EFFECTS OF D-GALACTOSAMINE ON NUCLEOTIDE METABOLISM AND ON MICROSOMAL MEMBRANES IN MOUSE LIVER

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1. Introduction

Parenteral administration of D-galactosamine causes acute hepatic injury in rodents, morphologically resembling viral hepatitis [1]. An inhibition of hepatic RNA synthesis due to D-galactosamine has been reported on the basis of decreased incorporation of radioactive precursors [2–4], and it has been considered to be the consequence of the depletion of the hepatic UTP pool by D-galactosamine, from which excessive formation of UDP-*N*-acetyl hexosamines and UDP-hexosamines occurs [5]. Little attention has been paid to the simultaneous inhibition of protein synthesis also reflected by decreased amino acid incorporation [6–8]. The reversal of the hepatotoxic action of D-galactosamine by D-galactose has been observed in this laboratory [8]. In the experiments presented here the in vitro incorporation of radioactive uridine and guanosine has been investigated, and a decrease of the specific radioactivity of UTP is demonstrated by a double labeling technique.

D-Galactosamine is known to be incorporated into glycogen [9] and the formation of atypical polysaccharides has been demonstrated by electron microscopy [3]. It is known that in vivo treatment of mice with D-galactosamine and D-glucose simultaneously causes an alteration of mouse liver microsomal membranes, which can then be pelleted through a 2 M solution of sucrose in the ultracentrifuge. This change can be reversed by treating the membranes with α-amylase. The effect of the combined treatment can be prevented by simultaneous administration of D-galactose. An interrelation between the synthesis of basic polysaccharide, damage of the endoplasmic reticulum and inhibition of protein synthesis is suggested.

2. Materials and methods

L-[2,3-^{3}H]Valine (279 mCi/mmol) and [U-^{14}C]-uridine (332 mCi/mmol) were purchased from the Isotope Institute of the Hungarian Academy of Sciences. D-[1-^{14}C]Galactosamine (58 mCi/mmol), [U-^{3}H]uridine (6.4 mCi/mmol) and [U-^{14}C]guanosine (281 mCi/mmol) were purchased from the Radiochemical Centre, Amersham. Unlabeled D-galactosamine HCl was the product of Serva, Heidelberg. Crystalline α-amylase prepared from pig pancreas was a kind gift of Dr M. Telegdi.

In each in vitro experiment slices from a single liver were prepared and incubated as described earlier [8]. Estimation of the protein and RNA bound radioactivity in the perchloric acid-insoluble material, and the isolation of labeled UDP-hexosamines were also performed as described [8].

For the separation of smooth and rough membranes the procedure of Lewis and Tata [10] was adopted. Mice were starved for 48 hr (glycogen content of the liver was less than 0.5 mg/g wet weight) and were injected with 1 ml of a sugar-containing solution. 120 min later the animals were decapitated. The livers were removed immediately and minced with scissors, and homogenized in 5 vol ice-cold Medium A (containing 0.35 M sucrose, 25 mM KCl, 10 mM $MgCl_2$ and 50 mM Tris–HCl buffer, pH 7.6) by thirty strokes in a hand-operated glass-Teflon homogenizer. The homogenate was centrifuged at 10 000 *g* for

20 min to obtain the postmitochondrial supernatant (PMS), which was layered over a discontinuous sucrose gradient and ultracentrifuged (see fig. 2). An aliquot of the PMS was deproteinized by adding perchloric acid (final concentration 0.4 N), the latter acid was then removed from the supernatant by neutralization with KOH. Glycogen was estimated in the supernatant with iodine according to the method of Krisman [11] using mouse liver glycogen prepared by the KOH method [11] as standard.

3. Results and discussion

3.1. *Incorporation of radioactive guanosine, uridine and valine in mouse liver slices*

Incorporation of [^{3}H]valine and [^{14}C]uridine into the perchloric acid-insoluble material of mouse liver slices was found to decrease in the presence of 10 mM D-galactosamine, whereas 1 mM D-galactosamine caused the decrease of uridine incorporation only (fig. 1A). Incorporation of [^{14}C]guanosine was inhibited by about 40% in the presence of D-galactosamine (10 mM), and this inhibition was not influenced by the addition of 20 mM uridine (fig. 1B), which has been shown to revert the inhibition of guanosine incorporation in vivo [12], and to increase the inhibition of valine incorporation in vitro [8].

3.2. *Estimation of the specific radioactivity of UTP in liver slices*

We have reported earlier that the inhibition of orotate incorporation by D-galactosamine was more complete than that of uridine incorporation [8]. This discrepancy prompted us to investigate the effect of D-galactosamine on the specific radioactivity of the UTP pool in the presence of tritiated uridine. As the direct estimation of this value seemed not feasible, an indirect approach was planned based on the assumptions that (*a*) the specific ^{3}H radioactivity of UDP-hexosamines is equal to that of the precursor UTP and (*b*) added D-galactosamine is the only precursor of the hexosamine moiety of non N-acetylated UDP-hexosamines [5,9], therefore the amount of the isolated UDP-hexosamines can be estimated from the ^{14}C radioactivity of the UDP-hexosamines and the specific radioactivity of D-galactosamine in the medium. Thus the specific radioactivity of UTP is equal to the ratio of the ^{3}H radioactivity and the amount of UDP-hexosamines. Details of an experiment of this type are presented in table 1.

The substantial decrease of the specific radioactivity of UTP with the increase of D-galactosamine concentration is thought to be due to enhanced uridylate synthesis induced by the UTP trapping effect of D-galactosamine [13], which is not compensated by increased utilization of externally sup-

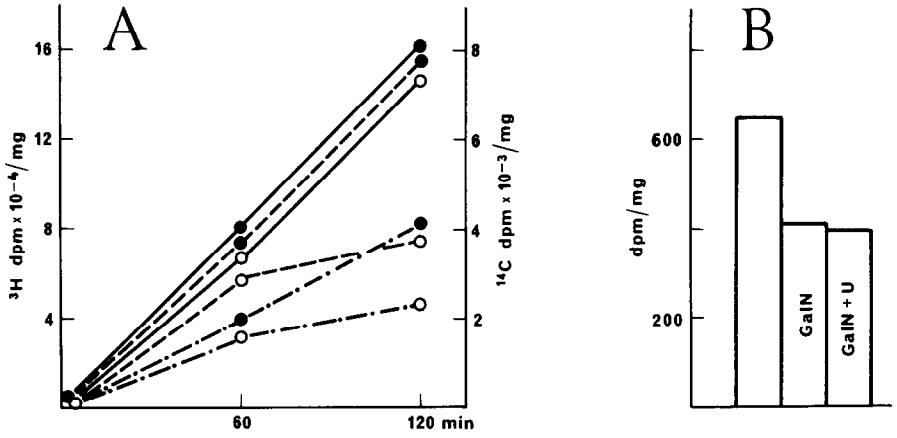

Fig. 1. Incorporation of radioactive precursors in mouse liver slices in the presence of D-galactosamine. Protein and RNA bound radioactivity is expressed as dpm per mg of perchloric acid insoluble material of the slices [8]. A: Incorporation of [^{3}H]valine (•) (concentration in the medium 10 μCi/ml) and [^{14}C]uridine (○) (1.3 μCi/ml) in the absence (——) and in the presence of 1 mM (– – –) and 10 mM (–·–·–·–) of D-galactosamine. B: Incorporation of [^{14}C]guanosine (0.4 μCi/ml) in liver slices incubated for 120 min in the presence of 10 mM D-galactosamine, or 10 mM D-galactosamine and 20 mM uridine.

Table 1
Estimation of the specific radioactivity of UTP from the radioactivity of double-labeled UDP-hexosamines

D-Galactosamine in the medium		Radioactivity in UDP-hexosamines		Specific radioactivity of UTP (mCi/mmol) $[\frac{a \times b}{c}]$
Concentration (mM)	Specific radioactivity (mCi/mmol (a))	^{3}H dpm (b)	^{14}C dpm (c)	
0.02	58.0	7 810	8 690	52.12
1.0	2.14	6 080	1 680	7.74
10.0	0.43	50 850	7 710	2.83

Mouse liver slices were incubated with [^{3}H]uridine (10 μCi/ml) at different concentrations of [^{14}C]galactosamine diluted with unlabeled D-galactosamine for 120 min. Non N-acetylated UDP-hexosamines were isolated from the perchlorid acid supernatant of the liver tissue by absorption onto charcoal followed by ion-exchange chromatography on Dowex-1 column using the formate gradient elution technique [8]. Calculation is discussed in the text.

plied uridine. It is concluded that the rate of orotate and uridine incorporation is not a reliable measure of RNA synthesis in galactosamine hepatitis [2–4], as has been pointed out by Keppler and coworkers recently, who assumed an increase of the specific radioactivity of UTP in the rat [12]. It is perhaps the rate of guanosine incorporation which reflects RNA synthesis more reliably [12]. Enzyme induction studies (another approach to the problem, ref. [4]) are complicated by the circumstance that protein synthesis is also inhibited.

3.3. *Alteration of the sedimentation pattern of microsomal membranes*

Mice starved for 48 hr were injected intraperitoneally with D-glucose (5 g/kg body weight), D-galactose (750 mg/kg) or D-galactosamine (250 mg/kg), and the microsomal membranes were fractionated (see Materials and methods, and fig. 2). There are different methods for the preparation of microsomal membranes, nevertheless there is agreement as regards the relative positions of the smooth (light) and rough (heavy) membranes after ultracentrifugation in discontinous sucrose gradients [14–16]; smooth membranes can be collected at the interface over 1.3 M sucrose, while rough membranes are found at the 1.3–2.0 M interface. D-Glucose, D-galactose and D-galactosamine in themselves each failed to alter this distribution, but a combined treatment with D-glucose and D-galactosamine produced the shift of the rough membranes from the 1.3–2 M interface to the pellet (tubes *a* and *b* in fig. 2) which was most marked in cases when the hepatic glycogen content was between 5 and 10 mg/g wet liver weight, as estimated in the PMS. Simultaneous administration of D-galactose to the animal, or a short treatment of the pellet with α-amylase reversed the sedimentation pattern to normal (fig. 2. *c, d*).

The pooled pellet obtained from the livers of two animals which were treated with D-glucose and D-galactosamine was resuspended in Medium A containing 0.5% (w/v) deoxycholate, and ultracentrifuged as before. The sediment containing the polysaccharide (and presumably free ribosomes) was resuspended in 1 ml of Medium A and mixed with the PMS of the liver of an untreated mouse. The mixture was ultracentrifuged according to the standard method. The rough membranes were pelleted in this case, too, similarly to the pattern obtained with preparations from animals treated with D-glucose and D-galactosamine.

Alteration of the sedimentation characteristics of the heavy microsomal membranes is due to the adherence of the anomalous glycogen-containing hexosamines [9] which is synthetized in the presence of D-glucose and D-galactosamine. The intracellular occurrence of such a complex is supported by the finding of Shinozuka and coworkers [3], who

Fig. 2. Altered sedimentation of microsomes after treatment of mice with D-glucose and D-galactosamine. Treatment of mice and preparation of the postmitochondrial supernatant was performed as described in Materials and methods. The PMS of one liver was layered gently over a discontinous sucrose gradient in 12 ml cellulose nitrate tubes (from bottom to top, 2 ml of 2 M sucrose, 2.5 ml of 1.3 M sucrose and 2 ml of 0.6 M sucrose each containing 10 mM $MgCl_2$), and centrifuged at 40 000 rpm in the SW 41 rotor of a Beckman L2-65B ultracentrifuge for 120 min. The distribution visible in tube *a* was obtained after treatment with various doses of D-glucose, D-galactose and D-galactosamine separately. Pattern *b* appeared after a combined treatment with D-glucose (5 g/kg) and D-galactosamine (250 mg/kg). Simultaneous administration of D-galactose (750 mg/kg) reversed the distribution to normal. The pellet obtained after the combined treatment (*b*) was suspended in 4 ml of Medium A and 0.1 mg α-amylase was added to an aliquot of 2 ml. Both samples were incubated at 37°C for 10 min after which no iodine staining could be demonstrated in the amylase treated sample. The samples were applied to a discontinuous sucrose gradient and ultracentrifuged. The control sample was pelleted as before (*c*), whereas the amylase-digested membranes were found at the 1.3–2 M interface (*d*).

demonstrated aggregates of ribosomes and a typical polysaccharide in galactosamine hepatitis. Our observation indicates the substantial alteration of the endoplasmic reticulum, which may involve the inhibition of protein synthesis.

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