TRAPPING OF URIDINE PHOSPHATES BY D-GALACTOSE IN ETHANOL-TREATED LIVER

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1. Introduction

Tygstrup and Keiding have shown, that ethanol greatly enhances galactose toxicity in rats [1]. Ethanol treatment increases the NADH/NAD ratio in liver [2–4] and by this inhibits the UDP-galactose 4-epimerase (EC 5.1.3.2) [3, 5]. Under these conditions oxidation [3] and elimination [6–8] of galactose are impaired as this sugar is predominantly metabolized by way of galactose-1-phosphate, UDP-galactose and UDPG [9–11]. The uridine phosphate moiety is easily liberated after formation of UDPG from UDP-galactose by glucosyltransfer [12], pyrophosphorolysis [13, 14], and phosphodiesterase type hydrolysis [15].

In this communication it is shown that combined galactose + ethanol treatment results in an accumulation of galactose-1-phosphate and UDP-galactose in rat liver. The formation of high amounts of UDP-galactose leads to a change in the distribution of liver uracil nucleotides. A marked decrease of UDPG, UTP, UDP, and UMP is followed by an increase of the sum of uracil nucleotides.

The trapping of uridine phosphates by UDP-galactose accumulation is analogous to that induced by D-galactosamine [16–18], 2-deoxy-D-galactose or D-glucosamine [18]. The decrease in UTP is considered to be the most essential primary mechanism of galactosamine-induced liver damage [18, 19] and is proposed to explain the increased toxicity of galactose by ethanol.

2. Experimental procedures

Female Wistar rats (150–165 g) received an oral dose of ethanol (130 mmole/kg body weight); one hr later D-galactose (2.7 mmole/kg) was administered intraperitoneally. Animals treated exclusively with galactose served as controls. The livers were freeze-clamped in situ under light ether anesthesia. The following metabolites were determined enzymatically: Uracil nucleotides [20], glucose-6-phosphate, ATP [21], ADP, AMP [22], lactate [23], pyruvate [24], glucose-1-phosphate [17], and galactose-1-phosphate [17].

3. Results

3.1. *Control parameters*

The lactate/pyruvate ratio, as an indicator of the cytoplasmic redox state [25] increased strongly two hr after oral administration of ethanol to rats (table 1). This ratio was not influenced significantly by the galactose treatment; however, as described earlier [25] it was increased in livers of starved rats (table 1).

Neither galactose alone nor galactose + ethanol changed the absolute and relative amounts of adenosine phosphates and of glucose monophosphates significantly (table 2).

3.2. *Galactose metabolites*

The UDP-galactose 4-epimerase reaction is indicated as the rate-limiting step of galactose metabolism in rat liver by the ratios of galactose metabolites (table 1, 2). A sufficient galactose load increased the hepatic levels of galactose-1-phosphate and UDP-galactose in fed (fig. 1), starved, and ethanol-treated animals without a significant change of the ratio of both metabolites (table 2); the UDPG/UDP-galactose ratio and the UDPG content, however, decreased

Table 1
Metabolite contents in normal, galactose-treated, and ethanol + galactose-treated liver; mean values as μmole per g fresh weight ± S.D.(*n*).

	Fed	Starved	Galactose (starved)	Ethanol + galactose (starved)
UDPGal	0.09±0.01 (19)	0.09 ±0.01 (5)	0.32 ±0.07 (10)	1.25 ± 0.16 (9)[a]
UDPG	0.32±0.04 (65)	0.29 ±0.05 (14)	0.24 ±0.09 (10)	0.15 ± 0.03 (10)[b]
UDPG/UDPGal	3.4 ±0.3 (10)	3.3 ±0.3 (5)	0.78 ±0.39 (10)	0.11 ± 0.02 (9)[a]
UTP + UDP	0.34±0.05 (36)	0.23 ±0.05 (13)	0.15 ±0.03 (10)	0.11 ± 0.02 (9)[b]
ΣUMP	1.24±0.13 (21)	1.04 ±0.11 (11)	1.12 ±0.12 (10)	1.61 ± 0.13 (9)[a]
Lactate	2.88±0.89 (6)	1.44 ±0.49 (5)	1.33 ±0.13 (5)	1.43 ± 0.21 (5)
Pyruvate	0.24±0.04 (6)	0.045±0.015 (5)	0.054±0.007 (5)	0.015± 0.004 (5)[a]
Lactate/pyruvate	11.9 ±2.9 (6)	31.9 ±5.6 (5)	25.0 ±5.9 (5)	103.2 ±31.0 (5)[a]

[a] $p < 0.001$ as compared to galactose-treated and untreated animals.
[b] $p < 0.01$ as compared to the other groups.

Rats were starved for 12 hr overnight. Ethanol (6 g/kg body weight = 130 mmoles/kg) was given orally as a 50% (v/v) solution, one hr later D-galactose (486 mg/kg = 2.7 mmole/kg) was injected intraperitoneally as a 1 M aqueous solution. Livers were removed one hr after galactose and two hr after ethanol administration. Quantitative enzymatic hydrolysis of all uracil 5′-nucleotides followed by specific measurement of 5′-UMP was used to determine ΣUMP [20]. The liver uracil nucleotide contents in fed rats are data from previous work [20].

Table 2
Liver metabolites after treatment with galactose or galactose + ethanol (μmole per g fresh weight ± S.D. (*n*)).

	Galactose	Ethanol + galactose
Galactose-1-P	0.18 ±0.04 (10)	0.69 ±0.11 (9)[a]
Glucose-1-P	0.010±0.004 (10)	0.011±0.005 (10)
Glucose-6-P	0.26 ±0.06 (5)	0.30 ±0.13 (5)
Glc-6-P/Glc-1-P	22.2 ±5.9 (5)	22.8 ±5.9 (5)
UDPGal/Gal-1-P	1.94 ±0.35 (10)	1.85 ±0.27 (9)
ATP	2.60 ±0.16 (9)	2.81 ±0.15 (10)
ADP	0.88 ±0.17 (10)	0.97 ±0.19 (10)
AMP	0.15 ±0.09 (10)	0.19 ±0.07 (10)
ATP/ADP	3.14 ±0.52 (9)	3.10 ±0.53 (10)
ΣAMP	3.55 ±0.43 (9)	4.38 ±0.41 (9)[a]

[a] $p < 0.001$ as compared to galactose-treated animals.

The experimental conditions were the same as described in table 1. Galactose-1-phosphate was assayed with galactose dehydrogenase and alkaline phosphatase [17], these values being 6% higher than determinations with UDPG:galactose-1-phosphate uridylyltransferase [17]. Glucose-1-phosphate was measured with UDPG-pyrophosphorylase and UDPG-dehydrogenase [17], 5′-AMP after hydrolysis of all acid soluble adenine 5′-nucleotides (ΣAMP) [20] specifically with adenylate kinase [22].

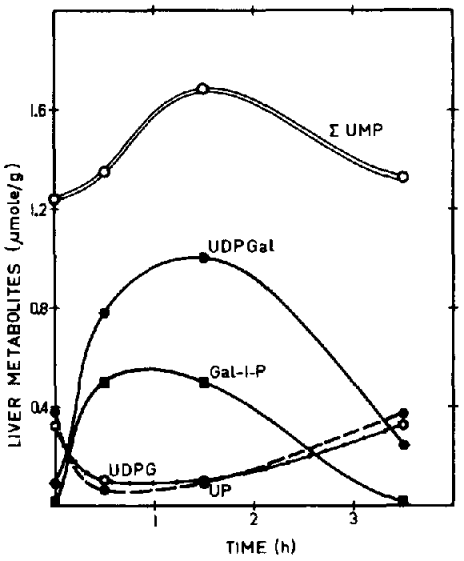

Fig. 1. Time-dependent changes of galactose metabolite and uracil nucleotide contents in livers of fed rats. D-galactose (2.7 mmole/kg) was injected at zero time, one hr after ethanol administration (130 mmole/kg). Uridine phosphates (UTP + UDP + UMP) are designated as UP, the sum of all acid soluble uracil 5′-nucleotides as ΣUMP.

(table 1). The decrease of UDPG was negatively correlated to the extent of UDP-galactose accumulation. The almost 4-fold increase of galactose-1-phosphate and UDP-galactose and the even stronger drop of the UDPG content in the ethanol-treated liver after a galactose load demonstrate the ethanol-induced inhibition of the UDP-galactose 4-epimerase (tables 1, 2).

3.3. *Changes in uracil nucleotides*

The formation and accumulation of high amounts of UDP-galactose strain the synthesis of UDPG and of uridine phosphates. The decrease of uridine phosphates was most pronounced after ethanol + galactose administration (table 1). The ratio (UTP + UDP):(UMP) remained in the normal range, corresponding approximately to the adenosine phosphate ratios [19]. Exclusive ethanol treatment did not affect the hepatic uracil nucleotide contents. Whereas the change in the distribution of acid soluble uracil nucleotides after galactose administration alone was not sufficient to provoke an increase of the sum of all uracil nucleotides (ΣUMP), this was observed in the ethanol + galactose-treated liver (table 1).

4. Discussion

A low potential of the NADH/NAD couple inhibits the UDP-galactose 4-epimerase, which was shown to require bound NAD [5]. Under conditions where ethanol did not increase the NADH/NAD ratio a normal oxidation of galactose was measured [8]. The pattern of galactose metabolites (table 1, 2) confirms the inhibitory effect of ethanol on galactose metabolism.

Galactose provokes pronounced alterations of the uracil nucleotide contents in liver, which are intensified by an inhibition of the UDP-galactose 4-epimerase. The turnover of UDP-galactose by epimerisation to UDP-glucose or by galactosyl transfer appears to be slower than necessary for the maintenance of normal hepatic UDPG and uridine phosphate levels after a galactose load. UDP-galactose 4-epimerase was also shown to be the rate-limiting enzyme of galactose metabolism in intact L-cells and HeLa-cells [26].

The toxicity of high amounts of galactose [27] which is increased by ethanol [1], was suggested to be the result of galactose-1-phosphate accumulation [28]. The proposed inhibition of phosphoglucomutase (EC 2.7.5.1) and of UDPG-pyrophosphorylase (EC 2.7.7.9) by galactose-1-phosphate [29], if it were metabolically significant should change the substrate/product ratios of these enzymes. Inspite of an almost 4-fold increase of galactose-1-phosphate (table 2) and of the galactose-1-phosphate/glucose-1-phosphate ratio, there is no indication to a significant inhibition of either enzyme as evidenced by substrate/product ratios in liver (tables 1 and 2).

The trapping of uridine phosphates provides a new approach to the mechanism of galactose toxicity.

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