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COMMENTARY

SIGNAL RECOGNITION BY PANCREATIC B-CELLS

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In the insulin secreting pancreatic B-cell, glucose is both fuel and physiological stimulus for the initiation of insulin secretion and the regulation of insulin biosynthesis. This dual function of glucose has been the central issue of pancreatic islet research since Grodsky *et al.* [1] and Coore and Randle [2] observed that insulin secretion *in vitro* is stimulated only by the metabolizable hexoses glucose and mannose. The argument in favor of a signal function of fuel metabolism [3] for initiation of insulin secretion by glucose and other nutrient secretagogues has gained considerable strength during the last few years. Meanwhile, the case for a low affinity glucose binding site in the pancreatic B-cell plasma membrane, the so-called B-cell plasma membrane glucoreceptor [4], has not been strengthened [5–9]. Nevertheless, proof of the nonexistence of such a plasma membrane glucoreceptor is practically impossible [6, 8].

The insulin secretory and biosynthetic activity of the pancreatic B-cell is regulated primarily by the glucose concentration in the circulation. As uptake of glucose into the pancreatic B-cells is not rate limiting for glucose utilization [9], variations in the rate of sugar metabolism in the pancreatic B-cell might transduce changes in the extracellular glucose concentrations, enabling physiological alterations of blood glucose concentration to produce corresponding adjustments to the rates of insulin secretion and biosynthesis. Identification of the enzymes at which metabolic regulation is exerted may lead to the elucidation of regulatory mechanisms of insulin secretion. It is important, therefore, to investigate whether the mechanisms underlying regulation of metabolic fluxes in other tissues [10–18] are also relevant for regulation of intermediary metabolism in pancreatic B-cells.

In this commentary we wish to provide a picture of the current understanding of the mechanism underlying recognition of glucose as an insulin secretagogue in the pancreatic B-cell and its relation to the insulin secretory action of hypoglycemic sulfonylureas.

GLUCOKINASE IN PANCREATIC B-CELLS

Pancreatic B-cells are freely permeable to glucose [19]. The intra-islet glucose concentration reflects the glucose concentration in the extracellular fluid [20, 21] and closely follows the blood glucose concentration when it changes in the normal physiological range. Thus, glucose transport into the islet cells is not rate limiting for glucose utilization [9]. The utilization of glucose in the pancreatic B-cells [22], as well as in the liver [23], increases in dependence on the glucose concentration in the millimolar concentration range. This is the range of glucose concentrations where insulin secretion from the pancreatic B-cell responds to glucose stimulation [6, 7]. This points to the phosphorylation of glucose as the concentration-dependent step in the utilization of glucose in the pancreatic B-cells. This, in turn, would increase glycolytic flux rate which may eventually result in the generation of a signal for the initiation of insulin secretion.

As in other tissues, hexokinases (ATP:D-hexose-6-phosphotransferases) catalyze the initial phosphorylation of glucose in the pancreatic islet cells. Indeed, there was early evidence for a low affinity component [20, 24] in pancreatic islets in addition to a high affinity component of this enzyme which is apparently composed of three hexokinase isoenzymes [25]. There are three fractions of glucose phosphorylating activity with high affinity which can be identified by chromatographic separation of rat pancreatic islet cytosol [25], corresponding to the hexokinase isoenzymes types I, II, and III which are also found in other tissues. More definite evidence for the existence of a hexokinase isoenzyme with low affinity (called hexokinase type IV or glucokinase; EC 2.7.1.2) in pancreatic islet cells was obtained using specific antibodies raised against liver glucokinase [26–29]. The low affinity enzyme activity in cytoplasmic fractions from rat [27] and mouse [29] pancreatic islets could be inhibited by this antibody. These antibodies recognized a single protein subunit with an apparent molecular weight of 56,500 in rat pancreatic islet cytosol protein [28]. This is similar to the presumed molecular weight of liver glucokinase [30]. Electrophoretic mobility of this islet cell protein as detected by immunoblotting was also identical to liver glucokinase [28]. This proves the existence of glucokinase protein both in pancreatic islets and liver. Significant expression of the glucokinase gene is apparently restricted to these two tissues. Glucokinase was undetectable in other tissues such as adipose tissue [28], kidney [28], brain [28], or exocrine pancreas†. Chromatographic resolution [25] and immunoblotting after two-dimensional gel electrophoresis [28] revealed two isoforms of the enzyme. However, while they were equally abundant

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in liver extract, the component with the higher pI value dominated in rat pancreatic islet extracts [28]. Glucokinase antibody inhibited enzyme activity equally well in cytoplasmic fractions from rat [27] and ob/ob mouse pancreatic islets [29] which, in contrast to rat islets, are composed virtually exclusively of B-cells [31]. This provides strong evidence for the existence of glucokinase (hexokinase type IV; EC 2.7.1.2) in pancreatic B-cells.

QUANTITATIVE DISTRIBUTION OF GLUCOKINASE IN PANCREATIC B-CELLS

Threshold, half-maximally, and maximally stimulating concentrations for glucose-induced insulin release from isolated islets have been shown to be in the millimolar concentration range, similar to those for the utilization of glucose [6, 7, 9, 32]. There is at least a 5-fold increase of these parameters when the glucose concentration is raised from 3 to 20 mM [6–9, 32]. Several investigators have published quantitative ratios between the hexokinase (types I–III) and glucokinase (type IV) activities in pancreatic islets [33, 34]. However, these ratios do not seem to support the contention that the increments of insulin secretion and glucose utilization are based on a corresponding contribution from glucokinase activity to the total glucose phosphorylation capacity of the pancreatic B-cell [35]. In hepatocytes it is generally accepted that glucokinase activity contributes 70–90% to the total glucose phosphorylation capacity [36]. With a photometric method using the NAD-dependent glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*, Burch *et al.* [33] and Bedoya *et al.* [37] reported contributions of glucokinase to the total glucose phosphorylation capacity in homogenates of islets from rats, mice, and hamsters and of human islets ranging from 15 to 40% only. Measuring glucose phosphorylation capacities with the same method in rat islet cytoplasmic supernatant fractions, authors from the same research group observed roughly equal contributions of hexokinase and glucokinase activities to the total glucose phosphorylation capacity [25]. With a radiometric method, Bedoya *et al.* reported conflicting results, a glucokinase contribution of 30% for rat islet homogenates [34] and of 80% for human islet homogenates [37]. Using the classical photometric method with the NADP-dependent glucose-6-phosphate dehydrogenase from Baker's yeast [38, 39], contributions of glucokinase to total glucose phosphorylation capacity were higher. Several authors reported contributions of glucokinase ranging from 50–80% to total phosphorylation capacity in homogenates [20, 40, 41] or cytoplasmic fractions [27, 29, 41] from rat [20, 27, 41] or mouse islets [29, 40]. Several variables may be responsible for these discordant hexokinase/glucokinase ratios.

In addition to the different assay methods, measurement of glucose phosphorylating enzyme activity in islet cell homogenates or cytoplasmic fractions is affected by the lack of stability of glucokinase [42], which presents a particular problem during storage. Indeed, when total homogenates are stored frozen before measurement of enzyme activity, losses of glucokinase enzyme activity as high as 10% per day may be encountered,* even if the sample has been stabilized by addition of a thiol protecting agent.* When glucokinase protein is stored in the form of a high speed cytoplasmic supernatant, loss of glucokinase activity is less likely.* As the high affinity hexokinase activity is less susceptible to deterioration during storage [42], this may well explain why the contribution of the low affinity enzyme component to the total glucose phosphorylation capacity of pancreatic islet cells is easily underestimated.

Table 1 shows the results of a direct comparison of hexokinase and glucokinase activities determined at 1 and 100 mM D-glucose, respectively, in homogenates from ob/ob mouse pancreatic islets and rat liver. Pancreatic islets and liver were homogenized directly after isolation of the tissue, and enzyme activities were measured immediately thereafter. Two photometric methods based on the formation of NADPH and NADH, respectively, in a coupled assay system [38, 39] were applied, one using the NADP-dependent glucose-6-phosphate dehydrogenase from Baker's Yeast, the other using the NAD-dependent glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*. With both methods the contribution of glucokinase to total glucose phosphorylation capacity was in the range of 50% in pancreatic islet homogenates and in the range of 70% in liver homogenates (Table 1). This is roughly 20% lower than in cytoplasmic fractions [29], because hexokinase is partially bound to the particulate fraction [43, 44]. A comparison of the enzyme activities expressed per mg of protein reveals that hexokinase activities were two to three times and glucokinase activities seven to eight times higher in cytoplasmic fractions [29] than in tissue homogenates (Table 1). Glucokinase activities were similar in tissue homogenates (Table 1) as well as in cytoplasmic fractions [29] of pancreatic islets and liver. Thus, even if glucokinase activities might be somewhat lower in the islet than in liver tissue [29] (Table 1), it is likely that a relative abundance of glucokinase in pancreatic islets twenty times lower

Table 1. Hexokinase and glucokinase activities in tissue homogenates of ob/ob mouse pancreatic islets and of rat liver

Enzyme	Method	Enzyme activities in tissue homogenates (mU/mg protein)	
		Pancreatic islets	Liver
Hexokinase	A	1.04 ± 0.19	0.61 ± 0.11
Glucokinase	A	0.91 ± 0.28	1.17 ± 0.16
Hexokinase	B	0.93 ± 0.20	0.47 ± 0.19
Glucokinase	B	0.82 ± 0.44	1.03 ± 0.10

Animals were fasted for 1 day before the experiment. Enzyme activities were determined with a photometric method using either glucose-6-phosphate dehydrogenase from Baker's Yeast (method A) or from *Leuconostoc mesenteroides* (method B) [29].

All values are means ± SEM for seven experiments.

* S. Lenzen, unpublished observation.

than in liver as reported by Iynedjian *et al.* [28] is an underestimate of the amount of glucokinase present in pancreatic islets.

KINETIC CHARACTERISTICS OF GLUCOKINASE

Kinetic characteristics of glucokinase for its only substrates, D-glucose and D-mannose, have been elucidated. Several authors have reported K_m values for glucokinase in homogenates or cytoplasmic fractions from normal and ob/ob mouse and rat islets in the range of 6.5 to 20 mM [20, 24, 25, 29, 37, 41, 45–47]. K_m values for hexokinase ranged from 50 to 150 μM [20, 24, 25, 29, 37, 41]. These values were obtained with a photometric assay using glucose-6-phosphate from Baker's Yeast [20, 25, 29, 41] or from *Leuconostoc mesenteroides* [25] or with a radiometric assay [24, 37, 47].

These K_m values are comparable to those in the liver [36]. For glucose from human islets, a single K_m value of 5 mM has been reported [37]. K_m values around 5 mM for glucokinase from purified fractions were somewhat lower [25], thus confirming an observation made with purified liver glucokinase [30]. Table 2 shows K_m values for rat and ob/ob mouse pancreatic islet and liver hexokinase and glucokinase originating from a direct comparative measurement in cytoplasmic fractions [29, 48]. All hexokinase K_m values were around 50 μM, and all glucokinase K_m values around 10 mM (Table 2).

Hexokinase from pancreatic islet tissue [49–52], as well as from liver [36], is severely inhibited by glucose-6-phosphate. Low K_i values in the range of 0.1 to 0.2 mM were reported [49, 50], as for liver hexokinase [36]. Such an end product inhibition is not evident for glucokinase from pancreatic islets [50] and liver [36]. For ATP a K_m value (vs glucose) of 0.5 mM has been reported for rat pancreatic islet glucokinase [25] similar to that for rat liver [53]. Thus, glucokinase in the pancreatic B-cell is not likely to be affected by variations in the physiological concentrations of ATP, ADP, and glucose-6-phosphate. Moreover, the enzyme is remarkably free of allosteric effects [30]. Hill coefficients somewhat greater than 1.0 for D-glucose and D-mannose as reported with glucokinase from islets [25, 35, 45] and liver [53, 54] are indicative of some degree of co-operativity [30]. A useful consequence of the co-operativity is a particular sensitivity of the reaction due to a sigmoidal shape of the saturation curve to small changes in the glucose concentration in the range of 5–10 mM. However, because glucokinase is a monomeric enzyme [30] and co-operativity is impossible for a single binding site at equilibrium [30], the co-operativity of glucokinase has been explained purely in kinetic terms [30, 53, 54]. Therefore, a "mnemonical mechanism" has been postulated to account for the slightly sigmoidal kinetics of glucokinase [55]. In order to explain co-operativity for an enzyme such as glucokinase on the basis of a "mnemonical mechanism", it is necessary to postulate the existence of two interconvertible forms of the free enzyme [30].

D-Mannose is a substrate with lower affinity for pancreatic islet glucokinase [29]. K_m values with D-mannose have been reported to be 50–100% higher than for D-glucose with glucokinase from ob/ob mouse islets [29], rat insulinomas [56] and liver [29, 56]. A K_m value determined for islet glucokinase was 22.7 mM [29] (Table 2). The relative velocity of the phosphorylation of D-mannose by glucokinase from pancreatic islets or liver was in the range of 20–25% of that of D-glucose [29]. Earlier values for D-mannose reported to be in the range of 80% of those of D-glucose are likely to be too high [56]. These high percentages would not agree with an insulin secretory potency of D-mannose, which is several times lower than that of D-glucose [6, 57]. Mammalian hexokinase displays a greater affinity for the α anomer of D-glucose but a higher maximal velocity with the β anomer of D-glucose [44, 50, 51]. Several studies suggest that glucokinase from rat islets, insulinomas and liver has a slightly higher affinity for the α anomers of D-glucose [45, 50–52, 58–60] and of D-mannose [45, 56]. However, the preference of glucokinase for α-D-glucose is restricted to glucose concentrations not exceeding 20 mM [45, 50, 60]. At higher concentrations of the hexose, the β anomer of D-glucose may be phosphorylated in preference to the α anomer [45, 50, 60]. It is possible, therefore, that factors other than the limited α anomeric specificity of pancreatic B-cell glucokinase may contribute to the α anomeric specificity of hexose metabolism in pancreatic islets and hexose-induced insulin release [61, 62]. For these reasons, Sener, Malaisse and their collaborators have concluded that glucose utilization and hence insulin secretion in response to this hexose may not [50, 52, 60] or not solely [52] be regulated by glucokinase. On the other hand, only the synthetic analogue of α-D-glucose, pseudo-α-D,L-glucose, but not its β-isomer, significantly inhibits glucose-induced insulin release and rat pancreatic islet glucokinase activity [63]. This observation provides additional independent evidence for a major contribution of pancreatic B-cell glucokinase to glucose recognition by pancreatic B-cells. Other sugars such as L-glucose, D-fructose, D-galactose, 3-*O*-methyl-D-glucose, and *N*-acetyl-D-glucosamine are not phosphorylated or not phosphorylated to any

Table 2. K_m values for hexokinase (HK) and glucokinase (GK) from pancreatic islets and liver from rats or ob/ob mice with the substrates D-glucose or D-mannose

		K_m values			
		Pancreatic islets		Liver	
Animal species	Method	HK (μM)	GK (mM)	HK (μM)	GK (mM)
		D-Glucose			
Rat	A	51	11.4	46	9.7
ob/ob Mouse	A	61	10.3	67	10.4
ob/ob Mouse	B	48	11.9	58	9.5
		D-Mannose			
ob/ob Mouse	A		22.7	75	20.9

Enzyme activities were determined with a photometric method using either glucose-6-phosphate dehydrogenase from Baker's Yeast (method A) or from *Leuconostoc mesenteroides* (method B) [29].

appreciable extent by pancreatic B-cell glucokinase [29]. D-Mannoheptulose and probably other sugars such as D-glucosamine which inhibit liver glucokinase [25, 29, 36, 64] also inhibit pancreatic B-cell glucokinase [25, 29]. When all the characteristics of glucokinase from pancreatic B-cells are compared with those from the liver [30, 36, 65], it can be concluded that there is a complete identity of the antigenic and kinetic properties of glucokinase from the two tissues.

The properties of pancreatic B-cell glucokinase and the characteristics of glucose-induced insulin secretion [6, 7] are congruent. This supports the view that glucokinase is a low affinity hexose phosphorylating enzyme which couples fluctuations of the blood glucose concentration to changes in the glycolytic flux rate, and hence finally to the rate of insulin secretion. The properties of B-cell glucokinase are: a narrow substrate specificity; high K_m for the two substrates, D-glucose and D-mannose, in the range of 10 and 20 mM respectively; higher V_{max} for D-glucose than for D-mannose; a slight preference for the α anomers of these two aldohexoses; and inhibition of the glucokinase activity by specific glucokinase antibodies. All of these features distinguish glucokinase in pancreatic B-cells from low K_m hexokinases.

ALLOXAN AS A TOOL FOR THE ELUCIDATION OF THE GLUCOSE RECOGNITION MECHANISMS OF PANCREATIC B-CELLS

Alloxan inhibits glucose-induced insulin secretion [66, 67] and the characteristics of its inhibitory action [68, 69] suggest that inhibition of glucokinase in pancreatic B-cells may contribute to its inhibitory action on insulin secretion. Experimental evidence supports this assumption. Alloxan inhibits glucokinase but not hexokinase from pancreatic B-cells [29, 70] and liver [29, 70, 71]. In experiments with glucokinase from pancreatic B-cells [29] or liver [29, 70], the half-maximal inhibitory concentration of alloxan is around 5 μM. The sensitivity of glucokinase to inhibition by alloxan is remarkably selective. Only phosphofructokinase is also prone to inhibition by alloxan, but much less so than glucokinase [71]. Other glycolytic enzymes such as pyruvate kinase and glucose-6-phosphate dehydrogenase are not inhibited by alloxan [71].

Only D-glucose and D-mannose protect glucokinase of pancreatic B-cells [29] and liver [29, 70] against inhibition by alloxan. The α anomers of these two aldohexoses provide significantly greater protection of glucokinase activity than the β anomers [29, 70]. L-Glucose, D-fructose, D-galactose, and 3-*O*-methyl-D-glucose do not protect glucokinase against inhibition by alloxan [29]. However, 3-*O*-methyl-D-glucose, which is not metabolized and without insulin secretory potency, does not protect glucokinase directly against inhibition by alloxan, but can protect glucokinase in the intact cell [29, 70] through inhibition of alloxan uptake by the cell [29]. Thus, D-glucose and 3-*O*-methyl-D-glucose provide protection through actions at different sites; D-glucose through interference of the binding of alloxan to a site on the enzyme, and 3-*O*-methyl-D-glucose through inhibition of alloxan transport into the B-cell [29]. A very important observation was that glucokinase activity is also protected by D-mannoheptulose [29], a sugar which by itself inhibits glucose-induced insulin secretion [2], as well as metabolism of D-glucose in pancreatic islets [22] and glucokinase activity [25, 29]. This observation showed that the substrates D-glucose and D-mannose as well as the inhibitors, D-mannoheptulose and the pyrimidine derivative alloxan, interact with the same site of the glucokinase, i.e. the active site of the enzyme. Using *N*-acetyl-D-glucosamine, Meglasson *et al.* [70] did not observe a significant protective effect of this sugar, because it* is much smaller than that provided by D-mannoheptulose [29]. For these reasons the authors concluded that the reactive site for alloxan was not the active site of the enzyme [70]. However, several facts appear to exclude this interpretation. For example, D-mannoheptulose protects glucokinase against alloxan inhibition [29]. Liver glucokinase which is identical to pancreatic B-cell glucokinase, contains only one glucose-binding site per molecule [30]. Thus, the substrates D-glucose and D-mannose, the inhibitory sugar D-mannoheptulose, and the pyrimidine derivative alloxan, all compete for this single binding site of the enzyme. The molecular mechanism underlying the binding to this site has not yet been elucidated. However, all of the characteristics of glucokinase inhibition by alloxan and the protective action of the various sugars are complementary to the inhibition of insulin secretion by alloxan [68, 69]. Thus, the alloxan action supports the hypothesis that glucokinase is a rate-limiting enzyme for pancreatic B-cell glycolysis which contributes significantly to the regulation of the metabolic flux rate through glycolysis and, thereby, finally to the generation of a signal for initiation of insulin secretion by D-glucose.

DIETARY AND HORMONAL REGULATION OF GLUCOKINASE

The only major factors that determine the activity of glucokinase are the concentration of glucose (see above) and the activity level of the enzyme. The activity state of glucokinase in liver and pancreatic islets is modulated by prior dietary history and hormonal status [35, 36, 72–74]. As the long-term regulation of insulin secretion is also under dietary [75, 76] and hormonal [77] control, it is reasonable to investigate the possible contribution of changes in the glucokinase activity level to the long-term regulation of glucose-induced insulin secretion. The influence of dietary and hormonal factors in the regulation of glucokinase makes it likely that changes in the activity level of this enzyme play a significant role in the adaptation of the pancreatic B-cell function to the varying nutritional and metabolic demands of the organism. For example, fasting induces a time-dependent decrease of the activities of hexokinase and glucokinase of rat [27, 33, 41, 48] and mouse [37] pancreatic islets.

All studies with the exception of that by Burch *et al.* [33] have observed a more pronounced reduction of glucokinase than of hexokinase activity during

* S. Lenzen, unpublished observation.

starvation [27, 37, 41, 48]. Meglasson and Matschinsky [35] reported that glucokinase contributes only 25% to the total glucose phosphorylation capacity of the rat pancreatic islet cell and that starvation reduces glucokinase activity by only 20–30% [35]. Such a small reduction of the pancreatic B-cell glucokinase activity during starvation (less than 10% of the total glucose phosphorylation capacity) would be unlikely to confer a significant role in the mediation of reduced glucose-induced insulin secretion during starvation. Refeeding reverses the starvation-induced reduction of hexokinase and glucokinase activities in pancreatic islets [27, 33, 48] and liver [27, 36, 48, 72–74]. Insulin treatment prevents the fall of the glucokinase activity in pancreatic islets [27, 48] and liver [36, 72–74]. Thus, it is evident that changes in the insulin concentration but not in the blood glucose concentration are responsible for the starvation-induced reduction and refeeding-induced restoration of glucokinase activity [27, 36, 48, 72–74]. At most glucose may play a permissive role in the induction of glucokinase in pancreatic islets and liver [73]. This also applies to hormones such as glucocorticoids and thyroid hormones, which do not affect significantly glucokinase activities in pancreatic islets* and liver [36, 72–74] from adult animals.

The functional significance of insulin for induction of glucokinase is further attested to by its low activity in the liver in other conditions of insulin deficiency such as diabetes induced by alloxan, streptozotocin, or pancreatectomy; by D-mannoheptulose which inhibits insulin secretion; and by administration of anti-insulin antibody [36, 72–74]. And the loss of most of the glucokinase activity in large chemically-induced as well as in radiation-induced transplantable B-cell tumors, which no longer respond to glucose stimulation with insulin secretion, is also the result of a loss of the ability of insulin to induce this enzyme [48]. Thus, insulin is the major factor that regulates the activity level of glucokinase in pancreatic B-cells and liver *in vivo*. At variance from this conclusion, Bedoya *et al.* [34] stated recently that glucose regulates the activity level of glucokinase in pancreatic islet cells, whereas insulin is the primary determinant for liver glucokinase. However, Bedoya *et al.* [34] used an experimental design which did not exclude the possibility that glucose-induced insulin secretion was responsible for the increase of the glucokinase activity levels in the pancreatic B-cells. The concentrations of insulin necessary to induce pancreatic islet and liver glucokinase are by one to three orders of magnitude lower than those which are presumably required for inhibition of insulin secretion from pancreatic islets by insulin [78].

It is likely therefore that the induction of glucokinase in pancreatic islets [24, 48] and liver [36, 72–74] is an action of insulin mediated through the insulin receptor in the plasma membrane of the pancreatic islet and liver cell. This is consistent with the decrease of glucokinase activity during starvation being slower in pancreatic islets than in liver [27, 48], because the pancreatic islet cell membrane is exposed to higher insulin concentrations *in vivo* than the liver cell membrane.

* S. Lenzen, unpublished observation.

MECHANISMS OF INSULIN SECRETORY ACTION OF GLUCOSE AND HYPOGLYCEMIC SULFONYLUREAS AND THEIR INTERRELATIONS

Hypoglycemic sulfonylureas such as tolbutamide and glibenclamide mediate their insulin secretory action presumably through interaction with a recognition site in the pancreatic B-cell plasma membrane [79]. Most recent information indicates that this may be the ATP-dependent K^+ channel in the B-cell plasma membrane [80, 81]. The same channel is likely to be modulated by changes in energy rich phosphates in the pancreatic B-cell [82–84] as brought about by a variety of nutrient insulin secretagogues, when they increase metabolic flux rates through glycolysis, citric acid cycle and respiratory chain [85]. The resultant depolarization of the pancreatic B-cell plasma membrane leads to an opening of voltage-dependent calcium channels. Increasing free cytoplasmic calcium concentration finally links the primary event of signal generation to exocytosis of insulin. Amino acids and keto acids with insulin releasing potency enter mitochondrial metabolism through transamination, decarboxylation, or activation of glutamate dehydrogenase [86, 87]. Concentrations of glucose which induce insulin secretion can increase intramitochondrial generation of energy rich phosphates only after phosphorylation by glucokinase and after increasing the metabolic flux rate through glycolysis [6].

Hypoglycemic sulfonylureas reduce the blood glucose concentration *in vivo*. The consequence is a reduction of glucose phosphorylation by glucokinase in pancreatic B-cells. However, hypoglycemic sulfonylureas such as glibenclamide can induce glucokinase in pancreatic islets and liver, when glucokinase activity is diminished [27]. This is not a direct effect of the sulfonylurea on pancreatic islet glucokinase [27]. It is apparently rather an inductive effect on the glucokinase of the pancreatic B-cells by insulin, which is released from these B-cells by sulfonylurea stimulation [27]. Such a positive feedback loop between an increasing extracellular insulin concentration and glucose-induced insulin secretion [27] can keep the glucose recognition system of the pancreatic B-cell sensitive for the initiation of insulin secretion and insulin biosynthesis by glucose. This may be of particular importance when glucokinase activity is low during consumption of a low carbohydrate diet [88]. Thus, induction of glucokinase in pancreatic B-cells by insulin *in vivo* offers a mechanism through which the mechanisms of insulin secretory action of glucose and hypoglycemic sulfonylureas can be interconnected, although they are primarily independent [89] and converge probably only at the level of the ATP-sensitive K^+ channel in the B-cell plasma membrane [81].

REGULATION OF METABOLIC FLUX RATE THROUGH GLYCOLYSIS IN PANCREATIC B-CELLS

Regulatory enzymes [10, 11] are usually positioned at strategically important positions for the control of flux through an enzyme sequence. As a general rule,

rate control is best achieved at the first non-equilibrium step of the pathway, and adjacent steps may participate in the regulatory control. As the optimal concentration for the substrate of a non-equilibrium reaction is at its K_m [13], it is likely that a regulatory enzyme with a K_m in the millimolar range is particularly suited to relate the extracellular glucose concentration to the rate of insulin secretion by controlling the rate of metabolic flux.

Pancreatic B-cells contain hexokinase, the glucose phosphorylating enzyme activity with a low K_m, which allows glucose utilization to adapt to the basal energy requirements of the B-cells. In addition, pancreatic B-cells contain glucokinase, the glucose phosphorylating enzyme activity with a high K_m, which relates glycolytic flux rate to physiological changes in the blood glucose concentration. However, the rate limitation of the metabolic flux through the glycolytic pathway in the pancreatic B-cell is not necessarily restricted to the initial phosphorylation of glucose by glucokinase [12, 13, 90, 91]. The rate of flux through glycolysis may also be controlled below this initial phosphorylation step in the pathway [12, 13, 90, 91].

In millimolar concentrations, the α anomers of D-glucose and D-mannose are more potent insulin secretagogues than the β anomers [61, 62] and the rate of glycolysis is higher when islets are exposed to the α anomers of these two aldohexoses [61, 92]. While glucokinase is an α-stereospecific enzyme, phosphofructokinase, the next kinase along the initial reaction sequence of glycolysis, shows a preference for β-fructose-6-phosphate as a substrate [93]. Some investigators have attributed the α anomeric preference of pancreatic islet glycolysis primarily to the α-stereospecificity [93, 94] of phosphoglucose isomerase [95]. However, in such a case the preferential insulin secretory response to α-D-mannose [61, 62] would require another explanation, because phosphomannose isomerase is a β-stereospecific enzyme [93]. The extremely rapid spontaneous mutarotation of glucose-6-phosphate [96] is another argument which can be raised against a rate-limiting role of phosphoglucose isomerase [45]. Preferential phosphorylation of α-D-glucose by glucokinase [35, 45, 59] and the higher rate of utilization of α-D-glucose by the pancreatic B-cell [61, 92] meet the greater demands imposed on the pancreatic B-cells through the greater insulin secretory and synthetic potency of α-D-glucose [61, 62] and thus may be responsible for lower intraislet concentrations of glucose-6-phosphate [50, 95, 97], when exposed to α-D-glucose. The resultant acceleration of the rate of the phosphofructokinase reaction [98–100] increases the removal of glucose-6-phosphate and may help to diminish the inhibition of hexokinase by glucose-6-phosphate. This is important in order to avoid that the increased pancreatic B-cell phosphorylation capacity through increased glucokinase activity at increasing glucose concentrations is nullified by a decreasing contribution to total pancreatic B-cell phosphorylation capacity by hexokinase due to inhibition by glucose-6-phosphate [13]. Phosphofructokinase, though β-stereospecific [93], is a complex allosteric enzyme with several stimulatory and inhibitory modulators [98–100] controlling the glycolytic flux rate at a secondary control point. Greater activation of the non-equilibrium reaction catalyzed by this kinase may be a more important determinant for the higher glycolytic flux rate with α-D-glucose than the α-stereospecificity of phosphoglucose isomerase [93]. The latter enzyme catalyzes an equilibrium reaction and has no cofactors [13, 93, 99]. The non-equilibrium reaction of pancreatic B-cell glycolysis catalyzed by phosphofructokinase may be considered, therefore, as part of the rate-limiting system of pancreatic B-cell glycolysis together with the initial phosphorylation of glucose by glucokinase. This conclusion is supported by the observation that phosphofructokinase is the only other glycolytic enzyme in addition to glucokinase, which is inhibited significantly by alloxan [71]. Furthermore, phosphofructokinase and pyruvate kinase like glucokinase are insulin-sensitive kinases [98, 99]. Their activities are reduced during starvation [33, 41], whereas pancreatic islet phosphoglucose isomerase is not reduced [41]. Decreased enzyme activities of these kinases at secondary control points of pancreatic B-cell glycolysis may contribute together with a decreased glucokinase activity to a decrease of the signal generating glycolytic flux rate for initiation of glucose-induced insulin secretion and thereby to the complex changes of pancreatic B-cell metabolism, which accompany a diminished insulin secretory response to glucose during starvation [75, 76]. The reduction of the insulin secretory response during starvation to amino acids and keto acids [75], whose metabolic fate is nearly exclusively intramitochondrial in contrast to that of glucose, indicates that further metabolic control points exist below the glycolytic chain [13, 101]. Regulation of the signal generating intramitochondrial metabolic flux rate [90] for initiation of insulin secretion helps the pancreatic B-cell to adapt to changing functional demands in dependence on the nutritional and metabolic status of the organism. Thus, it can be concluded that regulatory points below the initial phosphorylation of glucose by glucokinase contribute to the flux regulation of the signal generating metabolism of glucose [102] in the pancreatic B-cell.

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