Hexokinase and glucokinase binding in permeabilized guinea-pig hepatocytes

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The release of glucokinase (hexokinase IV) from digitonin-permeabilized hepatocytes from rat, guinea pig or mouse liver is inhibited by physiological concentrations of Mg^{2+} (> 0.25 mM). Preincubation of hepatocytes with fructose increases glucokinase release during permeabilization in the presence of Mg^{2+} but decreases glucokinase release in the absence of Mg^{2+}, suggesting that fructose causes translocation of glucokinase from the Mg^{2+}-dependent site. Glucose (25 mM) and sorbitol (1 mM) also induce translocation of glucokinase from the Mg^{2+}-dependent site in guinea-pig, as in rat hepatocytes, but glucose is less effective than fructose or sorbitol, and the concentrations of fructose and sorbitol that cause half-maximal activation (A_{50}) are 3-fold and 20-fold higher, respectively, in guinea-pig than in rat hepatocytes (170 μM and 257 μM, compared with 61 μM and 13 μM). Dihydroxyacetone and glycerol have no effect on fructose-induced or sorbitol-induced translocation in guinea-pig hepatocytes, in contrast with the potentiation and inhibition, respectively, by these substrates in rat hepatocytes. Some, but not all, of the differences between rat and guinea-pig hepatocytes could be due to the more reduced cytoplasmic NADH/NAD^+ redox state in guinea-pig cells. The activity of low-K_m hexokinases accounts for 30% of total hexokinase activity (low-K_m hexokinases + glucokinase) in guinea-pig hepatocytes. Of the low-K_m hexokinase activity, approx. 30% is released in the presence of Mg^{2+}, 9% shows Mg^{2+}-dependent binding and 60% shows Mg^{2+}-independent binding. There was no substrate-induced translocation of low-K_m hexokinase activity, indicating that translocation is specific for hexokinase IV.

INTRODUCTION

Four hexokinase isoenzymes (EC 2.7.1.1) are expressed in mammalian tissues. Hexokinase IV, commonly known as glucokinase, is expressed only in hepatocytes and in insulin-secreting β-cells, and differs from the other hexokinases (I–III) in its lower molecular mass (52 kDa compared with 100 kDa), its low affinity for glucose and its sigmoidal kinetics [1]. Hexokinases I–III have been described as ambiquitous enzymes [2], because studies on homogenates from several tissues have shown that these isoenzymes reversibly partition between a membrane-bound and a free state [3–8]. Hepatic glucokinase, in contrast, has been assumed to be present exclusively in the free state in the cytoplasm [1,8]. However, recent studies on digitonin-permeabilized rat hepatocytes have established that, in cells equilibrated with 5 mM glucose as sole carbohydrate substrate, glucokinase is present predominantly in a bound state by a Mg^{2+}-dependent mechanism, but certain substrates, and particularly sorbitol and fructose, cause translocation of glucokinase from the Mg^{2+}-dependent binding site [9,10]. Whether the binding characteristics or intracellular locations of glucokinase in the hepatocyte are analogous to those of the low-K_m hexokinase isoenzymes previously reported for extrahepatic tissues is as yet unestablished, since comparative studies have not been performed in the same experimental system. Rat hepatocytes have very low activities of the low-K_m isoenzymes in comparison with glucokinase [11]. Guinea-pig hepatocytes, in contrast, have a much higher activity of the low-K_m isoenzymes [12] than do rat hepatocytes, and are therefore a suitable model for comparative studies on binding of low-K_m hexokinases and glucokinase. This study examines the binding characteristics and substrate effects on low-K_m hexokinases and glucokinase in guinea-pig hepatocytes.

MATERIALS AND METHODS

Materials

Sources of materials were as described previously [9,10].

Hepatocyte isolation and monolayer culture

Hepatocytes were isolated by collagenase perfusion of the liver [12,13] from male Dunkin–Hartley guinea pigs (body wt. 300–400 g), male albino Wistar rats (body wt. 180–300 g) and male Balb/c mice (body wt. 22–25 g). All animals were fed *ad libitum*. The hepatocytes 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/cm^2 and incubated at 37 °C in a humidified atmosphere equilibrated with 5% CO_2 in air. After cell attachment (approx. 4 h), the medium was changed to serum-free medium containing 10 nM dexamethasone [9]. Incubations with digitonin for determination of enzyme-activity release were performed after between 8 h and 30 h in culture.

Incubation of hepatocyte monolayers

Glucokinase and hexokinase release during permeabilization of hepatocytes with digitonin was determined either on hepatocytes that had been maintained in the standard culture medium (containing 5 mM glucose) or after incubation of the cells (5–30 min) in medium supplemented with the substrates indicated.

Permeabilization of hepatocytes with digitonin

The hepatocyte monolayers were washed once with 150 mM NaCl and then permeabilized for 6 min in medium (400 μl/well)

Abbreviation used: A_{50}, concn. giving half-maximal activation.

containing 300 mM sucrose, 2 mM dithiothreitol, 3 mM Hepes, pH 7.2 at 20 °C, and digitonin and Mg^{2+} at the concentrations indicated as described previously [9]. Low-K_m hexokinase activity and glucokinase activity were assayed in the digitonin eluate aspirated on termination of the 6 min permeabilization and in the residual cell matrix extracted either in 100 mM KCl/25 mM Hepes/7.5 mM $MgCl_2$/4 mM dithiothreitol/0.05% (w/v) Triton X-100 (rat and guinea-pig hepatocytes) or in 100 mM KCl/25 mM Hepes/7.5 mM $MgCl_2$/4 mM dithiothreitol (mouse hepatocytes) as in [9]. Enzyme activities released in the digitonin eluates are expressed as a percentage of the total activity in the digitonin eluate plus cell-matrix extract [9].

Hexokinase and glucokinase activity

Hexokinase and glucokinase were determined as described previously with 0.5 mM or 100 mM glucose [12]. In rat and mouse hepatocytes there was negligible activity with 0.5 mM glucose relative to 100 mM glucose, and glucokinase activity was determined with 100 mM glucose. In guinea-pig hepatocytes low-K_m hexokinase and glucokinase were assayed with 0.5 mM (low-K_m) and 100 mM glucose (total hexokinase activity). The low-K_m hexokinase represents the activity determined at 0.5 mM glucose, and glucokinase activity represents the difference in activity between 100 mM and 0.5 mM glucose. In whole extracts of guinea-pig hepatocytes, the activity assayed at 0.5 mM glucose was 30 ± 2% (mean $\pm$ S.E.M., $n = 9$) of the activity at 100 mM glucose.

Resolution of guinea-pig hexokinase activity by f.p.l.c. on a Mono-Q Sepharose column [9] eluted with a linear NaCl gradient (0–0.7M) showed three peaks of low-K_m activity that were eluted between 0.1 M and 0.25 M NaCl, and glucokinase was eluted at 0.3 M NaCl. The low-K_m peaks eluted with increasing NaCl accounted for 3%, 15% and 16% respectively of total hexokinase activity (low-K_m + glucokinase activity).

Total hexokinase activity (low-K_m + glucokinase) was 11.8 ± 2.5 m-units/mg of protein for rat hepatocytes, 11.0 ± 2.5 m-units/mg for guinea-pig hepatocytes and 12.6 ± 2.4 m-units/mg for mouse hepatocytes, where 1 m-unit is the amount of enzyme converting 1 nmol of substrate/min. Protein was determined by an automated Lowry method [14].

Low-K_m hexokinase activity (assayed at 0.5 mM glucose) and glucokinase activity (as above) in the digitonin eluates is expressed as a percentage of total activity in the digitonin eluate plus cell matrix. Results are expressed as means $\pm$ S.E.M. for the number of cell preparations indicated. Statistical analysis for differences in incubation conditions was by the Student's paired t test, and for differences between species by the unpaired t test.

RESULTS

Effects of [digitonin] and [Mg^{2+}] on glucokinase release from permeabilized hepatocytes

We have shown previously that, when rat hepatocytes are pre-equilibrated in medium containing 5 mM glucose and permeabilized with digitonin in the presence of Mg^{2+}, glucokinase remains bound to the cell matrix at digitonin concentrations that cause release of several cytoplasmic enzymes. However, pre-incubation of hepatocytes with fructose increases the release of glucokinase during permeabilization in the presence of Mg^{2+} without affecting the release of other cytoplasmic enzymes [9]. Figure 1 shows the effects of increasing [digitonin] on glucokinase release in the absence or presence of 5 mM $MgCl_2$ in rat, guinea-pig or mouse hepatocytes preincubated without or with fructose. With increasing [digitonin], glucokinase release was higher during permeabilization in the absence of Mg^{2+} than in its presence for all three species at the digitonin concentrations studied. During permeabilization in the absence of Mg^{2+}, glucokinase release was slightly but significantly lower ($P < 0.05$, at 0.025 mg/ml digitonin, in rat and mouse cells; Figure 1) in fructose-pretreated compared with untreated cells. During permeabilization in the presence of Mg^{2+}, glucokinase release increased progressively with increasing [digitonin], but remained higher in fructose-pretreated than in control cells at all digitonin concentrations studied (Figure 1). In previous experiments on rat hepatocytes, a

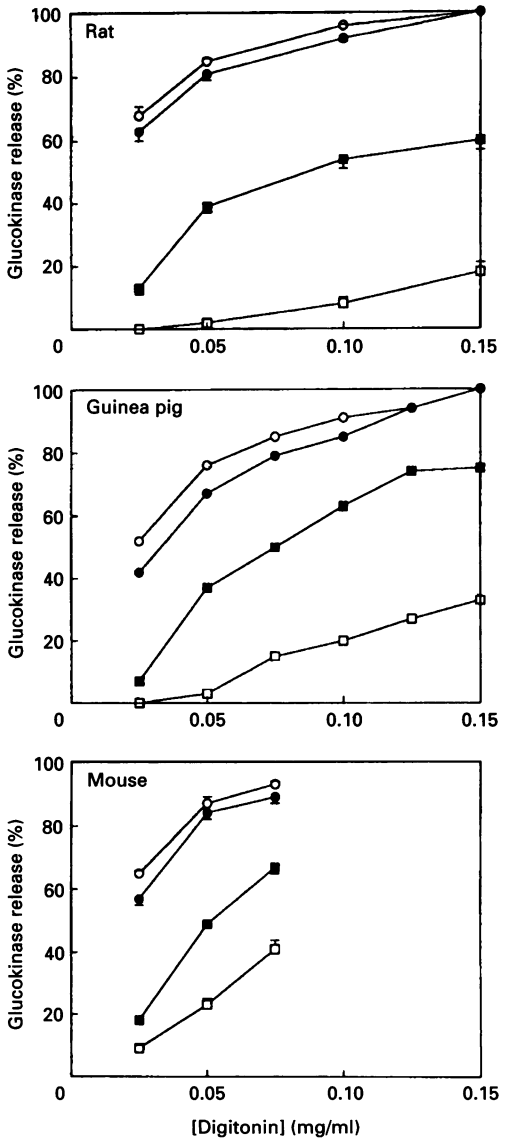

Figure 1 Effects of [digitonin] on glucokinase release from rat and guinea-pig hepatocytes

Rat or guinea-pig hepatocytes were preincubated for 30 min without (○, □) or with (●, ■) 4 mM fructose. They were then washed and permeabilized for 6 min in 300 mM sucrose/3 mM Hepes/2 mM dithiothreitol without (○, ●) or with 5 mM $MgCl_2$ (□, ■) at the [digitonin] indicated. Glucokinase activity released in the digitonin eluate is expressed as a percentage of total activity. Values are means $\pm$ S.E.M. for 8 (rat) or 4 (mouse) or means of 2 (guinea-pig) hepatocyte preparations.

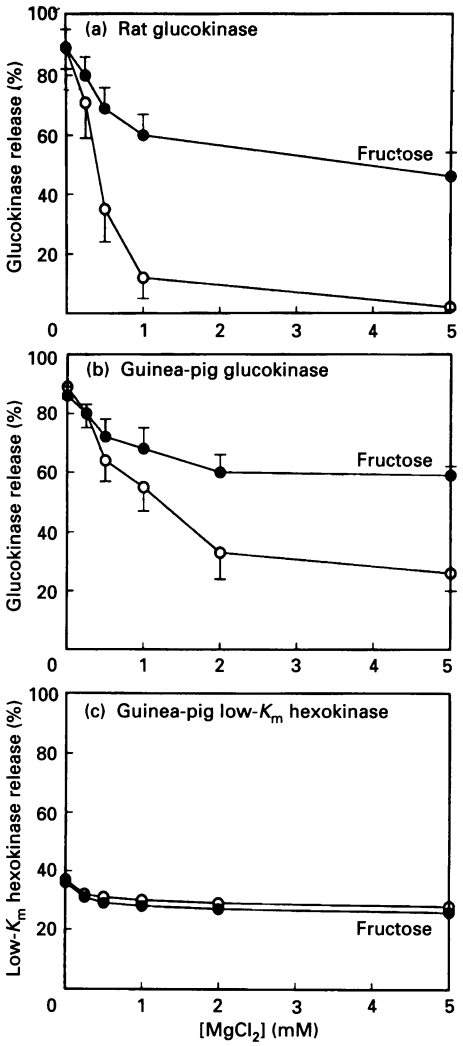

Figure 2 Effects of [Mg^{2+}] on glucokinase and low-K_m hexokinase release from permeabilized hepatocytes

Rat or guinea-pig hepatocytes were preincubated for 30 min without (○) or with (●) 4 mM fructose. They were then washed and permeabilized for 6 min in 300 mM sucrose/3 mM Hepes/2 mM dithiothreitol containing 0.075 mg/ml digitonin at the [Mg^{2+}] indicated. Glucokinase (**a** and **b**) or low-K_m hexokinase (**c**) activity released in the digitonin eluate is expressed as a percentage of total activity. Values are means ± S.E.M. for each of 4 hepatocyte preparations.

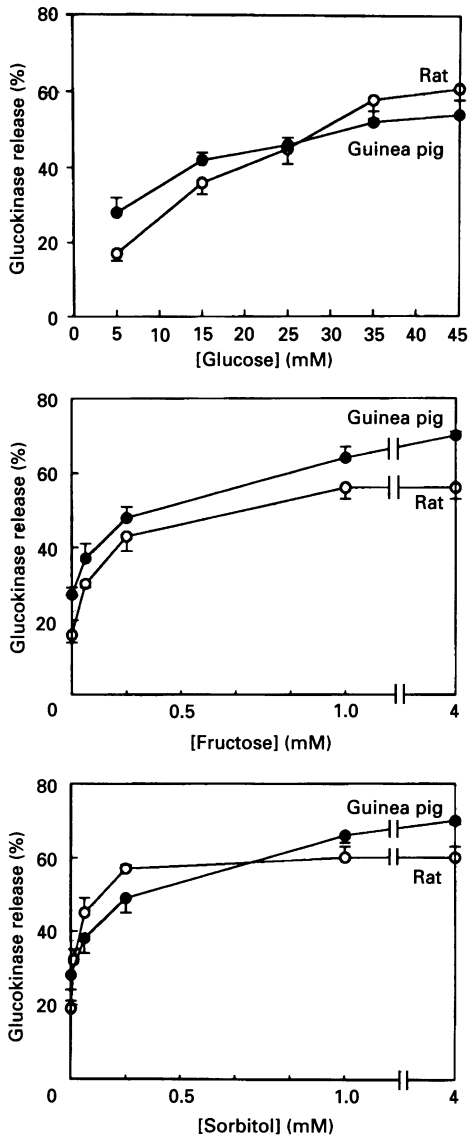

Figure 3 Effects of glucose, fructose or sorbitol on glucokinase translocation

Rat (○) or guinea-pig hepatocytes (●) were preincubated for 30 min with the concentrations of glucose, fructose or sorbitol indicated. The hepatocytes were then washed and permeabilized for 6 min in 300 mM sucrose/3 mM Hepes/2 mM dithiothreitol/5 mM $MgCl_2$ containing 0.075 mg/ml digitonin. Glucokinase activity released in the digitonin eluate is expressed as a percentage of total activity. Values are means ± S.E.M. for each of 4 hepatocyte preparations.

[digitonin] of 0.05 mg/ml was used because it caused near-maximal release of glucokinase in the absence of Mg^{2+}, and because higher digitonin concentrations caused release of glutamate dehydrogenase [9,10]. In studies on guinea-pig hepatocytes, the low-K_m hexokinase activity accounted for all or most of the hexokinase activity released at 0.05 mg/ml digitonin in the presence of Mg^{2+}. To minimize errors in determination of guinea-pig glucokinase (by subtraction of the low-K_m activity), a higher [digitonin] (0.075 mg/ml) was used in the rest of this study for both guinea-pig and rat hepatocytes. This resulted in higher enzyme release and slightly higher substrate A_{50} values (concn. giving half-maximal activation) (by about 10%) than at lower [digitonin]. The [Mg^{2+}] that caused half-maximal inhibition of glucokinase release during permeabilization of guinea-pig hepatocytes with 0.075 mg/ml digitonin was similar (0.5–0.7 mM) to that in rat hepatocytes (Figures 2a and 2b).

Effects of [Mg^{2+}] on low-K_m hexokinase binding to guinea-pig hepatocytes

In the absence of Mg^{2+} at a [digitonin] that caused more than 85% release of glucokinase, there was 37% release of low-K_m hexokinase activity (Figure 2c). Increasing [$MgCl_2$] (0.25–5 mM) suppressed the release of low-K_m hexokinase from 37% to 28%, with half-maximal inhibition at 0.5 mM $MgCl_2$.

Table 1 Sensitivity of glucokinase translocation to various substrates in rat and guinea-pig hepatocytes

Rat and guinea-pig hepatocytes were incubated for 30 min with the substrates indicated at concentrations ranging from 10 μM to 4 mM in medium containing 5 mM glucose. They were then washed and permeabilized as in Figure 3. Values represent the concentration of substrate that causes half-maximum stimulation of glucokinase release (A_{50}). Values are means ± S.E.M. for the numbers of experiments shown in parentheses: * $P < 0.05$, ** $P < 0.005$ relative to rat hepatocytes.

		Substrate A_{50} (μM)	
Substrate	Hepatocytes ...	Rat	Guinea-pig
Fructose		61 ± 9 (9)	170 ± 25** (13)
Tagatose		50 ± 13 (4)	140 ± 30* (3)
Psicose		82 ± 20 (5)	> 400 (3)
Sorbitol		13 ± 3 (9)	257 ± 26** (11)

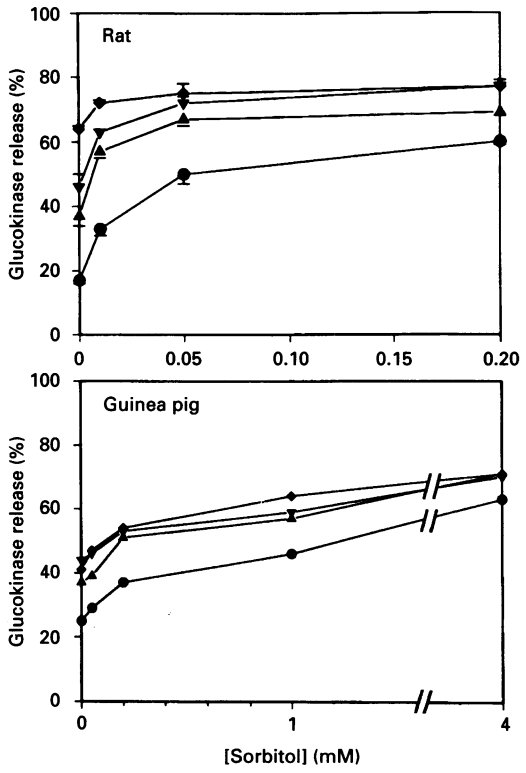

Figure 4 Interactions of glucose and sorbitol effects on glucokinase translocation

Hepatocytes were preincubated for 30 min with varying [sorbitol]: 10–200 μM for rat and 50 μM–4 mM for guinea pig. The [glucose] was 5 mM (●), 15 mM (▲), 25 mM (▼) or 35 mM (◆). They were then permeabilized as in Figure 3. Glucokinase activity released in the digitonin eluate is expressed as a percentage of total activity. Values are means ± S.E.M. for 3 experiments (rat hepatocytes) or means of 2 experiments (guinea-pig hepatocytes).

Substrate-induced glucokinase translocation in guinea-pig and rat hepatocytes

Figure 3 shows the effects of preincubation of hepatocytes with glucose (10–45 mM), fructose (50 μM–4 mM) or sorbitol (10 μM–4 mM) on glucokinase release during subsequent permeabilization of hepatocytes with digitonin in the presence of 5 mM $MgCl_2$. In rat hepatocytes the increment in glucokinase release caused by an increase in [glucose] from 5 mM to 45 mM was similar to that caused by maximally effective concentrations of fructose or sorbitol (1–4 mM). However, in guinea-pig hepatocytes, the increment caused by glucose was smaller than that caused by fructose or sorbitol. Table 1 shows the affinities of glucokinase translocation from the Mg^{2+}-dependent site in rat and guinea-pig hepatocytes for various substrates that are precursors of ligands of the glucokinase-binding protein [15,16]. The concentrations of fructose and tagatose that caused half-maximal activation (A_{50}) of glucokinase translocation were 3-fold higher in guinea-pig than in rat hepatocytes, whereas the A_{50} for sorbitol was 20-fold higher in guinea-pig hepatocytes (Table 1).

Interactions of sorbitol and glucose

In view of the very high affinity for sorbitol of glucokinase translocation in rat hepatocytes, we previously considered the possibility that the glucose effect may be mediated via conversion into sorbitol through aldose reductase [10]. Experiments with inhibitors of glucokinase and aldose reductase provided support for this hypothesis [10]. Because of the much lower affinity of glucokinase translocation for sorbitol in guinea-pig hepatocytes, the combined effects of sorbitol and glucose on glucokinase translocation were investigated (Figure 4). In rat hepatocytes in the combined presence of glucose (25–35 mM) and sorbitol (1 mM), glucokinase translocation was greater ($P < 0.05$) than with sorbitol (1 mM) and 5 mM glucose. Qualitatively similar results were observed in guinea-pig cells. Although this does not exclude the possibility that the effect of glucose may be in part mediated via conversion into sorbitol [10], it suggests that the glucose effect is in part (30%) independent of conversion into sorbitol.

We have shown previously that in rat hepatocytes the effects of maximally effective concentrations of fructose and sorbitol on glucokinase translocation are not additive, and the extent of translocation is identical in the combined presence of 1 mM fructose and 1 mM sorbitol to that with either substrate alone [10]. However, experiments determining the effects of fructose (50 μM–1 mM) at varying [glucose] in rat hepatocytes show similar partially additive effects (Figure 5) to the combined effects of sorbitol and glucose (Figure 4). This suggests that fructose and sorbitol induce glucokinase translocation through a common mechanism. However, the effects of high [glucose] (25–35 mM) are in part (~ 70%) mediated through a mechanism in common with the effects of sorbitol or fructose, but in part (~ 30%) through an independent mechanism, which is additive with the effects of maximally effective concentrations of sorbitol or fructose.

Effects of dihydroxyacetone, glycerol and ethanol on glucokinase translocation in guinea-pig hepatocytes

In rat hepatocytes, glucokinase translocation induced by glucose, or by sub-maximal concentrations of fructose or sorbitol, is potentiated by dihydroxyacetone (0.5–5 mM) and inhibited by glycerol (1 mM) and ethanol (0.5–5 mM) [10]. Higher concentrations of glycerol (5 mM) also inhibit the effects of maximally effective concentrations of fructose and sorbitol [10]. In guinea-pig hepatocytes there was no significant effect of either 5 mM dihydroxyacetone or 5 mM glycerol on fructose-induced or sorbitol-induced translocation (Table 2). Lower concentrations of dihydroxyacetone and glycerol (0.5–2 mM) and ethanol

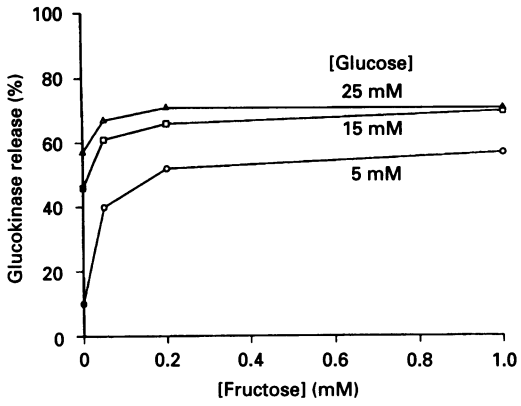

Figure 5 Interactions of glucose and fructose effects on glucokinase translocation

Rat hepatocytes were preincubated for 20 min with the concentrations of fructose and glucose indicated. They were then permeabilized for 6 min in 300 mM sucrose/3 mM Hepes/5 mM $MgCl_2$ containing 0.05 mg/ml digitonin. Glucokinase activity released in the digitonin eluate is expressed as a percentage of total activity. Similar results were obtained in two further experiments.

Table 2 Effects of dihydroxyacetone and glycerol on glucokinase translocation in guinea-pig hepatocytes

Guinea-pig hepatocytes were incubated for 30 min with the concentrations of fructose or sorbitol indicated in the absence or presence of 5 mM dihydroxyacetone (DHA) or 5 mM glycerol. They were then washed and permeabilized as in Figure 3. Glucokinase activity released in the digitonin eluate is expressed as a percentage of total activity. Values are means ± S.E.M. for 4 experiments.

	Glucokinase release (%)		
Additions	Control	5 mM DHA	5 mM glycerol
None	22±2	18±5	16±4
50 μM fructose	30±3	29±6	26±4
200 μM fructose	40±4	37±6	40±4
1 mM fructose	48±4	50±6	50±4
4 mM fructose	53±4	49±7	50±5
None	27±1	27±4	26±6
50μ M sorbitol	34±4	36±6	34±1
200 μM sorbitol	42±4	44±6	47±5
1 mM sorbitol	54±5	59±4	58±1

(0.5–10 mM) also had no effect on sub-maximally effective concentrations (200 μM) of fructose or sorbitol ($n = 4$; results not shown). Parallel experiments on rat hepatocytes ($n = 5$) using 0.075 mg/ml digitonin showed similar effects of ethanol, glycerol and dihydroxyacetone to those reported previously with 0.05 mg/ml digitonin [10].

Lack of effect of substrates on binding of low-K_m hexokinase activity

Preincubation of guinea-pig hepatocytes with glucose (15–45 mM) or fructose (50 μM–1 mM) or sorbitol (50 μM–4 mM) had no effect on the activity of low-K_m hexokinase activity recovered in the digitonin eluate or residual cell matrix after permeabilization of hepatocytes for 6 min with 0.075 mg/ml digitonin in 300 mM sucrose/3 mM Hepes/5 mM $MgCl_2$ (results of glucokinase experiments in Figure 3; results not shown), in contrast with the increased release of glucokinase. However, in hepatocytes preincubated with 4 mM fructose there was a diminished activity of low-K_m hexokinase activity in the digitonin eluate relative to controls. This was not associated with an increase in hexokinase activity in the residual cell matrix, but with a decrease in total hexokinase activity recovery in medium plus cell matrix. Thus the apparent decrease in percentage release of low-K_m hexokinase in fructose-treated (4 mM) as compared with control cells (Figure 2c) is due to a decrease in activity in the digitonin eluate without a corresponding increase in the cell matrix.

DISCUSSION

Guinea-pig hepatocytes have a higher activity of low-K_m hexokinases than do rat hepatocytes [11,12] and are therefore a good model to study the binding properties of low-K_m hexokinases and of glucokinase in the same experimental system. Only a small fraction (9%) of the low-K_m hexokinase activity showed Mg^{2+}-dependent binding at a [digitonin] at which > 80% of glucokinase activity is released in the absence of Mg^{2+}, but remains bound in the presence of Mg^{2+}. Variations in [digitonin] (0.025–0.15 mg/ml) had little effect on the fraction of Mg^{2+}-dependent bound activity. Of the remaining low-K_m activity, about 60% remains bound in the absence of Mg^{2+} and 30% is released in the presence of Mg^{2+}. The latter (30% fraction) may represent activity that is present in the 'free' state in the cytoplasm or is loosely bound, and the former (60% fraction) is presumably bound by a different mechanism from glucokinase. The relative activities of the three low-K_m hexokinase peaks that are eluted with increasing salt concentration from Mono-Q Sepharose were approximately in the proportions 1:5:5. It is possible therefore that the fractions of low-K_m activity that show different binding properties (9%, 60% and 30%) represent the three isoenzymes or different isoforms [8]. Although 9% of the low-K_m hexokinase activity showed Mg^{2+}-dependent binding with a similar affinity for Mg^{2+} ($A_{0.5}$ 0.5 mM) to glucokinase, neither this activity nor the Mg^{2+}-independent-bound activity showed substrate-induced translocation in response to fructose, sorbitol or an increase in [glucose], indicating that translocation by these substrates is specific for glucokinase. This supports the suggestion that the 62 kDa binding protein of glucokinase [15] may be involved in substrate-induced translocation [9,10], since this protein binds specifically to glucokinase and not to the other hexokinase isoenzymes [17].

In mouse and guinea-pig hepatocytes glucokinase shows similar Mg^{2+}-dependent binding properties to those in rat hepatocytes. Glucokinase remains bound to the cell matrix during permeabilization of hepatocytes in the presence of Mg^{2+} if hepatocytes have been pre-cultured with 5 mM glucose as sole carbohydrate substrate; however, it is released if the cells have been pre-cultured with fructose, indicating that fructose causes translocation from the Mg^{2+}-dependent binding site in all three species. Although substrate-induced translocation of glucokinase in guinea-pig hepatocytes showed similarities to the process in rat hepatocytes in its response to glucose, fructose, tagatose and sorbitol, there were three basic differences. First, in guinea-pig cells, glucose (35–45 mM) caused a smaller increment in glucokinase translocation in comparison with maximally effective concentrations of fructose or sorbitol (Figure 3). Secondly, the affinity for fructose and sorbitol was lower in guinea-pig than in

rat hepatocytes by 3-fold and 20-fold, respectively. Thirdly, dihydroxyacetone, glycerol and ethanol had no effect on substrate-induced translocation in guinea-pig cells. The latter finding was surprising, since dihydroxyacetone potentiates, and glycerol and ethanol inhibit, substrate-induced translocation in rat hepatocytes [10]. We have postulated that the inhibitory effects of ethanol and glycerol on glucokinase translocation may be due to a more reduced cytoplasmic redox state [10]. It is of interest that the cytoplasmic redox state is more reduced in guinea-pig than in rat liver [18], and, whereas during fasting the cytoplasmic redox state becomes more reduced in the rat [19], in the guinea-pig it either remains unchanged or becomes more oxidized [18]. The inhibition of glucokinase translocation in rat hepatocytes by substrates that reduce the cytoplasmic redox state may therefore be of physiological significance during fasting. Since substrates that cause a more reduced cytoplasmic redox state in rat liver decrease translocation induced by maximally effective concentrations of glucose as well as the sensitivity of translocation to fructose [10], the lower effectiveness of glucose and the lower sensitivity to fructose in guinea-pig hepatocytes might be due to the more reduced cytoplasmic redox state in this species. However, other mechanisms, for example differences in concentrations of phosphorylated intermediates or differences in the sensitivity of the regulatory protein of glucokinase to phosphorylated sugars [20], cannot be excluded.

The 20-fold lower affinity for sorbitol (as compared with 3-fold lower affinity for fructose and tagatose) in guinea-pig as compared with rat cells may be due to a difference in sorbitol metabolism by guinea-pig hepatocytes or to a difference in the affinity of the translocation mechanism for the products of fructose and sorbitol metabolism. Species differences in the affinity of the glucokinase-binding protein for various ligands have been reported [20]. The effects of sorbitol on glucokinase translocation are of particular interest, because in rat hepatocytes sorbitol is more potent than fructose at stimulating glucose conversion into glycogen and inducing glucokinase translocation [10]. Sorbitol is present in micromolar levels in the liver [21,22] and is therefore a potential physiological regulator of glucokinase [10]. Species differences in sensitivity to sorbitol could therefore be important in control of glucose metabolism. We postulated that conversion of glucose into sorbitol within the hepatocyte is a possible mechanism whereby glucose induces translocation of glucokinase [10]. However, the experiments determining the separate and combined effects of glucose (15–35 mM) and sorbitol (Figure 4) show that the effects of glucose are, at least in part, additive with the effect of sorbitol, suggesting that a component of the glucose effect (approx. 27%) is not mediated via conversion into sorbitol.

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