malaisse-Lagae, F., Malaisse, W. J., and Hers, H.-G.: Effects of glucose and glucagon on the fructose-2,6-bisphosphate content of pancreatic islets and purified pancreatic β-cells. Biochem. J. 1984; 221:759–64.

[22] Matschinsky, F. M., Meglasson, M., and Burch, P. T.: Glucokinase as pacemaker of glycolysis and glucose sensor of pancreatic β-cells. *In* Proceedings of the International Symposium on Effects of Hormones on Cellular Membrane Systems. Zeist, The Netherlands, September 18–22, 1983.

[23] Zawalich, W. S., and Matschinsky, F. M.: Sequential analysis of the releasing and fuel functions of glucose in isolated perifused pancreatic islets. Endocrinology 1977; 100:1–8.

[24] Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. 1965; 25:1375–84.

[25] Sokal, R. R., and Rohlf, F. J.: Biometry. San Francisco, Freeman, 1969:206–213, 226–246.

[26] Burch, P. T., Berner, D. K., Leontire, A., Vogin, A., Matschinsky, B. M., and Matschinsky, F. M.: Metabolic adaptation of pancreatic islet tissue in aging rats. J. Gerontol. 1984; 39:2–6.

[27] Neely, P., El-Maghrabi, M. R., Pilkis, S. J., and Claus, T. H.: Effect of diabetes, insulin, starvation, and refeeding on the level of rat hepatic fructose-2,6-bisphosphate. Diabetes 1981; 30:1062–64.

[28] Hue, L., and van de Werve, G.: Increased concentration of fructose-2,6-bisphosphate in livers of genetically obese mice. FEBS Lett. 1982; 145:263–66.

[29] Hue, L., van de Werve, G., and Jeanrenaud, B.: Fructose-2,6-bisphosphate in livers of genetically obese rats. Biochem. J. 1983; 214:1019–22.

[30] Hue, L.: Role of fructose-2,6-bisphosphate in the stimulation of glycolysis by anoxia in isolated hepatocytes. Biochem. J. 1982; 206:359–65.

[31] Claus, T. H., Schlumpf, J. R., El-Maghrabi, M. R., and Pilkis, S. J.: Regulation of the phosphorylation and activity of 6-phosphofructo 1-kinase in isolated hepatocytes by α-glycerolphosphate and fructose-2,6-bisphosphate. J. Biol. Chem. 1982; 257:7541–48.

[32] Grodsky, G. M.: Secretion of insulin. *In* Handbook of Experimental Pharmacology, Insulin 2. Hasselblatt, A., and Bruchhaussen, F. V., Eds. New York, Springer-Verlag, 1975:1–16.

[33] Hue, L., Blackmore, P. F., and Exton, J. H.: Fructose-2,6-bisphosphate: hormonal regulation and mechanism of its formation in liver. J. Biol. Chem. 1981; 256:8900–903.

[34] Richards, C. S., and Uyeda, K.: Hormonal regulation of fructose-6-P, 2-kinase and fructose-2,6-P_2 by two mechanisms. J. Biol. Chem. 1982; 257:8854–61.

[35] Ashcroft, S. J. H., Hedeskov, C. J., and Randle, P. J.: Glucose metabolism in mouse pancreatic islets. Biochem. J. 1970; 118:143–54.

[36] Hers, H.-G., and Van Schaftingen, E.: Fructose-2,6-bisphosphate 2 years after its discovery. Biochem. J. 1982; 206:1–12.

[37] Trus, M. D., Hintz, C. S., Weinstein, J. B., Williams, A. D., Pagliara, A. S., and Matschinsky, F. M.: A comparison of the effects of glucose and acetylcholine on insulin release and intermediary metabolism in rat pancreatic islets. J. Biol. Chem. 1979; 254:3921–29.

[38] Trus, M., Warner, H., and Matschinsky, F. M.: Effects of glucose on insulin release and on intermediary metabolism of isolated perifused pancreatic islets from fed and fasted rats. Diabetes 1980; 29:1–14.

[39] Matschinsky, F. M., Rutherford, C. R., and Ellerman, J. E.: Accumulation of citrate in pancreatic islets of obese hyperglycemic mice. Biochem. Biophys. Res. Commun. 1968; 33:855–62.

[40] Idahl, L.-A.: Glycolytic intermediates and signals for carbohydrate-induced insulin release. Horm. Metab. Res. (Suppl.) 1980; 10:20–26.

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