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Bile duct obstruction is associated with early postoperative upregulation of liver uncoupling protein-2 and reduced circulating glucose concentration in the rat

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Abstract

Objective: To evaluate whether upregulation of liver and muscle uncoupling protein 2 (UCP-2) is an acute phenomenon in obstructive jaundice and associated with secondary metabolic effects.

Methods: Male Sprague-Dawley rats were divided into four groups: bile duct ligated (BDL) and sham-operated pair-fed (PF), ad libitum fed (AL), and controls. BDL, PF, and AL rats were further divided into subgroups according to the interval postoperatively when they were reanesthetized and sampled for tissue and blood: 2, 4, and 8 d, respectively. Bilirubin, liver enzymes, glucose, free fatty acids, and insulin in blood plasma were analyzed. Liver and muscle tissue were sampled for UCP-2 and adenosine triphosphate analysis.

Results: The BDL rats showed an increase of the liver UCP-2 expression compared with PF and AL rats ($P < 0.05$) 4 d postoperatively. Liver adenosine triphosphate in BDL rats showed a decrease compared with sham-operated controls at all intervals ($P < 0.05$). Plasma glucose concentration in BDL rats was decreased compared with the other groups. Free fatty acids showed an initial increase 2 d postoperatively compared with sham-operated controls and PF and AL rats ($P < 0.05$) at the corresponding time point.

Conclusion: Obstructive jaundice is associated with an early upregulation of liver UCP-2, reduced liver adenosine triphosphate content, and decreased plasma glucose concentration, supporting the hypothesis that obstructive jaundice results in impaired energy homeostasis in the liver, which might cause decreased glucose output and hypoglycemia as a consequence.

Keywords: Bile duct ligation; Mitochondria; Obstructive jaundice; Uncoupling protein

Introduction

Experimental obstructive jaundice is associated with a concomitant decrease in insulin concentration and significant weight loss [1–3]. The weight decrease might be the result of reduced food intake, metabolic alteration, or a combination of both. The impaired mitochondrial function during obstructive jaundice indicates metabolic changes, although the exact mechanism is unknown [4,5]. Our group has shown that experimental obstructive jaundice for 1 wk resulted in increased expression of uncoupling protein-2 (UCP-2) in the liver [3]. UCP-2 is an inner membrane mitochondrial protein that increases the re-entry of protons into the mitochondrial matrix and reduces the half-life of respiratory reactive intermediates, thereby ''uncoupling'' oxygen consumption by the respiratory chain from adenosine triphosphate (ATP) synthesis, resulting in the production of heat instead of ATP [6–8].

Uncoupling protein-2 is expressed in several organs including the liver [8,9]. It has been suggested that a mild uncoupling of cellular respiration could be a process that prevents the accumulation of free oxygen radicals, whereas

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a longstanding upregulation of UCP-2 with increased energy waste could have a negative impact on the organism [10]. Whether the upregulation of liver UCP-2 during obstructive jaundice is an early or late phenomenon is, however, not known. Furthermore, the effect of obstructive jaundice on substrate energy metabolism is unknown.

We hypothesize that experimental obstructive jaundice in the rat causes an upregulation of liver UCP-2 with a concomitant decrease in mitochondrial energy production with secondary effects on energy-requiring metabolic processes in the liver such as gluconeogenesis.

In the present study we investigated whether obstructive jaundice is associated with an acute upregulation of UCP-2 in liver and muscle tissues and if these changes might cause metabolic alterations with secondary changes in liver ATP content, plasma glucose, free fatty acid (FFA), and insulin concentrations.

Materials and methods

Animals

Male Sprague-Dawley rats (2 mo old, mean ± standard error of the mean 241 ± 7 g; ALAB, Stockholm, Sweden) were housed individually in wire-bottom cages with a 12-h dark/12-h light cycle (lights on 07:00 to 19:00 h) and had free access to food and water before the experiments.

Rats were fed a mashed diet, known to minimize spillage [11]. The food was withdrawn immediately before the experiments.

All experiments had been approved by the regional ethical committee for animal experimentation and were performed according to international guidelines.

Experimental protocol and study design

The rats were randomly allocated to one of four groups: bile duct ligation (BDL), pair-fed (PF), ad libitum fed (AL), or control. In the BDL group, which constituted the experimental group, rats ($n = 27$) underwent a selective bile duct ligation. PF ($n = 27$), AL ($n = 28$), and control ($n = 18$) rats were subjected to a sham laparotomy. For each BDL rat, a corresponding PF rat was designated to exclude metabolic alterations due to starvation per se. Rats in the sham-operated control group, in contrast, were subjected to immediate sampling of tissue and blood to obtain an estimation of the impact of the surgical trauma on the experimental parameters. The BDL and AL groups were allowed food and water ad libitum postoperatively, whereas PF rats were allowed water ad libitum and food corresponding to the food intake of their respective BDL counterparts. Weight and cumulative food intake were registered daily for the BDL, PF, and AL groups. After 2, 4, and 8 d, respectively, the animals were reanesthetized and subjected to muscle and liver biopsies and blood sampling.

Anesthesia and surgical procedures

Anesthesia was provided by an intraperitoneal injection of pentobarbitone (40 mg/kg). The duodenum and the common bile duct were exposed through a midline incision. In BDL rats three ligatures were placed around the bile duct. The bile duct was then transected between the two sutures closest to the duodenum. In sham-operated rats (PF, AL, and control groups), a laparotomy was performed and the common bile duct exposed but not transected. In the control group tissue and blood sampling was performed immediately. Biopsies of the liver and soleus muscle tissue were freeze-clamped and put in liquid nitrogen. Thereafter blood samples were collected by heart puncture. All samples were stored at −70 °C until analysis. In PF and AL rats the abdomen was closed in two layers by running absorbable sutures. After surgery the animals were returned to individual cages placed under a heating lamp to maintain body temperature. There was no mortality in any group during the experimental period. At the time of tissue and blood sampling (2, 4, or 8 d postoperatively), rats were reanesthetized and treated as described above.

Blood chemistry

All samples were collected into ice-cold tubes containing aprotinin (400 KIU/mL; Trasylol, Bayer, Germany) and ethylenediaminetetra-acetic acid (5 mg/mL of blood). The samples were immediately separated in a refrigerated centrifuge, and the plasma was decanted and frozen at −70 °C for subsequent assay. Plasma glucose was determined enzymatically using a glucose analyzer model 2700 (Yellow Springs Instrument, Yellow Springs, OH, USA). Plasma concentrations of bilirubin, activities of alkaline phosphates and aminotransferases (aspartate and alanine aminotransferases) were determined with standard clinical laboratory methods. Plasma insulin was analyzed using a commercially available kit (Pharmacia Insulin RIA 100, Pharmacia-Upjohn, Uppsala, Sweden). Plasma amylase was measured using a quantitative colorimetric assay (Phadebas Amylase Test, Pharmacia-Upjohn). Plasma non-esterified fatty acids were determined by an enzymatic colorimetric method (NEFA-C, Wako Chemicals GmbH, Neuss, Germany).

Isolation of RNA and northern blot

Total cellular RNA was extracted from the liver and soleus muscle using the method of Chomczynski and Sacchi [12]. Briefly, 200 mg of tissue was immediately frozen in liquid nitrogen, followed by extraction with guanidium thiocyanate. The quality and concentration of RNA were determined by measuring the absorbance at 260 and 280 nm. Twenty micrograms of total RNA was separated on 1% agarose gel, containing 2% formaldehyde, and transferred to a nylon membrane (Zeta-Probe, Bio-Rad Laboratories, Hercules, CA, USA). The hybridization was carried out overnight at 65 °C for the UCP-2-probe and at 37 °C for the 18S probe.