Effects of supranormal liver glycogen content on hyperglucagonemia-induced liver glycogen breakdown

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Abstract The purpose of the present study was to test the hypothesis that a higher hepatic glycogen level is associated with higher glucagon-induced hepatic glycogen depletion. Four groups of anesthetized rats received three injections (at times 0, 30, and 60 min) of glucagon (intravenously, 20 μg/kg). Among these groups, hepatic glycogen levels had previously been manipulated either by an overloading diet (Fast-refed), a reduction in food intake (1/2-fast), or exercise (75 min of running, 26 m/min, 0% grade). A fourth group had normal hepatic glycogen levels. A fifth group of rats was injected only with saline (0.9% NaCl). Liver glycogen concentrations were measured every 30 min during the course of the 90-min experiment, using liver samples obtained from the open liver biopsy technique. Plasma glucagon concentrations were significantly higher ($P < 0.05$) in the glucagon-injected groups than in the saline-injected group. As expected, liver glycogen levels were significantly higher ($P < 0.01$; 1.6-fold) in the Fast-refed group than in all other groups. Glucagon-induced decreases in liver glycogen concentrations were similar in Fast-refed than in normally fed and exercised rats when the overall 90-min period was considered. However, during the course of the last 30-min period, liver glycogen was significantly ($P < 0.01$) decreased only in the Fast-refed group. The Fast-refed, normally fed, and exercised groups had a similar glucagon-induced hyperglycemia that was significantly more elevated ($P < 0.01$) than glucose levels measured in the saline-injected group. Glucagon-induced reactive hyperinsulinemia was observed only in the Fast-refed and normally fed rats, and not in the exercised and 1/2-fast rats. It is concluded that supranormal levels of liver glycogen may be associated with a larger hyperglucagonemia-induced liver glycogen breakdown.

Key words Insulin · Liver glycogenolysis · Hyperglycemia · Liver biopsies · Exercise

Introduction

The role of the liver as a source of glucose for the organism is well known. This function of the liver rests on the ability of this organ to produce glucose previously stored as glycogen and to make up glucose from gluconeogenic precursors. This function is particularly important when the demand for endogenous glucose is high, such as during fasting or prolonged exercise. The processes of hepatic glycogenolysis and gluconeogenesis are regulated by complex interactions of metabolic and hormonal stimuli (Bolleni et al. 1998). Among these stimuli, the specific effects of glucagon on hepatic glycogen breakdown deserve special attention since the hepatic glycogenolysis that occurs during fasting or moderate exercise is brought about mainly by glucagon (Exton 1987; Wasserman et al. 1989). Hepatic glycogenolysis accounts for nearly all of the initial (2 h) increase in glucose production in response to a physiological increment in plasma glucagon (Cherrington et al. 1981; Magnusson et al. 1995). Recently, it was reported that the liver of trained individuals is more responsive to a glucagon infusion than that of untrained individuals (Drouin et al. 1998). This suggests that training can sensitize the liver to the action of glucagon. On the other hand, trained individuals are more likely to have higher resting levels of liver glycogen, as they have for muscle glycogen (Adams and Koeslag 1989; Galbo et al. 1979). One factor that may regulate the effect of glucagon on the liver is the level of glycogen itself. By comparison, higher than normal initial levels of muscle and liver glycogen have been shown to increase muscle glycogenolysis and hepatic glucose production, respectively, during exercise (Hargreaves 1995; Vissing et al. 1989).
The purpose of the present study was to determine the influence of different initial levels of liver glycogen on glucagon-stimulated liver glycogen breakdown and related variables (plasma glucose and insulin levels) in rats. Our hypothesis was that supranormal initial hepatic glycogen levels brought about by dietary manipulations would result in a higher glucagon-induced liver glycogen breakdown.

Methods

Animal care

Male Sprague-Dawley rats (Charles River, Canada, St. Constant, Québec, Canada), weighing 240–260 g were housed in individual cages and allowed pellet rat chow and tap water ad libitum for 14 days after they were received into our laboratory. The lighting schedule was such that lights were on from 6:00 a.m. until 6:00 p.m., and the room temperature was maintained at 20–23 °C. During this time, rats underwent a habituation running protocol on a motor-driven rodent treadmill, consisting of ten sessions over 2 weeks, beginning with 15 min/day at 15 min/min, and progressively increased to 55 min/day at 30 min/min (0% grade), so that they were well accustomed to running and being handled. The last habituation session was held 48 h before the experiment for all rats.

Groups and surgery

After completion of their running habituation protocol, rats were randomly assigned to one of five experimental groups. The animals in four of these groups were injected with glucagon, and those in the fifth group were injected with saline (0.9% NaCl). Three of the four groups of glucagon-injected rats were subjected to different manipulations aimed at changing the initial liver glycogen content on the day (or 2 days) prior to the experiment. One group had its liver glycogen content overloaded (Fast-refed) by being subjected to a 24-h fast before access to the normal chow diet for 24 h. One group of rats (12–fast) received only 50% of their daily food intake on the night before experimentation. The third group of rats (Exercise) was normally fed but was subjected to 75 min of running exercise on a treadmill (26 m/min, 0% grade) 1 h before the beginning of the injections. The fourth group of glucagon-injected (Fed) and the saline-injected rats (Saline) were normally fed.

The entire experiment was conducted with the animals under anesthesia, so that on the morning of the experiment, rats in all groups were weighed and then anesthetized with pentobarbital sodium (40 mg/kg, administered intraperitoneally). For all rats, a catheter filled with sterile 0.9% NaCl was then implanted into the right jugular vein. A small mid-abdominal incision (2–2.5 cm) was then made to give free access to the liver. The incision was covered at all times with a sterile wet gauze. The whole surgery period lasted 15 min at most, and was followed by a 20-min recovery period before the beginning of the injections. Throughout the experiment, the body temperature of the animals was maintained with the aid of a heating blanket.

Experimental protocol

On the morning of the experiment, any remaining food was removed from the cages 2.5 h before the experiment, which was conducted between 9:00 a.m. and 12:00 a.m. After the surgical procedures described above, anesthetized rats received either three bolus injections (at times 0, 30, and 60 min) of glucagon (from porcine pancreas; Sigma-Aldrich Canada, Oakville, Canada) at a dose of 20 μg/kg dissolved in 1 ml diluting fluid (1.6% glycerine and 0.2% phenol) with saline (Geary et al. 1993), or an equivalent bolus of the vehicle only (saline), over a 1-min period. This dose of glucagon has been shown successfully to have effects on liver glycogen breakdown (Rao 1995). Each of the three glucagon injections was preceded by an open liver biopsy protocol, which itself was preceded by the removal of a jugular blood sample. A fourth liver biopsy sample was also taken at the end of the experiment (time 90 min), while blood was also sampled at times 15, 45, 75, and 90 min. Open liver biopsy samples were taken according to Daemen et al. (1986). After presenting the liver via the abdominal incision, a small piece of liver tissue (80–100 mg) was cut out of the edge of a liver lobe with a sharp scissors that had been cooled to liquid-nitrogen temperature. The use of a cooled scissors meant that there was almost no bleeding. The first three biopsy samples were taken from three different lobes, in the order: median, right, and left lobe. The last biopsy sample (end of the experiment) was taken from the median lobe. The whole biopsy procedure lasted for ≤20 s. In a pilot study, no significant (P > 0.05, one-way analysis of variance, ANOVA) effects of the open liver biopsy protocol on the catecholamine response were observed (Table 1). After the final blood and liver samples were taken, the soleus, gastrocnemius, and plantar muscles of the right leg were exposed. Samples of each muscle were removed, freeze clamped, and placed in liquid nitrogen.

Analytical methods

Blood was collected into 1-ml syringes containing ethylenediaminetetraacetic acid (7%). Major blood samples (1.3 ml) were obtained at times 0, 15, 30, 60 and 90 min. Minor blood samples (60 μl) were obtained at times 45 and 75 min, according to the protocol. 10 ml of blood was taken at the end of the experiment for the determination of catecholamine levels. Red blood cells obtained from blood centrifuged without preservatives were suspended in saline and reinjected into the animal. The fraction of blood (500 μl) to be used for glucagon determination was preserved in Trasylol (50 μl) before centrifugation. The fraction of blood (1.5 ml) to be used for catecholamine determination was transferred into tubes containing 50 μl of glutathione (60 mg/ml) and ethylene glycol-bis (β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (90 mg/ml), kept on crushed ice and centrifuged (4°C; at 1400 g, table Beckman GPRI centrifuge) within 30 min following collection. The remainder of the blood was also centrifuged at 6000 g (Eppendorf centrifuge, no. 5415), and the plasma was stored for subsequent determinations of glucose, insulin, free fatty acids (FFA), glyceral, and β-hydroxybutyrate. All tissue and blood samples were stored at −78 °C until analyses were performed.

Plasma glucose concentrations were determined with the aid of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, Ohio, USA). Insulin and glucagon concentrations were determined by commercially available radioimmunoassay kits (Radioassay System Laboratory; ICN Biomedicals, Costa Mesa, Calif., USA; distributed by Immunocorp, Montreal, Québec, Canada). FFA, glyceral, and β-hydroxybutyrate were assessed enzymatically with the aid of reagent kits from Boehringer Mannheim (distributed by Immunocorp). Catecholamines were extracted from plasma according to the procedure described by Répiec and Zaagsma (1986) and quantified by means of an isocratic high-performance liquid chromatography system (Waters Division, Millipore Corporation, Milford, MA). The mobile phase was a mixture of methanol, acetonitrile, and water (70:20:10), and the analytes were detected by ultraviolet detection (280 nm).

Table 1 Norepinephrine and epinephrine responses measured immediately after liver biopsy samples were taken, at different times during the course of the experiment in a pilot study. Values are presented as the means (SE) in ng/ml

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.24 (0.01) (n = 4)</td>
<td>0.57 (0.01) (n = 3)</td>
</tr>
<tr>
<td>30</td>
<td>0.21 (0.01) (n = 4)</td>
<td>0.53 (n = 1)</td>
</tr>
<tr>
<td>60</td>
<td>0.21 (0.01) (n = 4)</td>
<td>0.56 (0.02) (n = 4)</td>
</tr>
<tr>
<td>90</td>
<td>0.30 (0.07) (n = 4)</td>
<td>0.57 (0.03) (n = 5)</td>
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