Experimental and Toxicologic Pathology 66 (2014) 147–154

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Activity of glycogen synthase and glycogen phosphorylase in normal and cirrhotic rat liver during glycogen synthesis from glucose or fructose

Natalia N. Bezborodkina[a,*], Anna Yu. Chestnova[a], Sergey V. Okovity[b], Boris N. Kudryavtsev[a]

[a] Laboratory of Cellular Pathology, Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia
[b] Cathedra of Pharmacology, Saint Petersburg State Chemical Pharmaceutical Academy, St. Petersburg, Russia

ARTICLE INFO

Article history:
Received 15 February 2013
Accepted 3 December 2013

Keywords:
Glycogen
Glycogen synthase
Glycogen phosphorylase
Liver cirrhosis
Glucose
Fructose

ABSTRACT

Cirrhotic patients often demonstrate glucose intolerance, one of the possible causes being a decreased glycogen-synthesizing capacity of the liver. At the same time, information about the rates of glycogen synthesis in the cirrhotic liver is scanty and contradictory. We studied the dynamics of glycogen accumulation and the activity of glycogen synthase (GS) and glycogen phosphorylase (GP) in the course of 120 min after per os administration of glucose or fructose to fasted rats with CCl_4-cirrhosis or fasted normal rats. Blood serum and liver pieces were sampled for examinations. In the normal rat liver administration of glucose/fructose initiated a fast accumulation of glycogen, while in the cirrhotic liver glycogen was accumulated with a 20 min delay and at a lower rate. In the normal liver GS activity rose sharply and GP*a* activity dropped in the beginning of glycogen synthesis, but 60 min later a high synthesis rate was sustained at the background of a high GS and GP*a* activity. Contrariwise, in the cirrhotic liver glycogen was accumulated at the background of a decreased GS activity and a low GP*a* activity. Refeeding with fructose resulted in a faster increase in the GS activity in both the normal and the cirrhotic liver than refeeding with glucose. To conclude, the rate of glycogen synthesis in the cirrhotic liver is lower than in the normal one, the difference being probably associated with a low GS activity.

1. Introduction

Chronic hepatitis is a common human pathology. Its final and the most life-threatening stage is liver cirrhosis, which results in considerable reconstructions of the liver's structure and the changes of its metabolism (Bircher et al., 1999). Some characteristic features of this altered metabolism are: (1) energy is produced by oxidation of lipids rather than carbohydrates; (2) following nocturnal fasting, glucose is produced by gluconeogenesis rather than by glycogenolysis; (3) ketogenesis is enhanced (Petersen et al., 1999; Bechmann et al., 2012). Carbohydrate metabolism during liver cirrhosis is somewhat similar to that during diabetes, a disease primarily characterized by glucose and insulin intolerance (Schneiter et al., 1999; Kawaguchi et al., 2011). One of the causes of glucose intolerance in cirrhotic patients may be a decreased glycogen-synthesizing capacity of the liver.

Carbohydrates are the major component of the human diet. The most common dietary sugars are glucose and fructose, contained in the food both in the free form and as part of oligosaccharides, polysaccharides, glycosides and other derivatives. Contrary to glucose, absorption of fructose by the liver is not insulin-dependent. Therefore, fructose infusions to cirrhotic patients may not have the side effects associated with the infusion of glucose (Elliott et al., 2002). Fructose is also considered to bring about a faster accumulation of glycogen in the liver than glucose (Niewoehner et al., 1984a; Gitzelmann et al., 1989).

Glycogen accumulation in the cirrhotic liver is poorly studied. Information on its rates and the activity of the involved enzymes, glycogen synthase (GS) and glycogen phosphorylase (GP), is contradictory. Inconsistency of the results reported by different authors is mostly due to the fact that they were obtained from heterogeneous material (with various degrees of liver pathology expression and at various stages of the digestive cycle).

In this paper we report on the dynamics of glycogen accumulation and the activity of GS and GP in the normal and the cirrhotic

* Corresponding author at: Laboratory of Cellular Pathology, Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Avenue 4, 194064 St. Petersburg, Russia. Tel.: +7 812 2973796; fax: +7 812 2970341.
E-mail addresses: natalia_bezborodkina@mail.ru, bezbor74@mail.ru (N.N. Bezborodkina).

0940-2993/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.etp.2013.12.001

liver of fasted rats at different stages after per os administration of glucose or fructose.

2. Materials and methods

2.1. Animals

We used 102 outbred male white rats weighing 130–140 g in the beginning and 250–300 g in the end of the experiment. The rats were given a standard diet. In the beginning of the experiment the animals were separated into two groups, the experimental one and the control one. Liver cirrhosis in rats from the experimental group was produced by chronic inhalation of CCl_4 vapours (7 ml per 100 l of the closed chamber volume) in the course of six months; the animals were treated three times weekly for 20 min. Rats from the control group were untreated.

A week after the termination of treatment with CCl_4, 5 rats from the control and 5 rats from the experimental group were decapitated after nocturnal fasting. Their blood serum samples and pieces of liver were used for histological and biochemical research. To estimate the dynamics of glycogen accumulation in the liver, the rats were fasted for 48 h (water ad libitum) and then administered per os 30% solution of glucose or fructose (4 g per 1 kg of body weight). The rats were decapitated immediately after the termination of fasting and 10, 20, 30, 45, 60, 75, 90 and 120 min after the administration of monosaccharides.

The experiments described here were performed in accordance with the "Guide for the care and use of laboratory animals" (http://www.nap.edu/catalog/10498.html) and "On the statement of rules of laboratory practice" (http://www.zdrav.spb.ru/official_documents/MZ/list_mz2003.htm).

2.2. Histological methods

Pieces of rat liver were fixed in 10% neutral formaldehyde and embedded in paraffin blocks, which were cut with the Reichert microtome (Austria) into sections ca. 5 μm thick. The tissue sections were stained with haematoxylin–eosin (Biovitrum, Russia) after Romanovsky and picro-fuchsin after Van Gieson (Pearce, 1962). The stained sections were microscopically analyzed with the help of the Axiovert 200 M microscope (Carl Zeiss, Germany) equipped with 10 × 0.30 and 40 × 0.75 objectives. The relative volume of the connective tissue and the parenchyma of the rat liver in the sections was determined after (Weibel et al., 1969) using an eyepiece reticule (16 × 16), a 25 × 0.50 objective and an 8× eyepiece.

2.3. Biochemical study of the liver

The concentration of total bilirubin (TB), total protein and the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) in the rat blood serum was determined with the help of the Abbot-spectrum automatic biochemical analyzer (Abbott Laboratories S.A., USA).

2.4. Determination of the activity of antioxidant enzymes and the concentration of lipid peroxidation (LP) products in the liver

For this, we used 10% tissue homogenates made on 25 mM Tris–HCl buffer with 175 mM KCl (pH 7.4). The activity of superoxide dismutase (SOD) was estimated by the reduction of nitro blue tetrazolium with NADH and phenazine methosulphate (Nishikimi et al., 1972). Catalase activity was assessed by the rate of hydrogen peroxide decomposition (Luck and Catalase, 1971). The concentration of LP products, diene conjugates (DC) and malone dialdehyde (MDA) was determined, respectively, after Romanova and Stalnaya (1977) and after Uchigama and Michera (1978).

2.5. Determination of glucose concentration in the blood

Glucose concentration in the blood serum of rats was determined by an enzyme colorimetric assay using the standard kit (Olvex Diagnosticum, Russia). The assay is based on the glucose oxidation reaction in the presence of glucose oxidase with the formation of hydrogen peroxide, which, in turn, oxidizes o-toliudine in the presence of peroxidase with the formation of stained products. After adding perchlorate, experimental samples were centrifuged at 900 × *g* for 10 min. Working reagent was added to the obtained supernatant in the proportion of 1:200; after stirring, the mixture was incubated at room temperature for 30 min. Experimental and calibration samples were measured against the control sample at 500 nm using the Specol 11 spectrophotometer (Carl Zeiss, Germany) fitted with 1 cm cells.

2.6. Determination of glycogen content

Glycogen content was determined in the sediment formed after adding 1.2 volumes of ethanol to the lysate of liver tissues (Vilkova, 1982). Liver tissues were lysed in 30% KOH in a boiling water bath for 60 min. After centrifuging for 30 min at 1000 × *g*, the glycogen pellet was washed in an ascending ethanol series (70, 80 and 96%) and then centrifuged again. The pellet obtained after the centrifuging was hydrolysed in 2 N H_2SO_4 in a boiling water bath for 2.5 h. The hydrolysate was neutralized with 5 N NaOH up to pH 7.8–8.0 and the amount of the glucose formed was determined by glucose oxidase method using the standard kit.

2.7. Determination of GS and GP activity

Rat liver was homogenized in 50 mM Tris–HCl buffer (pH 7.4) with 5 mM ethylenediaminetetraacetic acid (EDTA), 200 mM sucrose, 0.01 M β-mercaptoethanol and 0.2 M phenylmethylsulfonyl fluoride on ice (1:10). The homogenate was centrifuged at 1000 × *g* and 4 °C for 10 min to remove incompletely destroyed cells and nuclei. The supernatant was then centrifuged at 14,000 × *g* and 4 °C for 10 min. The newly obtained supernatant was used to determine the activity of GS and GP by the substrate-labelled assay.

GS activity was estimated by the amount of [U-^{14}C]glucose included in the glycogen, using UDP-[U-^{14}C]glucose (300 mCi/mM) as a substrate (Vardanis, 1992). To determine the activity of the enzyme, 40 μl of the sample was added to 75 μl of 10 mM Tris–HCl buffer (pH 7.5) containing 0.17 mM EDTA, 100 mM NaF, 2.5 mM $MgSO_4$, 10% glycogen and 0.25 mM UDP-glucose (40,000 imp/min). The mixture was incubated for 10 min at 30 °C in the presence of 7.2 mM glucose-6-phosphate during determination of the activity of the enzyme's D-form and in the presence of 0.17 mM glucose-6-phosphate during determination of the activity of the enzyme's I-form. The reaction was arrested by application of 0.5 ml of 96% ethanol onto 1.5 cm^2 filters (Whatman 3 mm) with 50 μl of the reactive mixture. After the reaction arrest, the filters were rinsed twice (30 min each time) in 66% ethanol and once in acetone for 5 min in order to remove the non-included label. To count the impulses, dried filters were placed into vials with a liquid scintillator ZHS-7. The number of impulses was registered with a counter (Beckman, USA).

GP activity was determined using the inverse reaction of glycogen synthesis in vitro. The amount of [U-^{14}C]glucose incorporated into glycogen was measured using [U-^{14}C]glucose-1-phosphate (286 mCi/mM) as a substrate (Vardanis, 1992). The reaction was performed in 50 mM Tris–HCl buffer (pH 6.7) containing 100 mM NaF, 10% glycogen, 10 mM glucose-1-phosphate (35,000 imp/min).