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PRELIMINARY COMMUNICATION

INHIBITION OF GLUCURONIDE FORMATION BY D-GALACTOSONE OR D-GALACTOSAMINE IN ISOLATED HEPATOCYTES

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Glucuronide formation is a major pathway in the biotransformation and elimination of a wide variety of lipid soluble drugs, environmental chemicals and endogenous compounds such as bilirubin and steroid hormones (1). Many factors regulate glucuronide formation in the intact cell, for example the activity of UDP-glucuronosyltransferase (GT) and the intracellular supply of the cosubstrate UDP-glucuronic acid (UDPGA). Interestingly the latent enzyme, which is firmly incorporated into the microsomal membrane as an oligomeric complex, is activated by UDP-N-acetylglucosamine (UDPNAG) which has been suggested to act as a physiological modifier of GT activity (1-5). For example, UDPNAG activates GT activity allosterically and limits product inhibition by UDP (3,4). However, so far the evidence is based on studies with subcellular fractions (2-4) or on comparative studies with cellular and subcellular systems (5). Treatment of hepatocytes with the uridylate trapping agents galactosone and galactosamine offers a tool to investigate the effects of UDPNAG in the intact cell. Uridylate trapping agents are known to rapidly decrease UTP and UDPGA (6,7). The level of UDPNAG, however, is differentially affected by the two sugar analogues. Whereas galactosamine-treatment strongly increases the level of UDPNAG (6) galactosone-treatment markedly decreases the level of this nucleotide (7). Differential effects of the sugar analogues on the intracellular concentration of UDPNAG have been consistently found in the liver in vivo and in isolated hepatoma cells in vitro under a variety of conditions (D.O.R. Keppler, personal communication). Similar effects can therefore be assumed to occur in isolated hepatocytes. In the present study glucuronide formation was investigated in hepatocytes which were treated with the sugar analogues at concentrations decreasing UDPGA to a similar extent. At these equipotent concentrations glucuronide formation was more severely inhibited by galactosone. These results suggest that galactosone inhibits glucuronide formation more effectively than galactosamine after short-term treatment. This is probably due to the fact that galactosone, in contrast to galactosamine, lowers the intracellular concentration of UDPNAG.

Materials and Methods

D-Galactosamine-HCl was obtained from C.Roth (Karlsruhe, FRG). D-Galactosone (D-lyxo-2-hexosulose), synthesized according to Bayne, was generously supplied by Dr. K.A. Reiffen and Prof. F. Schneider, Department of Physiological

Table 1. Effects of D-galactosone and D-galactosamine on the content of UDP-glucuronic acid in isolated hepatocytes

Treatment	UDP-glucuronic acid[a] (nmol/10^6 cells)	
	(10 min)	(20 min)
None	1.98 ± 0.26	2.07 ± 0.37
+ D-Galactosamine (5 mM)	1.24 ± 0.38[b]	1.43 ± 0.24[b]
+ D-Galactosone (0.2 mM)	2.43 ± 0.54	1.51 ± 0.36[b]

[a] UDP-glucuronic acid was determined 10 and 20 min after addition of the sugar analogues. After 10 min incubation 1-naphthol (500 nmol/ml) was added as indicated in Fig. 1. The means ± SD of 5 determinations are listed.

[b] Significantly different from untreated controls, $p < 0.05$.

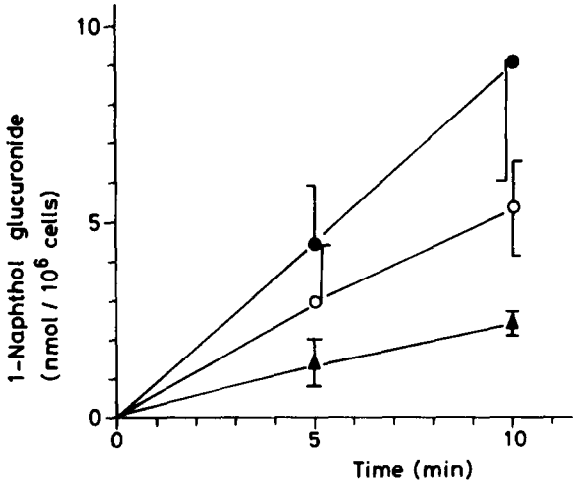

Fig. 1. Effects of D-galactosone (▲) and D-galactosamine (○) on 1-naphthol glucuronidation in isolated hepatocytes. Untreated controls (●). Hepatocytes (4 x 10^6 cells/ml) were treated with 0.2 mM galactosone or 5 mM galactosamine as in Table 1, and 1-naphthol (500 nmol/ml) was added after 10 min preincubation. Data represent the means ± SD of 4 experiments.

Chemistry, University of Marburg, FRG. D-Galactosone (purity 75%, w/v) is hygroscopic and unstable. It was stored at -20°C and dissolved in an aliquot of the incubation medium immediately before use.

Rat hepatocytes were prepared and 1-naphthol glucuronidation was determined as described (5,9) except that $MgSO_4$ in Krebs-Henseleit buffer, pH 7.4, was replaced by $MgCl_2$ in order to reduce 1-naphthol sulfation (10). The level of UDPGA in hepatocytes was determined enzymatically as described (9,11).

Results and Discussion

In rat hepatocytes a UDPGA content of 2.0 ± 0.3 nmol/10^6 cells was found (Table 1), in agreement with previous studies (9,12). 1 g liver represents about 130 x 10^6 liver cells (5) and this gives a cofactor content of 0.3 µmol/g liver which is similar to the content found in liver in vivo (6,7, 11). Treatment with galactosone or galactosamine markedly decreases the level of UDPGA in isolated hepatocytes, confirming previous studies in liver and hepatoma cells (6,7,11). After treatment with 2 mM galactosone for 20 min UDPGA could not be detected in hepatocytes (not shown). Treatment for 20 min with 0.2 mM galactosone or 5 mM galactosamine decreased UDPGA to a similar extent, by about 30% (Table 1). However at these equipotent concentrations glucuronide formation was more severely inhibited by galactosone (Fig. 1).

Both uridylate trapping agents powerfully decrease UDPGA but they markedly differ in their effects on the intracellular level of UDPNAG which is reduced by galactosone but increased by galactosamine (6,7). In galactosamine-treated hepatocytes reduced glucuronide formation due to the reduced level of UDPGA may be partly compensated by activation of GT due to the higher level of UDPNAG. In galactosone-treated hepatocytes UDPNAG activation of GT is substantially reduced. Hence the stronger inhibitory effect of galactosone on glucuronide formation is probably due to the reduced level of UDPNAG. Altered product inhibition by UDP can not explain the effect since it is of minor importance in the UDPNAG-activated state of the enzyme (3,4,13,14). In addition, galactosone or galactosamine do not inhibit liver microsomal GT activity in vitro up to a concentration of 5 mM (not shown).

The intracellular content of UDPGA and UDPNAG (about 0.3 µmol/g liver, 6,7) is in the range in which GT activity in vitro can be effectively controlled. Recently it could be ascertained, using borate chromatography of 4'-epimeric UDP-sugars, that UDPNAG was the major UDP-N-acetylhexosamine in hepatocytes even if the pool was greatly expanded after galactosamine-treatment (15). Liver membrane damage due to CCl_4 abolishes the latency of the enzyme and thereby the regulation of GT activity by UDPNAG (13,14). It is not known why UDPNAG is an allosteric modifier of GT activity nor is it known how the intracellular level of UDPNAG is regulated. One connection between UDPGA and UDPNAG is the fact that glucuronic acid and N-acetylglucosamine are repeating units of the carbohydrate side chains of proteoglycans such as hyaluronate, chondroitin sulfates and heparin. Although still at a preliminary stage the present study suggests a) that galactosone

is more effective than galactosamine as a short-term inhibitor of intracellular glucuronidation and b) that comparative studies with galactosone and galactosamine may be a valuable tool to investigate the interesting regulation of GT activity by UDPNAG in the intact hepatocyte.

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