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Metabolic Effects of Short Term Food Deprivation in the Rat

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Introduction

Amino acids are known to be utilized in postabsorptive states both as metabolic fuels and gluconeogenic substrates (Aikawa, Matsutaka, Yamamoto, Okuda, Ishikawa, Kawano and Matsunaga 1973; Exton 1972; Felig 1973). With starvation, other fuels, such as lipids and their derivatives are also preferentially used for energetic purposes (Herrera and Freinkel 1968; Owen, Felig, Morgan, Wahren and Cahill 1969). In the fasted state there is an increased flux of metabolites from peripheral tissues towards the liver (Blackshear, Holloway and Alberti 1974) which increases the rate of uptake of these materials (Nallathambi, Goorin and Adibi 1972). During food deprivation there is a net flux of amino acids and other metabolites from peripheral tissues towards the liver. With food deprivation, mammals develop an efficient mechanism for nitrogen conservation, particularly for essential amino acids. The metabolic changes during fasting, including the mechanisms for amino acids utilization, and the
Effect of Combined Adrenomedullation and Chemical Sympathectomy

In adrenomedullated and chemically sympathectomized rats, the plasma concentration of insulin was consistently higher and the plasma concentration of glucagon lower than in control rats. However, compared to controls, hepatic glycogen depletion during exercise was not significantly lower in adrenomedullated and chemically sympathectomized rats. In these rats, the blood glucose concentration did not change during exercise whether the rats were treated with glucagon antibodies or not.

Discussion

The present study has shown that in the rat, administration of antigen-stripped glucagon antibodies of high affinity decreases the exercise-induced hepatic glycogen depletion and the plasma insulin concentration after exercise (Table 1). Previous studies of hepatic glycogen depletion during exercise using glucagon antisera have given varying results (Galbo and Holst 1976; Galbo et al. 1978). However, the antibodies used in those studies were not antigen-stripped and only reduced the concentration of free glucagon to about 35% of control values, whereas the antigen-stripped glucagon antibodies used in the present study may reduce the plasma concentration to undetectable values (Holst, Galbo and Richter 1978). The more pronounced lowering of the free glucagon concentration possibly explains why, in the present study, could demonstrate a marked inhibition of hepatic glycogen depletion when glucagon antibodies were administered. Furthermore, in the present study, the application of a liver biopsy technique made it possible to determine the exercise-induced hepatic glycogen depletion in individual rats. Accordingly, we were probably able to measure hepatic glycogen depletion more accurately than in our previous studies. The fact that in the present study hepatic glycogen depletion was decreased by glucagon antibodies more markedly in rats with an intact sympatoadrenal system than in adrenomedullated and sympathectomized rats (Table 1) points to a concentration-dependent effect of glucagon on hepatic glycogen breakdown, since the concentration of plasma glucagon in the latter was lower than in the former (Table 1). Others have suggested that the effect of glucagon on hepatic glucose output during exercise is permissive: In pancreatectomized, insulin-infused dogs the hepatic glucose production increased in the absence of an increase in the plasma (gastrointestinal) glucagon concentration (Vranic and Kawamori 1979), and the increase was only diminished when the plasma glucagon concentration was decreased below the resting concentration by infusion of somatostatin (Isselzutz and Vranic 1980). Hepatic glycogen depletion has recently been shown to be reduced during exercise in adrenomedullated (Richter et al. 1980) and in adrenomedullated and chemically sympathectomized rats (Galbo et al. 1978) compared to controls. In the present study, combined adrenomedullation and chemical sympathectomy did not significantly reduce the exercise-induced hepatic glycogen depletion, although such a trend was observed (Table 1).

The difference in the plasma insulin concentration between glucagon-antibody treated and control rats was not due to a difference in the plasma glucose concentration (Table 1), neither could it be ascribed to altered sympatho-adrenal activity in antibody-treated rats compared to controls, since glucagon antibodies decreased the insulin concentration, also, in adrenomedullated and sympathectomized rats (Table 1). Thus, glucagon seems to stimulate insulin secretion in the exercising rat. Whether this stimulation of insulin secretion is due to a direct action of glucagon on the β-cell or is indirectly effected by glucagon, e.g., via glucagon-induced changes in the concentrations of metabolites influencing the β-cell, cannot be concluded from the present study.

A stimulating role of glucagon on insulin secretion at rest has previously been suggested (Epand and Douglas 1973; Samols, Mari and Marks 1965). Furthermore, in three dogs the concentration of glucagon and of insulin in plasma were higher and the glucose concentration lower during exercise with intact splanchnic nerves than during exercise after sectioning of the splanchnic nerves (Girardier, Seydoux, Berger and Viecsteinas 1978). It seems likely, that the lower plasma insulin concentration during exercise after cutting the splanchnic nerves was due to reduced stimulation of insulin secretion by glucagon, and not due to reduced splanchnic nerve enhancement of insulin secretion. This is so, since during exercise — a state where the overall sympathetic drive is increased — activity in the sympathetic fibers of the splanchnic nerves [which inhibit insulin secretion (Porte et al. 1973)] probably predominates relative to activity in the parasympathetic fibers of the splanchnic nerves [which stimulate insulin secretion (Porte, Girardier, Seydoux, Kanazawa and Pasternak 1973)]. Also, in vitro experiments have suggested a paracrine-stimulating effect of glucagon on insulin secretion (Sorensen, Eikle and Seybold 1979).

Combined adrenomedullation and sympathectomy increased and decreased the plasma concentration of insulin and of glucagon, respectively, after exercise (Table 1). These findings were not explainable by a difference in the plasma glucose concentrations between rats with and without an intact sympatho-adrenal system (Table 1), but may be explained by the lack of a suppressive and stimulatory effect of catecholamines on insulin and glucagon secretion, respectively (Richter et al. 1980; Unger, Dobbs and Orci 1978).

In conclusion, the present findings indicate that in exercising rats, pancreatic glucagon enhances hepatic glycogenolysis. Furthermore, the findings suggest that glucagon and the sympato-adrenal system increase and decrease, respectively, insulin secretion during exercise.

References


Materials and Methods

Female virgin Wistar rats weighing initially 174 ± 5 g were used. The animals were housed in collective cages with wire-mesh bottoms (in order to prevent coprophagia). The cages were kept in a temperature (23 °C) and light controlled (12 hours on/12 hours off) environment. The animals were randomly divided into groups and their food (rat chow pellets) was withdrawn at 0, 3, 6, 12, and 24 hours before the onset of the light cycle. During this period the animals were handled and internal circadian rhythms (Aro/a, Palou, Herrera and Alemany 1976) were studied in plasma protein concentration or in stored muscle water content. The hematocrit value only showed a significant transient increase at 12 hours of food deprivation.

Liver glycogen concentration (Table 2) had already decreased considerably after 3 hours of fasting — about 50% that of controls — dropping later to about 15% that of controls at 24 hours. Muscle glycogen content was considerably smaller and there were no significant differences versus controls during the 24 hour period studied in plasma protein concentration or in striated muscle water content. The hematocrit value only showed a significant transient increase at 12 hours of food deprivation.

Results

In Table 1 the changes in body weight experienced by rats subjected to food deprivation can be seen. Fasting produced a rapid and substantial decrease in liver weight, significant from 3 hours onwards. This effect seems to be more related to a loss of dry matter than to a loss of water. There were no significant changes in the 24 hour period studied in plasma protein concentration or in striated muscle water content. The hematocrit value only showed a significant transient increase at 12 hours of food deprivation.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>174.6 ± 5.0</td>
<td>171.6 ± 4.8</td>
<td>174.8 ± 5.2</td>
<td>169.8 ± 3.0</td>
<td>167.8 ± 4.8</td>
</tr>
<tr>
<td>Body weight loss (%)</td>
<td>0.0</td>
<td>3.5 ± 0.2*</td>
<td>5.6 ± 0.5*</td>
<td>5.7 ± 0.3*</td>
<td>8.0 ± 1.0*</td>
</tr>
<tr>
<td>Liver wet weight (in % of body weight)</td>
<td>5.26 ± 0.21</td>
<td>4.30 ± 0.12*</td>
<td>3.98 ± 0.08*</td>
<td>3.63 ± 0.15*</td>
<td>3.43 ± 0.10*</td>
</tr>
<tr>
<td>Liver dry weight (in % of body weight)</td>
<td>1.46 ± 0.08</td>
<td>1.19 ± 0.02*</td>
<td>1.10 ± 0.03*</td>
<td>1.02 ± 0.04*</td>
<td>0.96 ± 0.06*</td>
</tr>
<tr>
<td>Liver water content (%)</td>
<td>72.1 ± 0.4</td>
<td>71.2 ± 0.3</td>
<td>70.5 ± 0.4*</td>
<td>70.2 ± 0.4*</td>
<td>69.4 ± 0.2*</td>
</tr>
<tr>
<td>Striated muscle water content (%)</td>
<td>76.0 ± 0.6</td>
<td>76.0 ± 0.6</td>
<td>76.3 ± 0.3</td>
<td>76.8 ± 0.6</td>
<td>76.2 ± 0.3</td>
</tr>
<tr>
<td>Total plasma proteins (g/l)</td>
<td>69.0 ± 1.8</td>
<td>75.2 ± 0.9</td>
<td>70.4 ± 1.2</td>
<td>72.4 ± 1.0</td>
<td>71.9 ± 1.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.7 ± 1.1</td>
<td>43.6 ± 1.5</td>
<td>47.2 ± 0.9</td>
<td>49.1 ± 1.2*</td>
<td>46.6 ± 1.4</td>
</tr>
</tbody>
</table>

All data are the mean ± s.e.m. of 5–6 different animals per group. Significance of the differences versus fed controls (0 hours): * p < 0.05
Table 2 Glycogen stores in liver and striated muscle in rats subjected to short term food deprivation

<table>
<thead>
<tr>
<th>Hours of food deprivation</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen content in the liver (µmoles of glycosyl residues/g tissue)</td>
<td>238 ± 25</td>
<td>107 ± 22*</td>
<td>83 ± 10*</td>
<td>52 ± 7*</td>
<td>36 ± 6*</td>
</tr>
<tr>
<td>Mean glycogen content in whole liver (µmoles of glycosyl residues)#</td>
<td>2128</td>
<td>746</td>
<td>512</td>
<td>284</td>
<td>173</td>
</tr>
<tr>
<td>Cumulative loss (µmoles of glycosyl residues)</td>
<td>1382</td>
<td>1616</td>
<td>1844</td>
<td>1955</td>
<td>1955</td>
</tr>
<tr>
<td>Glycogen content in striated muscle (µmoles of glycosyl residues/g tissue)</td>
<td>46 ± 9</td>
<td>28 ± 6</td>
<td>23 ± 7</td>
<td>27 ± 7</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Mean glycogen content in whole striated muscle (µmoles of glycosyl residues)#</td>
<td>3350</td>
<td>2141</td>
<td>1813</td>
<td>1826</td>
<td>1191</td>
</tr>
<tr>
<td>Cumulative loss (µmoles of glycosyl residues)</td>
<td>1209</td>
<td>1537</td>
<td>1524</td>
<td>2159</td>
<td>2159</td>
</tr>
</tbody>
</table>

# corrected to the initial weight of controls
All data are the mean ± s.e.m. of 5-6 different animals per group
Significance of the differences versus fed controls: * = p < 0.05

content. The cumulative loss of liver glycogen during fasting is roughly equal to that of muscle glycogen.

Plasma urea, lactate and glucose concentrations (Figure 1) showed significant decreases within 3 hours, followed by a steady decline in concentration up to 24 hours.

There was a rapid initial increase in glycerol concentration during the first 3 hours, followed by a slower increase up to 24 hours. Plasma free fatty acids followed a similar pattern. There was a continuous increase in plasma acetoacetate and beta-hydroxybutyrate with fasting; the changes being significant from 3 hours onwards.

The changes in individual and composite amino acid concentrations with fasting can be seen in Figure 1 and 2. The drop in concentration of both total and essential amino acids with fasting was significant within 3 hours, with a transient recovery at 12 hours followed by a further small but significant drop at 24 hours. The non-essential amino acids did not show any significant change until 24 hours at which time they were significantly decreased.

Discussion

The experimental scheme used actually corresponds to a post-prandial situation, as the mean food transit time in the rat digestive system is similar to the shortest experimental food deprivation time used. However, important changes in plasma and liver composition of the rat are observed with so short a food deprivation time.

Liver and body size changes observed during food deprivation — that more directly affect changes in liver dry matter (Kaplan and Chaikoff 1936) — together with the absence of detectable hemoconcentration, suggest that liver protein content decreases substantially during fasting in agreement with the findings of Herrera, Knopp and Freinkel (1969).

The relative maintenance of plasma glucose concentration is based on the balance between the increase in liver glycolysis and gluconeogenesis, as well as a decrease in glucose utilization, mainly through substitution by other substrates.
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Fig. 2 Effects of short term food deprivation on the rat plasma levels of gluconeogenic amino acids, amino acids and basic and sulphur amino acids.

All values are the mean ± s.e.m. of 5-6 different animals; the results are expressed in µmoles/l.

The symbols used are the same as in Figure 1.

The provision of glucose, at least during the very first stages of food deprivation, depends mainly on liver glycogenolysis. However, when the total liver and muscle glycogen content are calculated, it is apparent that by 24 hours of food deprivation, both glycogen stores have contributed roughly to the same total amount of glucose or gluconeogenic precursors.

Gluconeogenesis from peripheral tissue released amino acids, lactate and glycerol is another important factor in maintaining an adequate supply of glucose during short periods of starvation. Gluconeogenesis from lactate is enhanced from 3 hours to 12 hours of fasting in the rat (Schimmel and Knobil 1970), coinciding with the maximal incidence of lactate transport and uptake by the liver. In spite of the increased lactate extraction (Krebsberg, Pennington and Boshell 1970), plasma lactate levels are relatively constant from about 3 to 6 hours until 24 hours, in agreement with the known actuation of the Cori cycle.

The availability of glycerol is considerably enhanced during fasting, due to an increased adipose tissue lypolysis (Knopp, Herrera and Freinkel 1970) that also frees fatty acids. In the rat, fasting induces a four to five-fold increase in glucose synthesis from glycerol (DeFreitas and Depocas 1970; Friedman, Goodman and Weinhouse 1970).

The extraordinary increase in plasma ketone bodies with fasting is mainly due to increased fatty acid metabolism by the liver (Bates, Linn and Huen 1976) favoring the use of ketone bodies for energetic purposes, thus freeing glucose for use by the obligatory glycolytic tissues (Cahill 1970).

Plasma urea concentration decreased throughout the 24 hour period of fasting in agreement with Owen et al. (1969) and Cahill (1970) as a consequence of the decrease in dietary amino acid availability, together with ketone body-induced acidosis counterbalanced by an increase in ammonia production (Herrera, Knopp and Freinkel 1969), which reduces nitrogen availability for urea synthesis.

From the data presented it can be postulated that amino acid utilization is increased during the first hours of fasting not being completely compensated then by amino acid release from peripheral tissues (Schimmel and Knobil 1970). With a more prolonged fast, a variety of mechanisms for nitrogen conservation prevent waste of amino nitrogen (Aoki et al. 1975), explaining the partial recovery during starvation of basal concentrations of several amino acids (Aoki et al. 1975; Palou, Alemany and Herrera 1978). The decrease in plasma urea and total nitrogen excretion is consistent with the activation of nitrogen conservation mechanisms (Cahill, Herrera, Morgan, Soeldner, Steinke, Levy, Reichard and Kipnis 1966; Cahill, Owen and Morgan 1968; Cahill 1970). In addition, increased free ammonia concentrations favor gluconeogenesis from lactate by the liver (Grumet and Katz 1978) thus sparing amino acids.

The flux of substrates from peripheral tissues towards the liver is increased in the fasted state (Felig 1973; Blackshear, Holloway and Alberti 1974), and the liver shows enhanced amino acid uptake (Nallathambi, Goorin and Adibi 1972); during fasting it is likely that a major fraction of amino acids released by peripheral tissues donate their alpha-amino nitrogen for synthesis of alanine, glutamine, glutamate, serine and glycine (Aikawa et al. 1973; Felig 1973). The partial rectification of glucose carbon in the form of certain amino acids, such as alanine, through the glucose alanine cycle (Felig 1973) can explain the relative stability in concentration of these gluconeogenic amino acids.

The changes in amino acid concentrations in rat plasma during short term food deprivation are qualitatively different from those found during longer fasting in rats (Henderson, Schurr and Elvejam 1949; Palou, Alemany and Herrera 1978).

The general metabolism of the rat subjected to short term food deprivation, as observed by its plasma composition, shows important changes and adaptations to the lack of food, that are detectable from 3 hours onwards. These adaptations imply the exhaustion of glycogen stores, the progressive use of lipid stores and the beginning of the metabolism of amino acids from peripheral tissues.

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