INHIBITION OF GLUCURONIDE SYNTHESIS BY PHYSIOLOGICAL METABOLITES IN LIVER SLICES

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1. Introduction

The synthesis of glucuronides (β-D-glucopyranosiduronic acids) is catalyzed by rather poorly characterized UDPglucuronyltransferase(s?) (UDPglucuronate glucuronyltransferase (acceptor unspecific), (EC 2.4.1.17). No specific inhibitor is known, which could be used to block the glucuronide synthesis in living cells. The glucuronide synthesis is insensitive to the various metabolites of the glucuronic acid pathway [1].

The synthesis of the glucuronyl donor, UDPglucuronic acid, by UDPglucose dehydrogenase (UDPglucose: NAD oxidoreductase, EC 1.1.1.22) can, however, be inhibited effectively at least *in vitro*, by UDPgalactose [2] and UDPxylose [3]. Furthermore, the level of UDPglucose, which is a precursor of UDPglucuronic acid, can be lowered in the liver by perfusion with a D-galactosamine containing medium [4].

In this study data are presented, which indicate that UDPgalactose, D-galactose, UDPxylose and D-galactosamine can be used to block glucuronide synthesis from phenolic aglycone in liver slices. D-galactose, UDPxylose and D-galactosamine have no effect on the oxygen uptake of the slices. None of these compounds inhibited the glucuronide synthesis in isolated microsomal membranes.

2. Experimental procedures

Twenty male Wistar rats (three months old) were used. The *o*-aminophenyl-β-D-glycopyranosiduronic acid synthesis was followed in 0.25 mm thick liver slices (Mickle Laboratory Engineering-microtome) incubated in D-glucose fortified Ringer-bicarbonate medium as described previously [5]. The total volume of the reaction mixture in conical centrifuge tubes was, however, 0.4 ml and the incubation was carried out (under 95 percent oxygen, 5 percent carbon dioxide atmosphere) in a shaker oscillating (27 mm) 150 times per minute. The respiration of slices in Ringer-phosphate medium (total volume 2.1 ml) was determined by using the direct Warburg method [6]. The microsomal fraction of the rat liver was isolated, treated with digitonin and used in studies of *p*-nitrophenyl-β-D-glucopyranosiduronic acid biosynthesis as described previously [1].

The uridine diphosphate sugars and UDPglucose dehydrogenase (type III, from bovine liver, 140,000 units/g) were purchased from Sigma Chemical Company.

3. Results

UDPgalactose caused a marked inhibition of glucuronide synthesis in liver slices (fig. 1A), when it was added to the incubation medium at a concentration ten times the K_i value for UDPglucose dehydrogenase inhibition *in vitro* [3]. Its effect was completely reversible. After transfer of the slices to fresh medium lacking UDPgalactose, glucuronide synthesis took place at the same rate as in slices incubated from the very beginning in a UDPgalactose-free medium. UDPgalactose did not inhibit glucuronide synthesis *in vitro* catalyzed by the isolated liver microsomal fraction (table 2).

The UDPgalactose precursor, D-galactose also inhi-

Table 1
The effect of D-galactose, UDPxylose and D-galactosamine on the respiration of rat liver slices.

Metabolite (mM)	O_2 uptake (mmoles/min × g (dry weight)	CO_2 release	RQ
Control	5.8	3.3	0.57
D-Galactose (10 mM)	5.8	3.2	0.55
UDPxylose (0.4 mM)	5.6	3.3	0.59
D-Galactosamine			
(1.0 mM)	5.6	2.9	0.52
(10 mM)	5.8	3.0	0.52

Table 2
The effect of UDPgalactose, D-galactose, UDPxylose and D-galactosamine on the *p*-nitrophenyl-β-D-glucopyranosiduronic acid synthesis by isolated (untreated and digitonin treated) microsomal fraction of rat liver.

Metabolite (mM)	*p*-Nitrophenyl-β-D-glucopyranosiduronic acid synthesized (μmoles/min × g of protein)	
	Untreated	Digitonin treated
Control	0.26	2.4
UDPgalactose (1.5 mM)	0.27	2.4
D-Galactose (10 mM)	0.26	2.4
UDPxylose (0.4 mM)	0.27	2.5
D-Galactosamine (10 mM)	0.27	2.6

bited glucuronide synthesis in liver slices, and this inhibition was reversible (fig. 1B). It did not inhibit the oxygen uptake of the slices (table 1), or the UDPglucuronyltransferase activity of the isolated microsomal fraction of rat liver (table 2). D-galactose (10 mM) slightly inhibited (15%) purified UDPglucose dehydrogenase, but not the glucuronide synthesis by the microsomal fraction in a medium generating UDP-glucuronic acid from UDPglucose with the aid of UDP-glucose dehydrogenase (table 3).

Table 3
The effect of D-galactose and D-galactosamine on the *p*-nitrophenyl-β-D-glucopyranosiduronic acid synthesis by the digitonin treated microsomal fraction of rat liver in the presence of UDPglucuronic acid generating system (UDPglucose 1.7 mM, NAD 0.8 mM, and UDPglucose dehydrogenase 35 units per reaction mixture). The microsomal fraction was added after a 30 min preincubation period.

Metabolite (mM)	*p*-Nitrophenyl-β-D-glucopyranosiduronic acid synthesized (μmoles/min × g of protein)
Control	0.60
D-Galactose (10 mM)	0.62
D-Galactosamine (mM)	0.63

The potent UDPglucose dehydrogenase inhibitor, UDPxylose, also effectively inhibited glucuronide synthesis in liver slices (fig. 2 A). A prolonged depression of glucuronide synthesis was observed in slices even after transfer into a UDPxylose-free medium in contrast to the inhibition caused by UDPgalactose and D-galactose (fig. 2 B). UDPxylose had no effect on the respiration of the slices (table 1). It was also without an effect on glucuronide synthesis in a reaction mixture containing UDPglucuronic acid and untreated or digitonin treated rat liver microsomal fractions (table 2).

In experiments with liver slices, D-galactosamine was also a potent inhibitor of glucuronide synthesis. Its effect was concentration dependent and irreversible, during the incubation times used (fig. 3A and 3B). It neither depressed the oxygen uptake of the slices (table 1), nor had any effect on glucuronide synthesis by microsomal fractions incubated with UDPglucuronic acid (table 2). D-galactosamine (10 mM) caused about 15 percent inhibition of purified UDPglucose dehydrogenase. It was, however, without any effect on glucuronide synthesis catalyzed by the isolated liver microsomal fraction, when a UDPglucuronic acid generating system was added instead of UDPglucuronic acid (table 3).

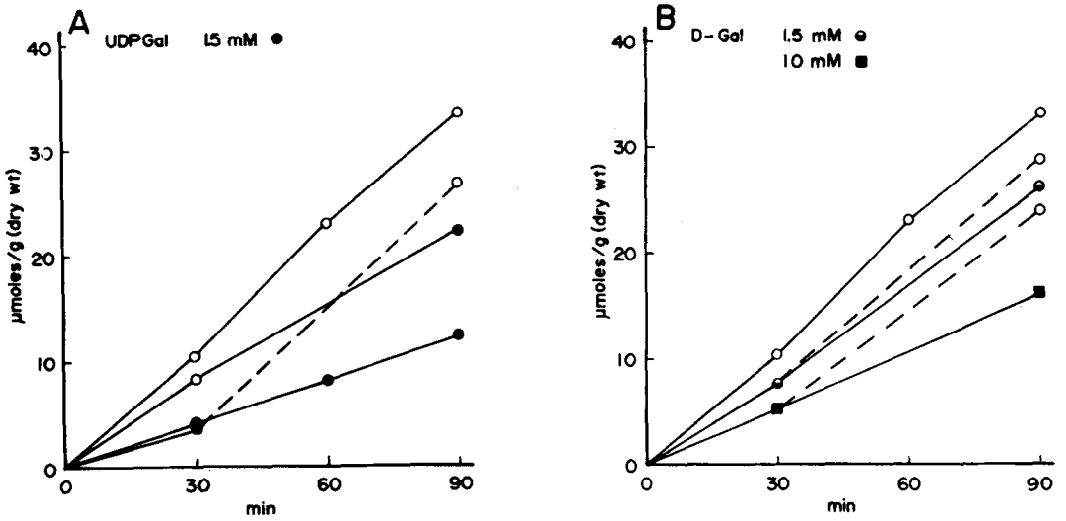

Fig. 1. The effect of UDPgalactose (A) and D-galactose (B) on the *o*-aminophenyl-β-D-glucopyranosiduronic acid synthesis in rat liver slices. Circles represent the synthesis in the absence of the metabolites in both figures, A: spots in the presence of 1.5 mM UDPgalactose, spot-and-circle first in the presence of UDPgalactose and then in its absence after the transfer of slices into a UDP-galactose free medium, and circle-and-spot UDPgalactose added during the incubation. B: the synthesis in the presence of 1.5 and 10 mM D-galactose, half spots and black squares, respectively, half spot-and-circle and black square-and-circle first in the presence of D-galactose and then in its absence after the transfer of slices into a D-galactose free medium.

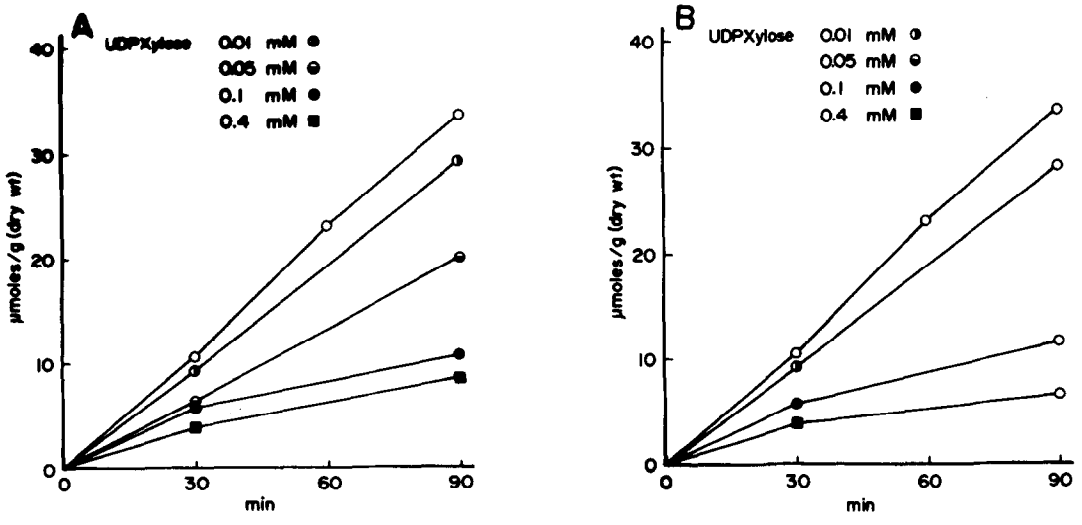

Fig. 2. The effect of various concentrations of UDPxylose on the *o*-aminophenyl-β-D-glucopyranosiduronic acid synthesis in rat liver slices (A). B: the synthesis first in the presence of UDPxylose and then in its absence after the transfer of slices into a UDP-xylose free medium. Circles represent the synthesis in UDPxylose free medium in both figures.

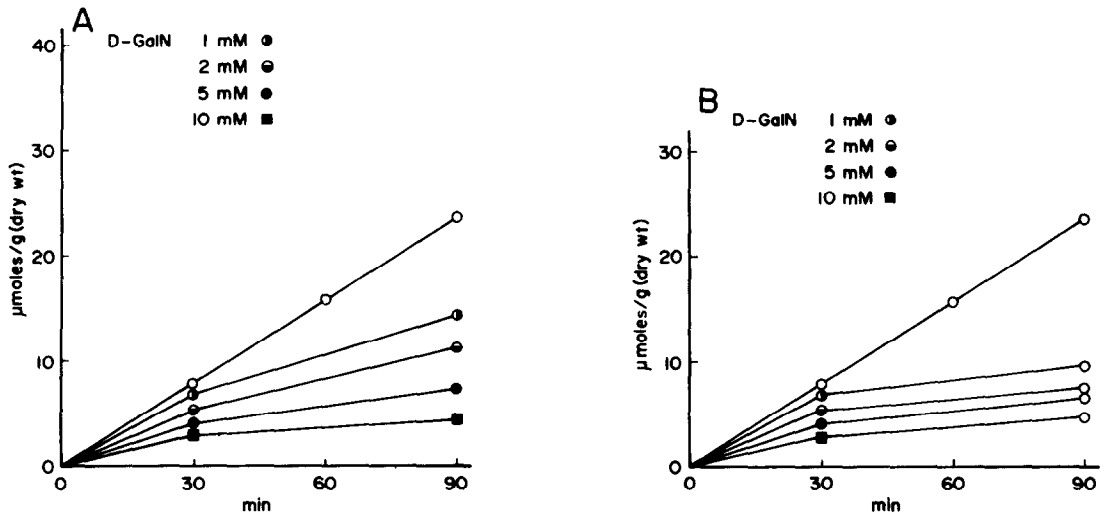

Fig. 3. The effect of various concentrations of D-galactosamine on the *o*-aminophenyl-β-D-glucopyranosiduronic acid synthesis in rat liver slices (A). B: the synthesis first in the presence of D-galactosamine and then in its absence after the transfer of slices into a D-galactosamine free medium. Circles, the synthesis in D-galactosamine free medium in both figures.

4. Discussion

The results indicate that glucuronide synthesis can be inhibited in living cells. Rather big polar compounds, eg. UDPgalactose and UDPxylose together with D-galactose and D-galactosamine enter the liver cells rather easily under the conditions used. Increasing the concentration of UDPxylose to one hundred times that of the K_i value observed for UDPglucose dehydrogenase *in vitro* [2] did not, however, block the glucuronide synthesis in liver slices completely.

Most probably UDPgalactose and D-galactose are readily consumed by the liver cells, since the inhibition of glucuronide synthesis is completely reversible. UDP-xylose, on the other hand, causes a prolonged inhibition, which may indicate a slow metabolism of this compound. Normally its concentration in the liver is very low, or it is completely absent [7]. A similar prolonged effect was observed with D-galactosamine, but in this case the concentrations needed were much higher. D-Galactosamine consumes nearly all available UTP for synthesis of UDPgalactosamine, and thus UDP-glucose levels are effectively depressed [4]. The metabolism of aminosugars is probably slow enough to result in a lengthy depression of glucuronide synthesis in liver slices.

Apparently the compounds have no acute toxic effects on cells, since the oxygen uptake remains unchanged in conditions under which the glucuronide synthesis has almost ceased.

Experiments with the isolated rat liver microsomal fraction (normal and digitonin activated) showed that UDPglucuronyltransferase itself was not inhibited by the compounds tested. The purified UDPglucose dehydrogenase was slightly inhibited by D-galactosamine and also by D-galactose, but these compounds had no effect on glucuronide synthesis when used together with UDPglucuronic acid generating UDPglucose - UDPglucose dehydrogenase system in a cell free reaction mixture.

There are some data suggesting that different UDP-glucuronyltransferases can conjugate even two phenolic aglycones, *o*-aminophenol and *p*-nitrophenol [8]. The conjugation of *o*-aminophenol by the isolated microsomal fraction was found, however, to be equally insensitive to the metabolites used in this study, when it was used as an aglycone instead of *p*-nitrophenol.

The results of the experiments with high UDPxylose and D-galactosamine concentrations indicate that the amount of UDPglucuronic acid present in the liver slices at the beginning of incubation was approximately two μmoles/g of dry weight. According to Flood-

gaard [7] the guinea-pig liver contains 1.4 μmoles/g of UDPglucuronic acid.

Acknowledgement

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