Control of glucokinase translocation in rat hepatocytes by sorbitol and the cytosolic redox state

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In rat hepatocytes cultured in 5 mM glucose, glucokinase activity is present predominantly in a bound state, and during permeabilization of the cells with digitonin in the presence of Mg^{2+} less than 20% of glucokinase activity is released. However, incubation of hepatocytes with a higher [glucose] [concn. giving half-maximal activation (A_{50}) 15 mM] or with fructose (A_{50} 50 μ M) causes translocation of glucokinase from its Mg^{2+} -dependent binding site to an alternative site [Agius and Peak (1993) *Biochem. J.* **296**, 785–796]. A comparison of various substrates showed that sorbitol (A_{50} 8 μ M) was 6-fold more potent than fructose at causing glucokinase translocation, whereas tagatose was as potent and mannitol was > 10-fold less potent (A_{50} 550 μ M). These substrates also stimulate glucose conversion into glycogen with a similar relative potency, suggesting that conversion of glucose into glycogen is dependent on the binding and/or location of glucokinase within the hepatocyte. Ethanol and glycerol inhibited the effects of fructose, sorbitol and glucose on glucokinase translocation, whereas dihydroxyacetone had a small additive effect at sub-maximal substrate

stimulation. The converse effects of glycerol and dihydroxyacetone suggest a role for the cytosolic NADH/NAD⁺ redox state in controlling glucokinase translocation. Titrations with three competitive inhibitors of glucokinase did not provide evidence for involvement of glucokinase flux in glucose-induced glucokinase translocation: *N*-acetylglucosamine inhibited glucose conversion into glycogen, but not glucose-induced glucokinase translocation; glucosamine partially suppressed glucose-induced and fructose-induced glucokinase translocation, at concentrations that caused total inhibition of glucose conversion into glycogen; D-mannoheptulose increased glucokinase release and had an additive effect with glucose. 3,3'-Tetramethylene-glutaric acid (5 mM), an inhibitor of aldose reductase, inhibited glucokinase translocation induced by glucose, but not that by sorbitol or fructose, suggesting that glucose may induce glucokinase translocation by conversion into sorbitol. Sorbitol generated from glucose intrahepatically or extrahepatically in hyperglycaemic conditions may be a physiological regulator of hepatic glucokinase translocation.

INTRODUCTION

Glucokinase (hexokinase IV) catalyses the ATP-dependent phosphorylation of glucose to glucose 6-phosphate and is expressed in hepatocytes and in the insulin-secreting pancreatic islet cells. It differs from the other hexokinase isoenzymes in its lower molecular mass (52 kDa versus 100 kDa), its low affinity for glucose, sigmoidal kinetics and lack of inhibition by physiological concentrations of glucose 6-phosphate [1,2]. We have shown that, in rat hepatocytes cultured with 5 mM glucose, glucokinase is present predominantly in a bound state when hepatocytes are permeabilized with digitonin in the presence of Mg^{2+} . The proportion of bound activity is affected by treatment of hepatocytes with cytochalasin-D or phalloidin, which alter the conformation of the microfilament cytoskeleton, and we postulated that glucokinase may occur in association with the microfilament cytoskeleton [3]. Incubation of hepatocytes with an increase in [glucose] (15–35 mM) or with fructose (50 μ M) causes translocation of glucokinase from its Mg^{2+} -dependent binding site to an alternative site. This substrate-induced translocation is inhibited by cyanide, suggesting involvement of an ATP-dependent mechanism, but not by cytochalasin-D or colchicine, and is probably therefore not dependent on dynamic changes in actin or tubulin polymerization. Because fructose induces glucokinase translocation at much lower concentrations than glucose, a metabolite generated via fructokinase [4] may be involved in enzyme translocation.

Recent studies have identified a 62 kDa regulatory protein that binds to glucokinase and decreases its affinity for glucose

[5–7]. This protein binds both fructose 1-phosphate and fructose 6-phosphate [8,9]. The latter increases the affinity of the binding protein for glucokinase and thereby inhibits the enzyme, whereas fructose 1-phosphate causes dissociation of the binding protein from glucokinase. Several other ligands of the regulatory protein have been identified which mimic the effects of either fructose 6-phosphate or fructose 1-phosphate [8,9]. The present study demonstrates that various substrates that can give rise to ligands of the regulatory protein induce glucokinase translocation, and of these sorbitol is the most potent. Experiments with inhibitors of glucokinase and of aldose reductase suggest that glucose-induced translocation of glucokinase is probably mediated not through glucokinase flux but possibly via conversion into sorbitol.

MATERIALS AND METHODS

Materials

Substrates and glucokinase inhibitors were from Sigma Chemical Co. (Poole, Dorset, U.K.). Sources of other reagents were as described previously [3].

Hepatocyte isolation and monolayer culture

Hepatocytes were isolated by collagenase perfusion of the liver from male Wistar rats (body wt. 220–280 g) fed *ad libitum* [3,10]. They were suspended in Minimum Essential Medium containing 5% neonatal-calf serum and inoculated in 24-well plates at a cell density of 6×10^4 cells/cm² and incubated at 37 °C in a humidified

atmosphere equilibrated with 5 % CO₂/air. After cell attachment (approx. 4 h) the medium was changed to serum-free medium containing 10 nM dexamethasone. Incubations with digitonin for determination of enzyme activity release were performed after between 8 h and 16 h of culture.

Incubation of hepatocyte monolayers with substrates and inhibitors

Hepatocyte monolayers were incubated in Minimum Essential Medium supplemented with substrates and inhibitors for the time intervals indicated before permeabilization with digitonin. Unless indicated otherwise, the standard medium contained 5 mM glucose.

Permeabilization of hepatocytes with digitonin

After incubation with substrates, the hepatocyte monolayers were washed once with 150 mM NaCl and then permeabilized for 6 min in medium (400 μ l/well) containing 300 mM sucrose, 3 mM HEPES, 2 mM dithiothreitol, 5 mM MgCl₂, 0.05 mg/ml digitonin, pH 7.2, at 20 °C in unstirred conditions as described previously [3]. Glucokinase activity was assayed [3] in the digitonin eluate aspirated on termination of the 6 min permeabilization and in the residual cell matrix extracted in 100 mM KCl/25 mM HEPES/7.5 mM MgCl₂/4 mM dithiothreitol/0.05 % Triton X-100 as in [3]. The glucokinase activity in the digitonin eluate is expressed as a proportion of the total activity in the eluate and cell matrix [3]. Incubations with substrates and metabolic inhibitors did not affect the total activity of glucokinase recovered in the eluate plus cell matrix. Variations in total activity recovery within an experiment were within 10 %. Throughout this study, the total glucokinase activity was between 8 and 13 m-units/mg of cell protein.

Glycogen synthesis

Glycogen synthesis was determined from 3 h incubations in standard Minimum Essential Medium supplemented with 10 mM [U-¹⁴C]glucose and other substrates as indicated. The incorporation of ¹⁴C label into glycogen was determined by ethanol precipitation [10] and is expressed as nmol of [¹⁴C]glucose incorporated per mg of cell protein. The cellular glycogen content was determined enzymically [10] and is expressed as nmol of glucosyl units per mg of cell protein [11].

RESULTS

Effects of sorbitol, tagatose and mannitol on glucokinase release and glycogen synthesis

Phosphorylated derivatives of fructose, sorbitol, tagatose, mannitol and L-sorbose are ligands of the regulatory binding protein of glucokinase. Sorbitol 6-phosphate, mannitol 1-phosphate and fructose 6-phosphate increase the affinity of the binding protein for glucokinase, whereas fructose 1-phosphate, tagatose 1-phosphate and L-sorbose 1-phosphate have the converse effect [8]. Figure 1 shows the effects of preincubation of hepatocytes with sorbitol, fructose, tagatose and mannitol on glucokinase release during subsequent permeabilization of hepatocytes with digitonin in the presence of MgCl₂. Sorbitol was more potent [concn. giving half-maximal activation (A_{50}) 8 ± 2 μ M] than either

fructose (A_{50} 52 ± 7 μ M) or tagatose (A_{50} 44 ± 13 μ M), and mannitol was least potent (A_{50} 550 ± 80 μ M). L-Sorbose had no effect at concentrations up to 0.2 mM, and a small, not statistically significant, effect at 1 mM (results not shown). Sorbitol, fructose, tagatose and mannitol (at 50 and 200 μ M) stimulated conversion of [¹⁴C]glucose (10 mM) into glycogen with a similar relative potency as for stimulation of glucokinase release (sorbitol > fructose = tagatose > mannitol), and the percentage stimulation of glucose conversion into glycogen correlated with the proportion of glucokinase release determined in parallel experiments (Figure 2). The effects of maximally effective concentrations (1 mM) of fructose and sorbitol on glucokinase release were not additive [control, 14 ± 4 ; 1 mM fructose, 66 ± 4 ; 1 mM sorbitol, 64 ± 3 ; 1 mM fructose + 1 mM sorbitol, 66 ± 6 %

Figure 1 Effects of fructose, tagatose, sorbitol and mannitol on glucokinase release

Hepatocytes were incubated for 30 min with the concentration of fructose ($\blacktriangle$), tagatose ($\blacktriangledown$), sorbitol ($\bullet$) or mannitol ($\blacklozenge$) indicated. They were then permeabilized with digitonin as described in the Materials and methods section, and the glucokinase activity release is expressed as a percentage of total activity. Values are means $\pm$ S.E.M. for 7 experiments.

Figure 2 Correlation between substrate-induced glucose conversion into glycogen and glucokinase translocation

Glucose conversion to glycogen was determined in 3 h incubations with 15 mM [U-¹⁴C]glucose in the absence or presence of either 50 μ M or 200 μ M fructose (F), tagatose (T), sorbitol (S) or mannitol (M). The incorporation of [¹⁴C]glucose into glycogen is expressed as a percentage of control values with glucose alone. Glucokinase release was determined from parallel 90 min incubations with the same [substrate] and is expressed as the percentage of glucokinase activity released. Values are means of 5 experiments.

Figure 3 Effects of [ethanol] on substrate-induced glucokinase release

Hepatocytes were incubated for 30 min with (a) 200 μ M fructose (●) or 50 μ M sorbitol (▲), or (b) 5 mM (◆), 15 mM (▼), 25 mM (▲) or 35 mM (●) glucose, and the concentrations of ethanol indicated. Glucokinase release determined during subsequent permeabilization with digitonin is expressed as a percentage of total activity. Values are means $\pm$ S.E.M. for 5 experiments (a) or means of 2 experiments (b).

glucokinase release, means $\pm$ S.E.M., $n = 4$), indicating that they share a common mechanism. Despite the higher affinity of glucokinase release for sorbitol compared with fructose (Figure 1), high concentrations of sorbitol (30 mM), unlike fructose, had only a small biphasic inhibitory effect on glucokinase release (10 mM fructose, $59 \pm 3\%$; 30 mM fructose, $40 \pm 3\%$, $P < 0.02$ relative to 1 mM fructose; 10 mM sorbitol, $62 \pm 2\%$; 30 mM sorbitol, $59 \pm 3\%$). This suggests that there is little net conversion of exogenous sorbitol into fructose by sorbitol dehydrogenase (EC 1.1.1.14) [12].

Effects of ethanol on the fructose-, sorbitol- and glucose-induced translocation

The higher affinity of glucokinase release for sorbitol compared with fructose raises the question whether the stimulatory effect of fructose on glucokinase release could be mediated by conversion into sorbitol. Since the interconversion of fructose and sorbitol via sorbitol dehydrogenase is dependent on the cytosolic NADH/NAD⁺ redox couple [12], we determined the effects of ethanol on fructose- and sorbitol-induced glucokinase release. Increasing [ethanol] would be expected to increase the conversion of fructose into sorbitol by increasing the NADH/NAD⁺ ratio. Initial experiments showed that 10 mM ethanol inhibits the

effects of sub-maximally effective fructose concentrations (50 and 200 μ M), but had little effect on the stimulation by 1 mM fructose. We subsequently determined the effects of varying [ethanol] on the stimulation of glucokinase release by 50 μ M sorbitol and 200 μ M fructose (Figure 3). These concentrations were selected because they cause a similar stimulation of glucokinase release (Figure 1). High concentrations of ethanol (2.5–10 mM) caused a greater suppression of sorbitol-induced than of fructose-induced glucokinase release, but in neither case did ethanol fully counteract the substrate-stimulation. The concentration of ethanol that caused half-maximum inhibition was 1 mM, and was similar with fructose (200 μ M) and sorbitol (50 μ M) as substrates (Figure 3a). In further experiments, where the effects of ethanol were determined in the presence of equimolar concentrations of fructose and sorbitol (200 μ M), ethanol inhibited to the same extent with both substrates (results not shown). The similar inhibitory effect of ethanol on fructose-induced and sorbitol-induced glucokinase release does not support the hypothesis that the effects of fructose are mediated via conversion into sorbitol, but suggests that the redox state probably interacts with the fructose and sorbitol effects at a distal and possibly common metabolic site. Ethanol also partially suppressed the stimulation of glucokinase release by glucose (Figure 3b).

Effects of dihydroxyacetone and glycerol

Fructose is metabolized by fructokinase to fructose 1-phosphate, which is hydrolysed by aldolase-B to dihydroxyacetone-phosphate and glyceraldehyde. Since dihydroxyacetone phosphate is also formed from glucose via hydrolysis of fructose 1,6-bisphosphate, it is a potential intermediate that may be involved in the fructose- and glucose-induced translocation. A comparison of effects of dihydroxyacetone (which is phosphorylated via triokinase) and of glycerol (which is metabolized to dihydroxyacetone phosphate via glycerokinase and glycerol-3-phosphate dehydrogenase) on glucokinase translocation showed that these substrates had converse effects. Dihydroxyacetone (5 mM) stimulated glucokinase release at sub-maximum concentrations of glucose, sorbitol or fructose, but not at maximally effective concentrations of these substrates (Figure 4). The concentration that caused half-maximal stimulation was ~ 1 mM, and the maximum effect of dihydroxyacetone (at 5 mM; Figure 5) was smaller than the maximum effects of glucose, fructose or sorbitol (Figure 4). However, glycerol (5 mM) potently inhibited glucose-, sorbitol- and fructose-induced glucokinase release (Figure 4). Half-maximum inhibition was at 0.7 mM glycerol (Figure 5). The opposite effects of dihydroxyacetone and glycerol on glucokinase release are of interest, because dihydroxyacetone stimulates and glycerol inhibits the conversion of 10 mM [U-¹⁴C]glucose into glycogen {control, 20 ± 2 ; 5 mM dihydroxyacetone, 56 ± 5 ; 5 mM glycerol, 7 ± 1 (means $\pm$ S.E.M., $n = 4$; nmol of [¹⁴C]-glucose incorporated/2 h per mg of protein)}. Since glycerol, like ethanol, increases the cytosolic NADH/NAD⁺ redox state [12], the opposite effects of dihydroxyacetone and glycerol could be due to the redox changes.

Effects of glucokinase inhibitors

If glucose induces glucokinase translocation through a metabolic product formed via glucokinase, then glucokinase inhibitors would be expected to counteract the effect of glucose on glucokinase translocation in parallel with inhibition of glucose metabolism. We therefore examined the effects of three com-

Figure 4 Effects of dihydroxyacetone and glycerol on substrate-induced glucokinase release

Hepatocytes were incubated for 30 min with the concentrations of glucose (a), sorbitol (b) or fructose (c) indicated in the absence (○) or presence of 5 mM dihydroxyacetone (▲) or 5 mM glycerol (◆). Values are means $\pm$ S.E.M. of either 4 (b) or 5 (a, c) experiments.

petitive inhibitors of glucokinase (glucosamine, *N*-acetylglucosamine, *D*-mannoheptulose [13]) on glucokinase translocation and glycogen synthesis.

D-Mannoheptulose in the presence of 5 mM glucose stimulated glucokinase release (A_{50} 2.6 ± 0.5 mM; mean $\pm$ S.E.M., $n = 4$). It showed a similar time course ($t_{1/2}$ 5 min) to that for glucose- and fructose-induced glucokinase release (Figure 6), and was likewise counteracted by 4 mM cyanide (results not shown), suggesting that it probably involves ATP-dependent phosphorylation [3]. Since *D*-mannoheptulose is a substrate for fructokinase [4], its effect on glucokinase release might be due to its phosphorylation product or metabolites thereof. When the effects of mannoheptulose were determined in the presence of 20 mM glucose,

Figure 5 Effects of [dihydroxyacetone] and [glycerol] on glucose-induced glucokinase release

Hepatocytes were incubated for 30 min with the concentrations of dihydroxyacetone (DHA; ○, ●) or glycerol (□, ■) indicated, in the presence of either 10 mM glucose (○, □) or 20 mM glucose (●, ■). Glucokinase release determined during subsequent permeabilization with digitonin is expressed as a percentage of total activity. Values are means $\pm$ S.E.M. for 3 experiments.

Figure 6 Effects of *D*-mannoheptulose on glucokinase release

Hepatocytes were incubated for 60 min with various concentrations of *D*-mannoheptulose (a) or with 5 mM *D*-mannoheptulose for various time intervals (b). Glucokinase release during subsequent permeabilization is expressed as a percentage of total activity. Values are means $\pm$ S.E.M. for either 4 (a) or 3 (b) experiments.

Table 1 Effects of glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc) on glycogen deposition and glucokinase release

Hepatocytes were incubated with the substrates and inhibitors indicated either for 3 h for determination of cellular glycogen content (expressed as nmol of glucosyl units/mg of cell protein) or for 90 min for subsequent determination of percentage glucokinase release by permeabilization with digitonin. Values in the bottom line represent values for cellular glycogen content and percentage glucokinase release in control incubations with 5 mM glucose and no inhibitors. Values are means $\pm$ S.E.M. for 4 experiments: * $P < 0.05$, ** $P < 0.005$, relative to controls with no inhibitor.

Inhibitor	Substrate ...	Glycogen content (nmol/mg)		Glucokinase release (%)		
		40 mM-glucose	20 mM-glucose	40 mM-glucose	20 mM-glucose	1 mM-fructose
None		431 $\pm$ 18	335 $\pm$ 25	55 $\pm$ 1	45 $\pm$ 2	38 $\pm$ 3
GlcNAc						
5 mM		427 $\pm$ 9	345 $\pm$ 22	55 $\pm$ 2	47 $\pm$ 1	36 $\pm$ 2
10 mM		427 $\pm$ 16	316 $\pm$ 15	55 $\pm$ 1	45 $\pm$ 3	38 $\pm$ 2
20 mM		351 $\pm$ 22*	269 $\pm$ 15*	53 $\pm$ 2	47 $\pm$ 2	37 $\pm$ 2
40 mM		272 $\pm$ 11**	198 $\pm$ 9**	54 $\pm$ 2	47 $\pm$ 2	37 $\pm$ 2
GlcN						
5 mM		238 $\pm$ 18**	138 $\pm$ 9**	51 $\pm$ 1	39 $\pm$ 1	35 $\pm$ 3
10 mM		162 $\pm$ 13**	111 $\pm$ 5**	49 $\pm$ 1*	35 $\pm$ 2*	34 $\pm$ 3
20 mM		122 $\pm$ 7**	75 $\pm$ 7**	39 $\pm$ 2**	32 $\pm$ 2*	31 $\pm$ 2*
40 mM		69 $\pm$ 13**	53 $\pm$ 5**	32 $\pm$ 3**	28 $\pm$ 2**	28 $\pm$ 2*
	No substrates ...	75 $\pm$ 7	85 $\pm$ 9	16 $\pm$ 2	16 $\pm$ 2	14 $\pm$ 3

mannoheptulose and glucose had additive effects ($P < 0.05$) on glucokinase release [control, 13 $\pm$ 2; 20 mM mannoheptulose, 49 $\pm$ 3; 20 mM glucose, 51 $\pm$ 5; mannoheptulose + glucose, 65 $\pm$ 4 (% glucokinase release, $n = 4$)]. Mannoheptulose (20 mM) inhibited glycogen deposition from 40 mM glucose by more than 80% (results not shown), consistent with inhibition of glucokinase [13–15].

Glucosamine and *N*-acetylglucosamine, unlike mannoheptulose, did not induce glucokinase translocation during incubation with 5 mM glucose (results not shown). Table 1 shows the effects of these inhibitors on glucokinase release and glycogen deposition during incubation with 20 mM or 40 mM glucose. *N*-Acetylglucosamine (20–40 mM) inhibited glycogen deposition by 27–55% during incubation with 20 mM glucose and by 22–45% during incubation with 40 mM glucose. However, it did not inhibit glucose-induced glucokinase release at either 20 mM or 40 mM glucose (Table 1). Glucosamine was more potent at inhibiting glycogen deposition than was *N*-acetylglucosamine. At 20 mM glucosamine caused total inhibition of glycogen deposition from 20 mM glucose, and 40 mM glucosamine caused total inhibition of glycogen deposition from 40 mM glucose. Glucosamine (20–40 mM) suppressed, but did not abolish, glucose-induced glucokinase release, and it suppressed the fructose effect with a similar potency, suggesting that it inhibits at a site common to both substrates.

Effects of 3,3'-tetramethyleneglutaric acid

The lack of inhibition of glucose-induced glucokinase translocation by *N*-acetylglucosamine, despite inhibition of glycogen deposition, suggests that the effects of glucose are probably mediated by a mechanism that does not involve flux through glucokinase. A possible mechanism is that glucose may be converted into sorbitol via aldose reductase (EC 1.1.1.21) [12]. Liver aldose reductase has low activity with glucose as a substrate [12,16,17]. It is noteworthy, however, that the intracellular [glucose] in the liver cell is similar to the extracellular concentration. Since stimulation of glucokinase release by 20 mM glucose is approximately equivalent to that caused by 6 μ M sorbitol, low activities of aldose reductase [12] may enable sufficient conversion of glucose into sorbitol to stimulate gluco-

Table 2 Effects of 3,3'-tetramethyleneglutaric acid on substrate-induced glucokinase release

Hepatocytes were incubated for 20 min with the substrates indicated in the absence or presence of 5 mM 3,3'-tetramethyleneglutaric acid (TMG), which was added 10 min before addition of substrates. Glucokinase release was determined during 6 min permeabilization with digitonin as described in the Materials and methods section, and enzyme activity released is expressed as a proportion of total activity. Values are means $\pm$ S.E.M. of 5 experiments: * $P < 0.05$ for TMG versus respective control.

Substrate	Glucokinase release (%)	
	Control	5 mM TMG
None	8 $\pm$ 2	3 $\pm$ 1*
+ 10 mM glucose	34 $\pm$ 2	16 $\pm$ 1*
+ 20 mM glucose	46 $\pm$ 3	32 $\pm$ 2*
+ 1 mM fructose	53 $\pm$ 3	49 $\pm$ 3
+ 1 mM sorbitol	49 $\pm$ 3	48 $\pm$ 3

kinase translocation. Sorbitol accumulation in liver has been demonstrated in diabetic rats [18]. To investigate the possible role of conversion of glucose into sorbitol, the effects of 3,3'-tetramethyleneglutaric acid, an inhibitor of aldose reductase [19,20], were examined. 3,3'-Tetramethyleneglutaric acid (5 mM) inhibited the stimulation of glucokinase release caused by 10–20 mM glucose, but had no effect on stimulation by 1 mM fructose or 1 mM sorbitol (Table 2).

DISCUSSION

Substrate-induced translocation

This study and our previous paper [3] demonstrate that a variety of substrates induce glucokinase translocation in rat hepatocytes, including glucose, mannose, fructose, tagatose, sorbitol, mannitol and D-mannoheptulose, whereas other substrates, including galactose, 3-*O*-methylglucose and 2-deoxyglucose, are ineffective [3]. Tagatose is a substrate for fructokinase [4], and tagatose 1-phosphate, like fructose 1-phosphate, binds to the glucokinase-binding protein and decreases its affinity for glucokinase [8]. The similarities in the effects of tagatose and fructose (Figure 1)

therefore support the hypothesis that the binding protein of glucokinase may be involved in fructose-induced translocation. Sorbitol and mannitol are not substrates for fructokinase [4]. Sorbitol 6-phosphate and mannitol 1-phosphate bind to the glucokinase-binding protein, and like fructose 6-phosphate, they increase the affinity of the binding protein for glucokinase, as determined from inhibition of glucokinase activity [8]. The potency of sorbitol 6-phosphate is greater than that of fructose 6-phosphate or of mannitol-1-phosphate [8]. It cannot be established from the present study whether sorbitol and mannitol are metabolized by hepatocytes to sorbitol 6-phosphate and mannitol 1-phosphate or to other derivatives. However, two points are noteworthy. First, that the effects of sorbitol are not mediated by conversion into fructose via sorbitol dehydrogenase; secondly, that the effects of sorbitol, mannitol, fructose and tagatose on glucokinase release correlate with the stimulation of glucose conversion into glycogen. Thus, although sorbitol 6-phosphate has a potent inhibitory effect on glucokinase activity via the binding protein [8], the stimulation of glucokinase translocation by sorbitol is associated with corresponding stimulation of glucose conversion into glycogen (Figure 2).

Although dihydroxyacetone increases glucokinase release, the maximum effect is smaller than that induced by fructose, sorbitol, tagatose or glucose. Dihydroxyacetone is phosphorylated to dihydroxyacetone phosphate via triokinase and further metabolized either to fructose 1,6-bisphosphate or through the glycolytic pathway. The small effect of dihydroxyacetone compared with fructose or glucose would suggest that neither fructose 1,6-bisphosphate nor a distal glycolytic intermediate is likely to be a key intermediate at inducing glucokinase translocation. An increase in [dihydroxyacetone phosphate] might decrease aldolase-B-catalysed hydrolysis of fructose 1-phosphate through a mass-action effect and thereby potentiate the effect of low, but not of maximally effective, concentrations of fructose. Alternatively, the effects of dihydroxyacetone may be due to a more oxidized cytosolic NADH/NAD⁺ redox state. The inhibitory effects of ethanol and glycerol on substrate-induced translocation of glucokinase also suggest a role for the cytosolic redox state. One possibility is that the active intermediates that mediate the effects of glucose, fructose or sorbitol are substrates/products of NADH/NAD⁺-coupled dehydrogenases. Another possibility is that glucokinase may bind to the cell matrix or cytoskeleton by associating with a dehydrogenase. Several glycolytic enzymes associate with cytoskeletal proteins through either direct binding or through indirect interaction via enzyme–enzyme–cytoskeleton interactions [21]. Phosphoglycerate mutase binds to microfilaments only in conjunction with lactate dehydrogenase [22], and triose phosphate isomerase binds to actin by interaction with aldolase or glyceraldehyde-3-phosphate dehydrogenase [23]. Binding of aldose to the cytoskeleton is influenced by the substrate and also by NADH [24].

Glucose-induced translocation

The glucokinase inhibitors used in this study had different effects on glucokinase translocation. D-Mannoheptulose caused translocation of glucokinase and did not counteract the stimulation by glucose, although it inhibited glycogen deposition. N-Acetylglucosamine did not cause glucokinase translocation, and concentrations which inhibited glucose conversion into glycogen did not counteract glucose-induced glucokinase translocation. Glucosamine did not cause glucokinase translocation, but it inhibited both glucose-induced and fructose-induced glucokinase translocation. The lack of inhibition of glucose-induced trans-

location by mannoheptulose and N-acetylglucosamine argues against a mechanism that involves flux through glucokinase. The lower potency of glucosamine on glucokinase translocation compared with inhibition of glycogen deposition also does not support a role for glucokinase flux in inducing glucokinase translocation. Furthermore, the similar inhibition by glucosamine of the glucose-induced and fructose-induced effects suggests that the effect of glucosamine is at a site that is common to both substrates. Since low concentrations of fructose are more likely to be metabolized via fructokinase than via glucokinase [1,4], the inhibitory effect of glucosamine is probably not via glucokinase.

The inhibitory effect of 3,3'-tetramethyleneglutaric acid, an inhibitor of aldose reductase [19,20], on glucose-induced, but not fructose- or sorbitol-induced, glucokinase translocation suggests that the glucose effect may be mediated via conversion into sorbitol. However, whether this inhibitor might have other non-specific effects cannot be excluded from the present study.

Physiological implications

Of the substrates tested, sorbitol is by far the most potent at stimulating glucokinase translocation and glucose conversion into glycogen. Glycerol was the most potent inhibitor of substrate-induced translocation, and it also inhibits glucose conversion into glycogen. Aldose reductase, which catalyses the conversion of glucose into sorbitol, is expressed in several tissues, including skeletal muscle, brain, kidney, nerve, blood vessels and erythrocytes [25]. Because the affinity of aldose reductase for glucose is low, there is little conversion of glucose into sorbitol at physiological [glucose]; however, increased amounts of sorbitol are formed in hyperglycaemic conditions, and sorbitol levels in erythrocytes correlate with blood [glucose] [26]. Glycerol is produced by lipolysis in adipose tissue in the fasting state and in response to stress hormones when glucose needs to be conserved for brain or muscle tissue respectively. It might be speculated that sorbitol formed in either liver or extrahepatic tissue acts as a positive modulator of hepatic glucokinase translocation and glucose conversion into glycogen in hyperglycaemic conditions, whereas glycerol acts as a negative modulator when glucose needs to be conserved for extrahepatic tissues.

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