CHANGES IN URIDINE NUCLEOTIDES DURING LIVER PERFUSION WITH D-GALACTOSAMINE

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1. Introduction

The first steps of galactosamine metabolism in liver follow the pathway of D-galactose [1,2]. Galactosamine-1-phosphate is converted by UDPG: galactose-1-phosphate uridylyltransferase (EC 2.7.7.12) to UDP-galactosamine [2], whose epimerisation to UDP-glucosamine was shown by Maley and Maley [3]. UDP-N-acetylgalactosamine and UDP-N-acetylglucosamine were identified as further metabolites of galactosamine in rat liver [4].

In this communication we report that the perfusion of isolated rat livers with galactosamine leads to a strong decrease in several uridine nucleotides (UDPG, UTP, UDP). Evidently this is due to a trapping of uridine phosphates by formation of UDP-aminosugars. The investigation of these effects in the isolated perfused liver contributes to an understanding of galactosamine-induced hepatitis which was shown to be closely related to human viral hepatitis [5,6].

2. Experimental procedures

Livers from fed, male Sprague Dawley rats (145–180 g) were perfused as described previously [7]. After an equilibration period of 40 min, 200 μmoles galactosamine HCl (C.Roth, Karlsruhe) were added to the medium (100 ml). Liver samples (300–800 mg) were taken before, and 8, 20 and 35 min after galactosamine addition. The tissue samples were obtained by freeze-stop technique and homogenized in about 4 vol 0.9 N perchloric acid. The neutralized supernatants were analysed enzymatically for adenine [5,8] and uridine [2] nucleotides. U-5′-MP was measured in the UTP/UDP-assay [2] by further addition of nucleoside monophosphate kinase (EC 2.7.4.4) from beef liver.

3. Results and discussion

As shown in table 1, neither the contents nor the respective ratios of the adenosine phosphates change significantly during liver perfusion with D-galactosamine. In contrast, UTP and UDP (table 2) decrease drastically during the uptake of galactosamine by the liver (about 0.4 μmoles × g liver^{-1} × min^{-1} as measured by the disappearance of galactosamine from the medium). A concomitant decrease of U-5′-MP can be derived from the corresponding ratios of adenosine phosphates and uridine phosphates [9]. The UDPG

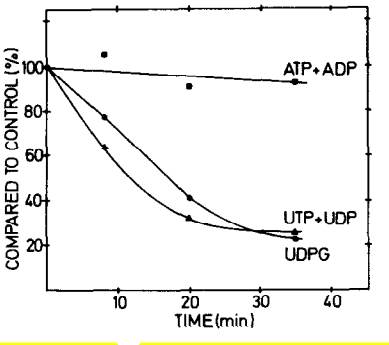

Fig. 1. Time-dependent decrease of uridine nucleotides in isolated perfused rat livers after galactosamine addition to the medium (200 μmoles/100 ml). No significant changes of adenine nucleotides are observed. For absolute values see tables 1 and 2.

Table 1
Adenine nucleotides (μmoles/g fresh liver).

Minutes after galactosamine addition	0	8	20	35
ATP				
Controls	2.42 ± 0.50	2.28 ± 0.49	2.77 ± 0.45	2.47 ± 0.48
GalN[a)]	2.54 ± 0.43	2.62 ± 0.35	2.37 ± 0.23	2.36 ± 0.23
ADP				
Controls	1.08 ± 0.12	1.07 ± 0.22	0.89 ± 0.22	1.09 ± 0.34
GalN[a)]	0.94 ± 0.18	0.91 ± 0.14	0.94 ± 0.06	0.93 ± 0.12
AMP				
Controls	0.28 ± 0.06	0.31 ± 0.14	0.17 ± 0.02	0.18 ± 0.04
GalN[a)]	0.30 ± 0.18	0.20 ± 0.05	0.21 ± 0.12	0.32 ± 0.23

Each value represents the mean of 4 samples ± S.D.
a)D-galactosamine (200 μmoles/100 ml).

Table 2
Uridine nucleotides (μmoles/g fresh liver).

Minutes after galactosamine addition	0	8	20	35
UDPG				
Controls	0.26 ± 0.07	0.31 ± 0.07	0.29 ± 0.09	0.26 ± 0.05
GalN[a)]	0.26 ± 0.06	0.24 ± 0.03	0.12 ± 0.05[b)]	0.06 ± 0.02[c)]
UTP + UDP				
Controls	0.35 ± 0.07	0.41 ± 0.20	0.34 ± 0.07	0.28 ± 0.03
GalN[a)]	0.36 ± 0.03	0.26 ± 0.05	0.11 ± 0.04[b)]	0.07 ± 0.02[c)]

Each value represents the mean of 4 samples ± S.D.
a) D-galactosamine (200 μmoles/100 ml).
b) $P < 0.005$ as compared with the controls.
c) $P < 0.0005$ as compared with the controls.

level is as low as 23% of the controls 35 min after galactosamine addition (fig. 1).

The effects of galactosamine on uridine nucleotide levels in liver can best be explained by two peculiarities of galactosamine metabolism:

(1) UDPG is a necessary substrate for the conversion of galactosamine-1-phosphate to UDP-galactosamine [2]: α-D-galactosamine-1-phosphate + UDPG ⇌ UDP-galactosamine + glucose-1-phosphate. In contrast to the metabolism of galactose, however, UDPG is not regenerated by the UDP-galactose 4-epimerase reaction. The uridine phosphate moiety remains bound in UDP-galactosamine, UDP-glucosamine, and also in the UDP-N-acetylhexosamines. It was shown [4], that galactosamine is a precursor of UDP-N-acetylhexosamines as well. In accordance with this difference of galactose and galactosamine metabolism no change in UDPG (0.32 ± 0.07 (S.D.)) and UTP + UDP (0.33 ± 0.05 (S.D.) μmoles/g) were observed when livers were perfused with an equimolar dose of D-galactose.

(2) In addition, galactosamine-1-phosphate was found to inhibit strongly the synthesis of UDPG from glucose-1-phosphate and UTP [2,10], the low level of UTP may further decrease the rate of UDPG formation.

Evidence for the trapping of uridine phosphates by formation of UDP-aminosugars was also obtained by the determination of total U-5′-MP after acid hydrolysis (1.3 N HCl, 20 min, 100°C) of the liver supernatants. 0.91 ± 0.17 (S.D.) μmoles UMP/g fresh weight were found in galactosamine-perfused livers as compared to 0.68 ± 0.21 (S.D.) in controls. This excludes an enhanced degradation of uridine phosphates after galactosamine treatment and is in agreement with measurements *in vivo*. Here it was found, that UDPG drops to 16% and UTP + UDP to 43% of the controls [2] and that U-5′-MP after acid hydrolysis increases 5-fold due to a formation of UDP-aminosugars. The galactosamine-induced qualitative and quantitative alterations in uridine nucleotides provide new aspects for the mechanism of galactosamine toxicity.

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