Involvement of glucokinase translocation in the mechanism by which resorcinol inhibits glycolysis in hepatocytes

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Proglycosyn and resorcinol stimulate glycogen synthesis and inhibit glycolysis in hepatocytes. The former effect is attributed to inactivation of phosphorylase mediated by glucuronidated metabolites. This study investigated the mechanism by which resorcinol inhibits glycolysis. Resorcinol (150 μ M) inhibited glycolysis in hepatocytes incubated with glucose (15–35 mM) but not with dihydroxyacetone (10 mM). The inhibition of glycolysis at elevated glucose concentration was associated with inhibition of glucose-induced dissociation of glucokinase and aldolase. The resorcinol concentration that caused half-maximal inhibition (20–43 μ M) increased with increasing glucose concentration (15–35 mM). Resorcinol inhibited the translocation of glucokinase and the stimulation of detritiation of [2- 3 H]glucose and [3- 3 H]glucose caused by sorbitol (10–200 μ M), but it potentiated the stimulation of glycogen synthesis. The inhibition of glycolysis by resorcinol could not be accounted for by diversion of substrate

to glycogen. The glucose 6-phosphate content correlated with the free glucokinase activity. Resorcinol counteracted the increase in glucose 6-phosphate and fructose 2,6-bisphosphate caused by elevated glucose concentration or by sorbitol. The suppression of glucose 6-phosphate at high glucose concentration (15–35 mM) could be explained by the low activity of free glucokinase. However, the suppression at 5 mM glucose was due in part to an independent mechanism. The effect of resorcinol on glucokinase translocation was partly counteracted by galactosamine, which suppresses UDP-glucose and inhibits glucuronide formation, and was mimicked by phenol and *p*-nitrophenol but not by *p*-nitrophenylglucuronide. It is concluded that resorcinol inhibits glycolysis at elevated glucose concentration or when stimulated by sorbitol through increased glucokinase binding. The results indicate a link between glucuronidation and glucokinase translocation.

INTRODUCTION

Phenacyl imidazolium compounds have anti-hyperglycaemic effects in animal models of non-insulin-dependent diabetes mellitus [1,2]. Proglycosyn (LY177507), a representative of these compounds, stimulates glycogen synthesis and inhibits glucose production from various substrates in rat hepatocytes [3]. Other effects of proglycosyn in hepatocytes include inhibition of glycolysis from exogenous glucose and endogenous glycogen, the inhibition of fatty acid synthesis, stimulation of fatty acid oxidation and a lowering of the concentration of fructose 2,6-bisphosphate [3–7]. The inhibition of gluconeogenesis is associated with sequential inactivation of phosphorylase and activation of glycogen synthase, and has been explained by diversion of glucose 6-phosphate derived from gluconeogenic precursors to glycogen [3,4]. Likewise, the inhibition of glycolysis from glucose is presumed to be due to metabolite diversion to glycogen [3,7].

Recent studies on the mechanism of action of proglycosyn have shown that resorcinol and other phenol derivatives mimic the effect of proglycosyn on the stimulation of glycogen synthesis and the lowering of the fructose 2,6-bisphosphate content [8,9]. The effect of these compounds on glycogen metabolism was paralleled by the intracellular accumulation of their glucuronide derivatives and was counteracted by galactosamine, which inhibits glucuronide formation. It was thus inferred that the sequential inactivation of phosphorylase and activation of synthase is mediated by the glucuronidated metabolites of these compounds. Of the compounds tested, resorcinol was the most potent at stimulating glycogen synthesis [8]. This effect of resorcinol is due, at least in part, to inhibition of phosphorylase kinase by the glucuronidated metabolite [9]. The decrease in fructose 2,6-bisphosphate content caused by resorcinol (unlike that caused by glucagon) is not associated with inactivation of 6-

phosphofructo-2-kinase and was suggested to be due to a decrease in glycogenolysis and an increase in glycogen synthesis as well as to the consumption of hexose phosphate by glucuronidation [9].

The present study investigated the effects of resorcinol on glucokinase translocation and aldolase binding in hepatocytes. The results demonstrate that resorcinol is a potent inhibitor of glucokinase translocation induced by elevated glucose concentration or sorbitol, and that the inhibition by resorcinol of glycolysis and the suppression of glucose 6-phosphate and fructose 2,6-bisphosphate at elevated glucose concentration are due to inhibition of glucokinase translocation, whereas the suppression of glucose 6-phosphate by resorcinol at 5 mM glucose is in part due to an independent mechanism. The results indicate a link between glucuronidation and glucokinase translocation.

MATERIALS AND METHODS

Materials

[2- 3 H]Glucose and [3- 3 H]glucose were from Amersham International (Little Chalfont, Bucks., U.K.) or from New England Nuclear (Boston, MA, U.S.A.) and were purified before use [10]. [U- 14 C]Glucose was from New England Nuclear. Resorcinol, *m*-nitrophenol, *p*-nitrophenol, *p*-nitrophenylglucuronide and Amberlite IRA-400 were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sources of other materials were as described previously [10].

Hepatocyte isolation and monolayer culture

Hepatocytes were isolated by collagenase perfusion of the liver [11] from male Wistar rats (body weight 160–210 g) fed *ad libitum*. They were suspended in minimum essential medium

containing 5% (v/v) neonatal calf serum, inoculated in 24-well plates at a density of 6×10^4 cells/cm² and incubated at 37 °C equilibrated with air/CO₂ (19:1). After attachment (approx. 4 h) the hepatocytes were cultured for 16 h in serum-free medium containing 10 nM dexamethasone [10]. Experiments were performed after between 16 and 20 h of culture. Unless indicated otherwise, the minimum essential medium contained 5 mM glucose.

Determination of glucose metabolism, glycogen synthesis and lactate formation

The hepatocytes were incubated in minimum essential medium (250 µl per well) with the glucose concentration and other additions indicated, plus [2-³H]glucose, [3-³H]glucose or [U-¹⁴C]glucose (4.5 µCi/ml ³H or 1.5 µCi/ml ¹⁴C). Incubations were for between 90 min and 3 h as indicated. On termination of the incubations the medium was collected for determination of ³H₂O and lactate, and the hepatocyte monolayers were washed in 150 mM NaCl and extracted in 0.1 M NaOH for determination of label incorporation into glycogen by precipitation with ethanol [11]. For determination of ³H₂O formation from [3-³H]glucose, 50 µl aliquots of culture medium were added to 1.0 ml volumes of Aberlite IRA-400 (borate form) and equilibrated for 10 min, after which 200 µl of supernatant was removed and the resin was washed twice with 500 µl of water. The washes were pooled and the radioactivity was determined in 5 ml of scintillation cocktail (Ultima Gold, Packard) with a Tri-Carb 2700TR Beta-counter (Packard). Cell protein was determined [12]; rates of glycogen synthesis ([U-¹⁴C]glucose incorporation into glycogen) and detritiation of glucose (formation of ³H₂O) are expressed as nmol of glucose metabolized/mg of cell protein during the incubation. The accumulation of lactate in the medium was determined enzymically [13] and is expressed as nmol/ml.

Permeabilization of hepatocytes with digitonin for determination of free and bound activities of glucokinase and aldolase

In experiments where both glucokinase and aldolase binding were determined (see Figure 1) the permeabilization medium contained 300 mM sucrose, 2 mM MgCl₂, 3 mM Hepes, 2 mM dithiothreitol and 75 µg/ml digitonin, pH 7.2, as described previously [14]. In experiments where only glucokinase binding was determined, the permeabilization medium contained 300 mM sucrose, 5 mM MgCl₂, 3 mM Hepes, 2 mM dithiothreitol and 37.5 µg/ml digitonin, pH 7.2, as described previously [10]. The hepatocyte monolayers were washed once with 150 mM NaCl and then incubated with 300 µl of permeabilization medium for 8 min. The plate was then shaken gently and the medium removed for determination of free enzyme activity. The residual cell matrix was extracted as described previously [10,14] for determination of the bound enzyme activity. Glucokinase [10] and aldolase [14] activity were determined as described previously, and the enzyme activity released during permeabilization with digitonin (free activity) is expressed as a percentage of total (free + bound) activity [10,14]. Substrates and inhibitors altered the distribution but not the total enzyme activity. Total glucokinase activity was between 8 and 14 m-units/mg of protein and aldolase activity was between 30 and 40 m-units/mg of protein, where 1 m-unit is the amount of enzyme converting 1 nmol of substrate/min at 37 °C.

Determination of intermediary metabolites

Hepatocytes were extracted in 3% HClO₄ for determination of glucose 6-phosphate and UDP-glucose and in 50 mM NaOH for

fructose 2,6-bisphosphate. Glucose 6-phosphate and UDP-glucose were determined fluorimetrically in assay cocktail containing 50 mM Tris/HCl, pH 8.0, and either 80 µM NADP, 0.025 unit/ml glucose 6-phosphate dehydrogenase and 1 unit/ml 6-phosphogluconate dehydrogenase (glucose 6-phosphate), or 200 µM NAD, 0.5 mM EDTA and 0.025 unit/ml UDP-glucose dehydrogenase (UDP-glucose). Fructose 2,6-bisphosphate was determined as described previously [15].

Expression of results

Results are expressed as means ± S.E.M. for the numbers of experiments (hepatocyte preparations) indicated. Statistical analysis was by Student's paired *t* test.

RESULTS

Effects of resorcinol on glucokinase and aldolase binding and lactate formation at varying glucose concentrations

Glucokinase and aldolase reversibly partition between bound and free states in the hepatocyte. Maximum binding occurs at low extracellular glucose concentration [10,14]. Glucokinase binds to its 68 kDa regulatory protein [16], which inhibits its activity [17], and aldolase binds to microfilaments and the bound state is also a kinetically less active state [18].

Resorcinol (150 µM) inhibited the effects of increasing glucose concentration on translocation of bound glucokinase and dissociation of aldolase, determined from the enzyme released during permeabilization of the hepatocytes with digitonin (Figures 1A and 1B); it also inhibited the detritiation of [2-³H]glucose and the formation of lactate (Figures 1C and 1D). The effects of resorcinol concentration are shown in Figure 2. The IC₅₀ increased with increasing glucose concentration, being 12, 15 and 20 µM for inhibition of lactate formation and 20, 28 and 43 µM for inhibition of glucokinase translocation at 15, 25 and 35 mM glucose respectively. The higher proportion of bound glucokinase and aldolase in the presence of resorcinol indicates a higher fraction of kinetically less active enzyme.

Lactate formation from dihydroxyacetone

In contrast with the inhibition of lactate formation from 15 to 35 mM glucose, resorcinol (150 µM) did not inhibit lactate formation in incubations with 5 mM glucose and 10 mM dihydroxyacetone as substrate (control, 305 ± 35 nmol/h per mg; resorcinol, 294 ± 43 nmol/h per mg; *n* = 6). In these incubations, resorcinol did not inhibit aldolase dissociation (control, $62 \pm 6\%$; resorcinol, $62 \pm 6\%$). The failure of resorcinol to inhibit lactate formation and aldolase dissociation with dihydroxyacetone indicates a site of action on glycolysis at or before the aldolase step.

Effects of resorcinol on glucose 6-phosphate and fructose 2,6-P₂ content

Studies on hepatocyte suspensions have shown that resorcinol lowers the content of glucose 6-phosphate and fructose 6-phosphate (which is in equilibrium with glucose 6-phosphate) and it also lowers the fructose 2,6-bisphosphate content [9]. The lowering of glucose 6-phosphate content was attributed to the inactivation of phosphorylase and the activation of glycogen synthase [9]. Because binding of glucokinase to its regulatory protein is potentiated by fructose 6-phosphate [17], the question arises whether the inhibition of glucokinase translocation by resorcinol is secondary to changes in hexose phosphate content

Figure 1 Effects of resorcinol on enzyme binding and glucose metabolism at various glucose concentrations

Hepatocytes were incubated for 90 min with [²⁻³H]glucose at the glucose concentrations indicated in the absence (○) or presence (●) of 150 μM resorcinol. On termination of the incubations the hepatocytes were permeabilized with digitonin for determination of free and bound enzyme activities. The free activity of glucokinase (A) and aldolase (B) are expressed as a percentage of total activity. Detritiation of [²⁻³H]glucose (C) is expressed as nmol/90 min per mg of protein and the accumulation of lactate in the medium (D) is expressed as nmol/ml. Values are means ± S.E.M. for six (A, B, C) or four (D) experiments. **P* < 0.05; ***P* < 0.005; resorcinol compared with respective control (paired *t* test).

Figure 2 Effects of resorcinol concentration on glucokinase binding and lactate formation

Hepatocytes were incubated for 90 min with the concentrations of resorcinol indicated, at 15, 25 or 35 mM glucose as shown in parentheses. For further details see the legend to Figure 1. Values are means ± S.E.M. for four experiments. Effects of resorcinol of 50 μM or greater were significantly different (*P* < 0.05) from corresponding control values.

or alternatively whether changes in hexose phosphate content are the consequence of changes in glucokinase binding because the free form of glucokinase represents the active form of the enzyme. To distinguish between these alternative possibilities the effects of resorcinol on glucose 6-phosphate content were determined.

There was an increase in glucose 6-phosphate and fructose 2,6-bisphosphate contents with increasing glucose concentration

(Figures 3A and 3B). Resorcinol (150 μM) suppressed (*P* < 0.05) the contents of both metabolites. Because a decrease in hexose phosphate content would be expected to increase the free glucokinase activity (contrary to the decrease observed in Figure 1A), the results establish that the inhibition of glucokinase translocation by resorcinol cannot be secondary to the observed change in hexose phosphate content. Figure 3(C) shows a correlation between glucose 6-phosphate content and glucokinase translocation (free glucokinase activity) in both the absence (○) and presence (●) of resorcinol. Because the free glucokinase represents the active form of the enzyme (which is not inhibited by the regulatory protein), the correlation suggests that the lowering of glucose 6-phosphate content by resorcinol at elevated glucose concentration can be explained by the inhibition of glucokinase translocation. The relation between fructose 2,6-bisphosphate and glucose 6-phosphate levels (Figure 3D) suggests that the lowering of fructose 2,6-bisphosphate content might be secondary to the changes in glucose 6-phosphate content.

Counteraction by resorcinol of the stimulation of glucose metabolism by sorbitol

Dissociation of glucokinase from its regulatory protein and its translocation [16,17] are induced by precursors of fructose 1-phosphate (fructose and sorbitol) [19,20]. To investigate further the mechanism by which resorcinol inhibits glucokinase translocation the effects of resorcinol on the stimulation of glucokinase translocation and glucose metabolism by sorbitol (10 μM to 1 mM) were determined. During incubation with 5 mM glucose, 150 μM resorcinol inhibited (*P* < 0.05) the effects of sorbitol (10–200 μM) on glucokinase translocation, the detritiation of [²⁻³H]glucose, and glucose 6-phosphate and fructose 2,6-bisphosphate levels (Figure 4). The inhibition of detritiation of [²⁻³H]glucose by resorcinol could be explained by inhibition of glucokinase translocation, as shown by the correlation between detritiation and free glucokinase activity (Figure 5A). There was a correlation between glucose 6-phosphate level and glucokinase translocation with increasing sorbitol concentration in both the absence and presence of resorcinol (Figure 5B). However, unlike detritiation of glucose, these correlations diverged at low sorbitol concentration, indicating that the suppression of glucose 6-phosphate content by resorcinol is due in part to the inhibition of glucokinase translocation and in part to an independent

Figure 3 Effects of resorcinol on glucose 6-phosphate and fructose 2,6-bisphosphate levels at various glucose concentrations

Hepatocytes were incubated for 60 min with the glucose concentrations indicated in the absence (○) or presence (●) of 150 μM resorcinol. Glucose 6-phosphate, fructose 2,6-bisphosphate, and glucokinase binding were determined in parallel incubations. (A) Glucose 6-phosphate; (B) fructose 2,6-bisphosphate; (C) glucose 6-phosphate compared with free glucokinase activity; (D) fructose 2,6-bisphosphate compared with glucose 6-phosphate. Values are means $\pm$ S.E.M. for four experiments. * P < 0.05; ** P < 0.005; resorcinol compared with respective control (paired t test).

Figure 4 Effects of resorcinol at increasing sorbitol concentrations on glucokinase binding, glucose metabolism and glucose 6-phosphate and fructose 2,6-bisphosphate contents

Hepatocytes were incubated for 90 min with the concentrations of sorbitol indicated in the absence (○) or presence (●) of 150 μM resorcinol. (A) Free glucokinase (percentage of total activity); (B) detritiation of [2-³H]glucose (nmol/90 min per mg of protein); (C) glucose 6-phosphate content (nmol/mg); (D) fructose 2,6-bisphosphate content (pmol/mg). Values are means $\pm$ S.E.M. for six experiments. * P < 0.05; ** P < 0.005; resorcinol compared with respective control (paired t test).

Figure 5 Effects of resorcinol at increasing sorbitol concentration on glucose metabolism and glucose 6-phosphate and fructose 2,6-bisphosphate contents

Data are from Figure 4, without resorcinol (○) and with 150 μM resorcinol (●). (A) Detritiation of [2-³H]glucose (nmol/90 min per mg of protein) compared with free glucokinase (percentage of total activity); (B) glucose 6-phosphate content (nmol/mg) compared with free glucokinase (percentage of total activity); (C) fructose 2,6-bisphosphate content (pmol/mg protein) compared with glucose 6-phosphate content (nmol/mg). Values are means $\pm$ S.E.M. for six experiments.

Figure 6 Effects of resorcinol at increasing sorbitol concentration on glycolysis and glycogen synthesis

Hepatocytes were incubated for 2 h with 10 mM glucose and the concentrations of sorbitol indicated either in the absence (○) or presence of 25 (▲) or 150 (■) μM resorcinol (Res). Rates of detritiation of [3-³H]glucose (A) and incorporation of [U-¹⁴C]glucose into glycogen (C) are expressed as nmol of glucose metabolized/2 h per mg of protein, and lactate accumulation (B) is expressed as nmol/ml. Values are means for three to four experiments. **P* < 0.05; ***P* < 0.005; resorcinol compared with respective control (paired *t* test). Effects of resorcinol on lactate formation are significant (*P* < 0.001).

Table 1 Interactions of galactosamine and resorcinol on glucokinase translocation and glycolysis

Hepatocytes were preincubated without or with 5 mM galactosamine and 5 mM glucose for 30 min. They were then incubated for 60 min with the glucose concentrations indicated either without or with 150 μM resorcinol. Lactate accumulation in the medium is expressed as nmol/ml of medium and the free glucokinase activity as a percentage of total activity. Values are means ± S.E.M. for six (glucokinase) or three (lactate) experiments: **P* < 0.05, ***P* < 0.005 relative to control with no additions; †*P* < 0.05, ††*P* < 0.005 relative to resorcinol alone; ‡*P* < 0.05 relative to galactosamine alone.

Additions	Free glucokinase (% of total)			Lactate accumulation (nmol/ml)		
	[Glucose] (mM) ... 5	15	25	5	15	25
None	22 ± 3	43 ± 2	56 ± 3	62 ± 12	131 ± 24	201 ± 27
150 μM resorcinol	22 ± 3	33 ± 2**	42 ± 2**	59 ± 15	74 ± 13*	105 ± 13*
Galactosamine	23 ± 3	44 ± 4	59 ± 3	60 ± 11	123 ± 18	188 ± 29
Galactosamine + 150 μM resorcinol	24 ± 3	45 ± 3††	55 ± 3††‡	54 ± 12	97 ± 20*‡	149 ± 26*†‡

mechanism that predominates at 5 mM glucose in the absence of sorbitol. The relation between fructose 2,6-bisphosphate and glucose 6-phosphate levels (Figure 5C) was similar to that at varying glucose concentration (Figure 3D), suggesting that changes in fructose 2,6-bisphosphate content are probably secondary to changes in glucose 6-phosphate content.

Effects of resorcinol on glycogen synthesis

The inhibition of lactate formation by resorcinol at 15–35 mM glucose (Figure 1) cannot be explained by the diversion of glucose to glycogen, because resorcinol stimulated (*P* < 0.05) glucose conversion to glycogen at 10 mM glucose (control, 23 ± 2 nmol/3 h per mg of protein; 25 μM resorcinol, 35 ± 3 nmol/3 h per mg; 150 μM resorcinol, 34 ± 3 nmol/3 h per mg; *n* = 3) but not at 30 mM glucose (control, 129 ± 10 nmol/3 h per mg; 25 μM resorcinol, 140 ± 8 nmol/3 h per mg; 150 μM resorcinol, 141 ± 10 nmol/3 h per mg). Resorcinol (150 μM) inhibited the detritiation of [3-³H]glucose (*P* < 0.05) at both 10 mM glucose (189 ± 17 to 80 ± 11 nmol/3 h per mg) and 30 mM glucose (554 ± 69 to 389 ± 46 nmol/3 h per mg) and this inhibition was greater than the increase in glycogen synthesis.

Figure 6 shows the effects of 25 or 150 μM resorcinol on glycolysis (lactate formation and detritiation of [3-³H]glucose) and glycogen synthesis in the presence of sorbitol (10–200 μM).

The sorbitol-induced translocation of glucokinase is associated with an increase in lactate formation, detritiation of [3-³H]glucose and glycogen synthesis. Resorcinol suppressed the stimulation of lactate formation and detritiation of [3-³H]glucose (Figures 6A and 6B) but it further stimulated glycogen synthesis (Figure 6C). The stimulation of glycogen synthesis was greater at 25 μM than at 150 μM resorcinol (*P* < 0.05), whereas the former inhibitory effects were greater at 150 μM resorcinol (*P* < 0.05). This further indicates that the inhibition of glycolysis cannot be explained by the diversion of substrate to glycogen.

Mechanism of action of resorcinol

The effect of resorcinol and other phenols on phosphorylase and glycogen synthase has been suggested to be mediated by glucuronidated derivatives of these compounds [8,9]. It was shown that galactosamine (5 mM) inhibited the conversion of phenol to its glucuronide in addition to its effects on phosphorylase and glycogen synthase [8]. To determine whether glucuronide formation is involved in the inhibition of lactate formation and glucokinase translocation by resorcinol, the effects of galactosamine were investigated. Preincubation of hepatocytes with 5 mM galactosamine partly counteracted the effects of 150 μM resorcinol on glucokinase translocation and on lactate formation (Table 1). In these experiments galactosamine lowered (*P* <

Table 2 Effects of phenol derivatives on glucokinase translocation

Hepatocytes were incubated for 60 min in minimal essential medium containing 5, 15 or 25 mM glucose and the additions indicated. On termination of the incubations, the hepatocytes were permeabilized with digitonin and the free and bound activities of glucokinase were determined. Free activity is expressed as a percentage of total activity. Values are means $\pm$ S.E.M. for three or four experiments; * $P < 0.05$ relative to no additions.

Additions	[Glucose] (mM)...	Free glucokinase (% of total)		
		5	15	25
None		20 $\pm$ 1	44 $\pm$ 4	61 $\pm$ 6
Resorcinol (150 μ M)		20 $\pm$ 1	31 $\pm$ 2*	41 $\pm$ 4*
Phenol (200 μ M)		20 $\pm$ 3	35 $\pm$ 6*	46 $\pm$ 6*
<i>m</i> -Nitrophenol (100 μ M)		22 $\pm$ 2	33 $\pm$ 4*	44 $\pm$ 5*
<i>p</i> -Nitrophenol (100 μ M)		17 $\pm$ 1	26 $\pm$ 3*	36 $\pm$ 4*
<i>p</i> -Nitrophenylglucuronide (200 μ M)		19 $\pm$ 2	44 $\pm$ 6	62 $\pm$ 9

0.01) the content of UDP-glucose (control, 2.57 ± 0.09 nmol/mg of protein; 5 mM galactosamine, 0.58 ± 0.14 nmol/mg; $n = 3$). Lower concentrations of galactosamine (0.5 and 1 mM) were less effective ($n = 5$, results not shown) and when galactosamine (5 mM) was added simultaneously with resorcinol it did not counteract the inhibition of glucokinase translocation ($n = 4$, results not shown), suggesting that resorcinol is metabolized rapidly by hepatocytes.

It has been suggested that the lowering of glucose 6-phosphate content by resorcinol might be due in part to the depletion of hexose phosphate by glucuronidation [9]. The effects of resorcinol on the UDP-glucose content were therefore determined. The inhibition of glucokinase translocation by resorcinol at various glucose concentrations (15–35 mM) was not associated with a change in total cellular UDP-glucose content (5 mM glucose, 2.67 ± 0.15 compared with 2.73 ± 0.13 nmol of UDP-glucose/mg of protein; 15 mM glucose, 2.70 ± 0.18 compared with 2.73 ± 0.16 nmol/mg; 25 mM glucose, 2.77 ± 0.18 compared with 2.67 ± 0.17 nmol/mg; 35 mM glucose, 2.55 ± 0.18 compared with 2.70 ± 0.17 nmol/mg; control compared with 150 μ M resorcinol; $n = 6$), suggesting that the decrease in hexose phosphate content cannot be explained by a decrease in total UDP-glucose content.

Table 2 shows the effects on glucokinase translocation of other phenol derivatives that are metabolized by glucuronidation. Phenol (0.2 mM), *m*-nitrophenol (0.1 mM) and *p*-nitrophenol (0.1 mM), like resorcinol, inhibited glucokinase translocation. However, *p*-nitrophenylglucuronide (0.2 mM) was ineffective (Table 2). These compounds did not cause leakage of lactate dehydrogenase into the medium or depletion of cellular ATP (results not shown).

DISCUSSION

Proglycosyn and resorcinol inhibit glycolysis from endogenous glycogen and exogenous glucose [3,8] and lower the fructose 2,6-bisphosphate content of hepatocytes [5,9]. Unlike the inhibition of glycolysis by glucagon, these effects are not associated with inactivation of phosphofructo-2-kinase [9]. Because these compounds also decreased the hexose 6-phosphate content of hepatocytes it was suggested that the suppression of fructose 2,6-bisphosphate might be explained by the decrease in hexose 6-phosphate content resulting from decreased glycogenolysis, increased glycogen synthesis and the consumption of hexose phosphate by glucuronidation [9]. However, the precise mechanism by which these compounds lower fructose 2,6-

bisphosphate or hexose phosphate levels remains unestablished [21].

The present study shows that resorcinol is a potent inhibitor of glucokinase translocation; it counteracts the stimulation caused by elevated glucose concentration (15–35 mM) and by sorbitol (10–200 μ M). Resorcinol also inhibits the dissociation of aldolase caused by elevated glucose concentration but not that caused by dihydroxyacetone. Aldolase dissociation is induced by substrates that are precursors of fructose 1,6-bisphosphate (glucose and mannose), fructose 1-phosphate (fructose and sorbitol) or triose phosphate (glycerol and dihydroxyacetone), and the effect of glucose is blocked by glucokinase inhibitors (*D*-mannoheptulose and *D*-glucosamine), indicating that it is due to metabolites distal to glucokinase [14]. The inhibition by resorcinol of lactate formation and aldolase dissociation by glucose (15–35 mM) but not by dihydroxyacetone establishes a site of action of resorcinol on glycolysis before the aldolase step.

The involvement of glucokinase translocation in the inhibition of glycolysis by resorcinol is supported by the following observations. First, in incubations at various glucose concentrations, resorcinol caused parallel inhibition of glucokinase translocation, glucose phosphorylation, aldolase dissociation and lactate formation (Figure 1). Secondly, for both glycolysis and glucokinase translocation the sensitivity to resorcinol decreased with increasing glucose concentration (Figure 2). Thirdly, in incubations with sorbitol (10–200 μ M), resorcinol inhibited glucokinase translocation, detritiation of [2- 3 H]glucose and [3- 3 H]glucose, and lactate formation (Figures 4 and 6). Fourthly, galactosamine partly counteracted the effects of resorcinol on both glucokinase translocation and lactate formation (Table 1). Finally, the inhibition of lactate formation or detritiation of [3- 3 H]glucose by resorcinol could not be explained by diversion of glucose 6-phosphate to glycogen.

It could be argued that these observations are also consistent with a hypothesis whereby resorcinol inhibits glycolysis by an independent mechanism and the change in glucokinase binding might be secondary to changes in metabolite levels as a result of other mechanisms. Glucokinase partitions between a bound (inactive) state in association with its regulatory protein and a dissociated or translocated state [16,17,22]. Binding of glucokinase to its regulatory protein is potentiated by fructose 6-phosphate, which is in equilibrium with glucose 6-phosphate [17]. Studies on isolated hepatocyte suspensions incubated with glucagon and gluconeogenic precursors showed that glucose phosphorylation by glucokinase decreased with increasing glucose 6-phosphate levels, suggesting increased glucokinase binding to its regulatory protein at increasing glucose 6-phosphate levels, although binding was not measured in that study [23]. However, the present study shows that when hepatocytes are incubated with substrates that induce glucokinase translocation (sorbitol and elevated glucose concentration) or with resorcinol, which inhibits glucokinase translocation, the glucose 6-phosphate content shows a direct correlation with the free glucokinase activity (Figures 3C and 5B) or an inverse correlation with bound activity. The most plausible explanation for a direct correlation between glucose 6-phosphate and free glucokinase is that the changes in glucose 6-phosphate are the consequence and not the cause of changes in free glucokinase, which is the active form of the enzyme [16,17]. It seems likely from these observations that in hepatocytes incubated with elevated glucose concentrations or sorbitol, fructose 6-phosphate does not have a dominant role in preserving binding of glucokinase to its regulatory protein and/or that the effects of glucose and fructose 1-phosphate (formed from sorbitol [20]), which cause dissociation [19], predominate over the effect of fructose 6-phosphate.

The correlation between glucokinase and glucose 6-phosphate levels at elevated glucose concentrations (Figure 3C) suggests that the lowering of glucose 6-phosphate content by resorcinol (at high glucose concentrations) is predominantly or exclusively due to the inhibition of glucokinase translocation, whereas the correlations at varying sorbitol concentrations in the presence of 5 mM glucose (Figure 5B) indicate that the lowering of glucose 6-phosphate content in these conditions is due in part to the inhibition of glucokinase translocation and in part to an independent mechanism, probably representing the inactivation of phosphorylase [8]. Thus at glucose concentrations above 15 mM, glucokinase is the exclusive source of glucose 6-phosphate, whereas at 5 mM glucose and varying sorbitol concentrations (10–50 μ M) phosphorylase is a contributing source of glucose 6-phosphate. It is noteworthy that resorcinol did not lower the UDP-glucose content; it therefore seems unlikely that the consumption of UDP-glucose by glucuronidation contributes to the decrease in glucose 6-phosphate content by resorcinol, although this possibility cannot be excluded. At low glucose concentration (5 mM), when the decrease in glucose 6-phosphate content caused by resorcinol is greater than can be explained by decreased glucokinase dissociation (Figure 3C), there was also a decrease in fructose 2,6-bisphosphate content (Figure 3B). Under these experimental conditions (5 mM glucose) resorcinol causes a greater inhibition of detritiation of [3-³H]glucose than of [2-³H]glucose (results not shown). This inhibition of glycolysis can be explained by the lower levels of fructose 2,6-bisphosphate, which in turn are related to the lower glucose 6-phosphate content (Figure 3D).

The correlation between glucose 6-phosphate and free glucokinase levels in the presence of compounds that stimulate (glucose and sorbitol) or inhibit (resorcinol) glucokinase translocation is of physiological interest because glucose 6-phosphate is an activator of glycogen synthase [24] and of glycolysis via an increase in fructose 2,6-bisphosphate content [25] and would thus act as an amplification mechanism for both pathways. The high flux-control coefficient of glucokinase for glycogen synthesis is consistent with amplification mechanisms [22]. The direct correlation between free glucokinase and glucose 6-phosphate levels is also of interest because it is the converse of that observed with gluconeogenic precursors [23]. Whether the glucose 6-phosphate in the latter situation might be in a different subcellular compartment is unclear. Evidence that glucose 6-phosphate derived from glucokinase is more effective than that from hexokinase I in activating glycogen synthase supports a role for compartmentation of glucose 6-phosphate in the hepatocyte [24]. The possibility that changes in total glucose 6-phosphate content as determined analytically might not reflect changes in glucose 6-phosphate content in the relevant subcellular compartment, or that glucose 6-phosphate might affect glucokinase translocation by additional as yet unidentified mechanisms, cannot be excluded.

The finding that galactosamine, which lowers UDP-glucose content and inhibits glucuronidation [8], counteracted the inhibition of both glucokinase translocation and glycolysis indicates that the glucuronidation of resorcinol is necessary not only for its effects on glycogen metabolism [8,9] but also for the inhibition of glucokinase translocation and glycolysis. Because glucokinase translocation was inhibited by various phenol derivatives but not by *p*-nitrophenylglucuronide, it seems likely that flux through glucuronidation (and/or metabolites that are affected by glucuronidation) rather than the glucuronide product is responsible for the inhibition. One possibility is that increased flux through UDP-glucose dehydrogenase (and/or reduction of NAD to NADH) might affect glucokinase translocation by an indirect or coupled mechanism.

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