HORMONAL EFFECTS ON GLUCONEOGENESIS FROM (U-14C)GLUTAMATE IN RAINBOW TROUT (SALMO GAIRDNERI)

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Abstract—1. Blood glucose was significantly decreased by insulin (4 IU/kg). Glucagon (1 mg/kg) and Cortisol (5 mg/kg) administration produced a significant hyperglycaemia.
2. Insulin administration did not modify liver glycogen levels. Glucagon showed a marked liver glycogen mobilization. Cortisol stimulated liver glycogen deposition.
3. Insulin and Glucagon showed a significant inverse effect on gluconeogenesis from (U-14C)glutamate, decreasing and increasing 14C-glucose formation respectively.
4. Hormonal treatments did not influence the very low levels of incorporation of (U-14C)glutamate into liver and muscle glycogen.

INTRODUCTION

The diet of carnivorous fish contains high levels of protein and, at the same time, little carbohydrate. As glucose is required as a primary fuel in certain tissues, gluconeogenesis from amino acids may be an important pathway when such fish take a normal high protein diet. The first direct evidence of gluconeogenesis in fish was provided by Hayashi and Ooshiro (1975) who showed it in perfused eel liver. Since then, there has been very little information available on the regulation of this process, especially in relation to the influence of diet composition and of hormones. Cowey et al. (1977a,b) showed that gluconeogenesis from alanine was markedly regulated by diet composition and insulin, both at enzyme level and by modifying substrate availabilities (concentrations). The gluconeogenic use of dietary amino acids can be basically regulated by substrate concentrations and also by a negative feedback effect provoked by glucose as the result of the gluconeogenic process. Endocrine mechanisms must exist to regulate both these, as well as the enzymes involved in the process.

The aim of this work was to discover the effect of insulin, glucagon and cortisol on the 'in totum' gluconeogenesis from glutamate, and amino acid, which plays a central role in amino acids metabolism. The hormones used in this work may affect the amino acids pool by decreasing, not altering or increasing substrate concentrations. The amino acids pool undergoes the same metabolic reactions as in mammals but at different rates and probably under different quantitative and qualitative regulating mechanisms. In that sense, the object of the present work is to throw some light on the endocrine regulation of gluconeogenesis in the whole animal, therefore the method of Friedman et al. (1965) was used. The hormonal effects on blood glucose and glycogen levels were also studied. Furthermore, as the trout is a carnivorous fish, in order to obtain maximal incorporation rate of (U-14C)glutamate into glucose, a high protein diet was used in the assays.

MATERIAL AND METHODS

Animals and maintenance

Rainbow trout, obtained from a local fish farm, of mean weight approximately 150 g, were randomly distributed in five fiberglass tanks (1.2 x 0.7 x 0.8 m), with ten fish/tank. Water, after being filtered, was kept at 15 ± 1°C, in an open circuit, at the rate of 1.5 l/min per kg biomass of fish. Trout were fed on a high protein diet of composition as shown in Table 1. The dry components of the diet were blended with sodium alginate (50 g/kg) and then thoroughly stirred with distilled water to get a homogeneous humidified mixture. Pellets were made by passing the diet mixture through an electric meat grinder equipped with dies of 2.5 mm hole size. After drying, the diet was kept in a deep freeze at -20°C. Trout were fed manually to satiation twice a day (9.00 and 17.00 hr). Light/dark period was 12/12 hr. After adapting to normal feeding, the trout were fed the experimental diet for 30 days, under the same conditions.

Glucogenesis from L-(U-14C)glutamate

L-(U-14C)glutamate, obtained from the Radiochemical Center of Amersham, was diluted with L-glutamate to give a solution containing 2.5 μCi/mmol in 1.0 ml 0.9% NaCl. After an overnight fast, trout were lightly anesthetized by placing them in water containing 0.1 g MS222 (ethyl-m-aminobenzoic acid methane sulphonate; Sandoz Ltd.)/l, for about 2 min. The fish were then given intraperitoneal injections of radioactive glutamate at the dose of 2.5 μCi/100 g body weight and placed back in the tank. In order to
determine the shortest time for maximal incorporation of glutamate into glucose, fish were killed, at a time course, by a blow on the head, and blood from the caudal vein was taken using a heparinized syringe. Blood was deproteinized by the method of Somogy (1945) and aliquots of the protein-free supernatant were passed through mixed-bed resin (Amberlite, MG-3 analytical grade, BDH Ltd.) columns (16 x 200 mm). Column eluate plus washings was concentrated to dryness at 35°C on a rotary film evaporator and redissolved with 5 ml distilled water. The amount of radioactive glucose in this solution was then measured using a liquid-scintillation spectrometer. The efficiency of counting was 83%. When glucose was isolated by thin layer chromatography on cellulose plates it was shown that more than 95% of the radioactivity was present in glucose. It was also confirmed that no ninhydrin-positive material was collected from the Amberlite column after passing an amino acids mixture solution containing glutamate through it. The glucose was quantitatively recovered from the eluate at the same time.

The amount of incorporation of glutamate into glucose was calculated from the relation given by Deodhar and Mistry (1969):

\[
\text{Incorporation (\%)} = \frac{\text{Body weight (g) \times diffusion space}}{\text{Blood glucose (disintegrations/min/ml) \times Glutamate injected (disintegrations/min)}} \times 100.
\]

Glucose diffusion space in trout was found to be 13.7% of the body weight (Cowey et al., 1977a), measured by the dilution principle after intravascular injection of a trace dose of (U-14C)glucose. The incorporation of 14C-glucose into liver and muscle glycogen was measured after glycogen hydrolysis using the relation given by Deodhar and Mistry (1969):

\[
\text{Incorporation (\%)} = \frac{\text{Disintegrations/min/g tissue}}{\text{Disintegrations/min injected}} \times 100.
\]

Hormonal treatments

Bovine insulin, glucagon and cortisol (Sigma Chemical Co. Ltd.) were administered at the rate of 4 I.U./kg fish (intravascularly), 1 mg/kg (intravascularly) and 5 mg/kg body weight (intramuscularly). After determining the time of maximal incorporation of (U-14C)glutamate into blood 14C-glucose, subsequent assays were carried out in order to determine the maximal effect for hormonal administration, in relation to the time for maximal rate of gluconeogenesis from glutamate, previously determined.

Chemical methods

For total glucose determinations trout were killed by a sharp blow on the head and blood taken from the caudal vein using heparin as anticoagulant. After centrifugation, a plasma aliquot was deproteinized with 3 N perchloric acid and glucose measured in the supernatant by a glucose oxidase method (Krebs et al., 1964). Liver and muscle glycogen were also measured by the glucose oxidase method after KOH (40% w/v) digestion, absolute ethanol precipitation and further hydrolysis with 4 N SO4H2.

RESULTS

Time-course of incorporation of glutamate into glucose. Hormonal actions time

Figure 1 shows the time-course of incorporation of (U-14C)glutamate into blood glucose in trout given a protein-free high carbohydrate diet in order to obtain a high rate of gluconeogenesis from amino acids. Each point represents the mean value for four fish. The incorporation reaches a plateau 6 hr after the injection of (U-14C)glutamate, therefore for experiments of hormonal effects on gluconeogenesis in trout, fish were examined 6 hr after intraperitoneal injection of substrate. This time is also helpful for comparison of these results with those obtained previously with alanine (Cowey et al., 1977a). The effect of a simultaneous intravascular injection of insulin on precursor incorporation is also shown in Fig. 1. Insulin reduced gluconeogenesis from glutamate to very low levels.

Figure 2 shows the time-course of incorporation of (U-14C)glutamate into glucose in trout fed the high protein diet after the administration of glucagon and cortisol. Each point represents the mean value for four fish at different times after hormonal treatment, the precursor always being injected 6 hr before deter-
maining each point of the time-course, in order to allow higher rates of incorporation. As can be observed from Fig. 2, the effect of glucagon on in totum gluconeogenesis is greater 6 hr after its administration. Cortisol maximal action took place 5 hr after its administration.

Effects of hormonal treatments on blood glucose and liver and muscle glycogen

Table 2 shows the influence of insulin (4 I.U./kg), glucagon (1 mg/kg) and cortisol (5 mg/kg) treatment on blood glucose in trout fed a high protein-free carbohydrate diet, in order to avoid interactions with glucose from dietary origin. Insulin produced a significant blood glucose decrease 6 hr after intravascular administration. On the contrary, glucagon and cortisol significantly enhanced blood glucose levels in the same proportion, at the doses employed.

Liver glycogen (Table 2) was not altered by insulin treatment. Nevertheless glycogen was significantly decreased by glucagon administration and significantly increased by cortisol. In muscle, glycogen levels were significantly diminished and enhanced by insulin and glucagon administration respectively.

Hormone effects on gluconeogenesis in the whole fish

Results of endocrine effects on (U-14C)glutamate incorporation into blood glucose are shown in Table 3. In the controls, which were given a sham injection of 0.9% NaCl, insulin significantly reduced the rate of incorporation. On the other hand, glucagon increased in totum gluconeogenesis from glutamate. Cortisol did not modify gluconeogenesis at the time of its maximal gluconeogenic action compared to controls.

The percentage of incorporation into liver and muscle glycogen was very low in all the experimental groups, giving significant differences (Table 3).

**DISCUSSION**

The relative capacity of trout to exert a certain metabolic control over gluconeogenesis when fed on different dietary regimes (Cowey *et al.*, 1977a; de la Higuera and Cardenas, 1985) contrasts with their inability to control blood glucose closely.

According to this work the glycaemia could be closely controlled by endocrine mechanisms if there were an adequate hormonal response to variations in glucose concentrations. This apparent endocrine imbalance is probably associated with a metabolic adaptation to diets normally low in available carbohydrates, where gluconeogenesis is the major pathway of glucose formation. Furthermore, no glucokinase activity could be found in trout liver even after prolonged feeding on high carbohydrate diets (Cowey *et al.*, 1977b) with the consequent inability to metabolize high glucose levels. On the contrary, trout gluconeogenesis can adapt to large changes in diet composition (Cowey *et al.*, 1977a; de la Higuera and Cardenas, 1985). Blood glucose levels in mammals is relatively closely regulated by endocrine mechanisms, mostly by insulin.

Rainbow trout insulin levels are of a similar order to those of mammals and vary greatly when animals are made to fast or fed a high protein diet (Thorpe and Ince, 1976), the apparent correlation between plasma amino acids and insulin indicates that the signal for insulin release is the circulating amino acids and not glucose (Tashima and Cahill, 1968; Thorpe and Ince, 1976), the apparent correlation between plasma amino acids and insulin indicates that the signal for insulin release is the circulating amino acids and not glucose (Tashima and Cahill, 1968; Thorpe and Ince, 1976). Other evidence comes from glucose intolerance tests (Palmer and Ryman, 1972). In any case, the regulating factors involved in insulin release remain unknown.

Gluconeogenesis from glutamate was maximal 6–8 hr after its administration. This time-course represents a certain delay and lower maximal incorporation rates than those obtained with alanine (Cowey *et al.*, 1977a) for a high protein diet. These results could be the consequence of a longer gluconeogenic pathway route towards glucose formation and its incorporation rate shows that glutamate is a poor gluconeogenic precursor, which could be the consequence of a number of factors such as deamination rate, implication in transdeaminating reactions etc. Glutamate incorporation into blood glucose was

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**Table 2. Effect of insulin, glucagon and cortisol treatment on blood glucose and muscle glycogen. (Mean ± SEM of six fish)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/100 ml blood)</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>167 ± 9</td>
<td>57.44 ± 3.34</td>
<td>6.31 ± 0.32</td>
<td>0.92 ± 0.25</td>
</tr>
<tr>
<td>Insulin</td>
<td>185 ± 23</td>
<td>32.58 ± 1.50*</td>
<td>6.19 ± 1.90</td>
<td>0.15 ± 0.04*</td>
</tr>
<tr>
<td>Glucagon</td>
<td>158 ± 16</td>
<td>101.71 ± 2.19*</td>
<td>4.95 ± 0.16f</td>
<td>1.85 ± 0.29f</td>
</tr>
<tr>
<td>Cortisol</td>
<td>115 ± 6</td>
<td>101.35 ± 5.72*</td>
<td>8.77 ± 0.41*</td>
<td>0.05 ± 0.13</td>
</tr>
</tbody>
</table>

*P < 0.001; †P < 0.01; ‡P < 0.05.

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**Table 3. Effect of insulin, glucagon and cortisol treatment on incorporation of U-14C-glutamate into blood glucose and liver and muscle glycogen. (Mean ± SEM of six fish)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Glycogen (mg/g tissue)</th>
<th>Blood glucose (% administered radioactivity)</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>128 ± 4</td>
<td>0.522 ± 0.060</td>
<td>0.025 ± 0.009</td>
<td>0.010 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>122 ± 5</td>
<td>0.225 ± 0.050*</td>
<td>0.046 ± 0.007</td>
<td>0.009 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Glucagon</td>
<td>153 ± 16</td>
<td>0.826 ± 0.050*</td>
<td>0.028 ± 0.006</td>
<td>0.007 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>123 ± 8</td>
<td>0.619 ± 0.096</td>
<td>0.020 ± 0.003</td>
<td>0.012 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.01.
markedly reduced by insulin administration. This effect should be attributed either to a lower substrate availability or to gluconeogenic enzymes modulation by insulin or a combination of both effects. It has been demonstrated that insulin decreases plasma amino acid levels (Inui et al., 1975; Cowey et al., 1977b; Ince and Thorpe, 1978) and by reducing amino acids degradation (Cowey et al., 1977b). Insulin control of enzyme activities is another mechanism to reduce gluconeogenesis. This hormone decreases liver phospho-enol-pyruvate-carboxy-kinase and pyruvate-kinase (Cowey et al., 1977b) and liver glutamate dehydrogenase and kidney aminotransferases (Cardenas, 1980) activities in rainbow trout.

Total liver glycogen levels were not significantly modified by insulin administration. The same results have been reported for trout (Cowey et al., 1977a,b) and Northern pike (Ince and Thorpe, 1976). The significantly lower muscle glycogen levels could be attributed to an increased glycogenolysis that might be due to an increase of y-amylase activity, accompanied by activation of the lysosomal activity, as observed in carp liver by Murat (1976). On the other hand, liver and muscle radioactive glycogen coming from (U-14C)glutamate was not modified by insulin administration.

The hyperglycaemia induced by glucagon administration has been demonstrated in trout (Morata et al., 1982a,b) and other fish (Ince and Thorpe, 1977; Murat et al., 1978). This hyperglycaemic effect of glucagon could be attributed, as in mammals, to an increased liver glycogenolysis together with gluconeogenesis activation. The relative importance of both mechanisms is not clear in fish where there are important interspecific differences. Murat et al. (1978) observed that in carp the hyperglycaemic effect of glucagon is mostly due to the stimulation of gluconeogenesis. Similar results were obtained by Renaud and Moon (1980) using isolated cell hepatocytes. Nevertheless, Birnbaum et al. (1976) have shown that in isolated goldfish hepatocytes, glycogenolysis was maximally activated by glucagon through increasing cyclic-AMP accumulation in the hepatocyte, as has been postulated for mammals (Johnson et al., 1972). Morata et al. (1982a), using a-cyano-3-hydroxy-cinamate as gluconeogenic inhibitor in trout, showed that glucagon exerts its hyperglycaemic effects through gluconeogenesis and not glycogenolysis. On the other hand, present results show that in trout the hyperglycaemic effect of glucagon is partly due to an activation of liver glycogenolysis and partly provoked by gluconeogenesis stimulation. The effect of glucagon on increasing muscle glycogen levels is not clear at present, but could represent an adaptation to starving, where glucagon, direct or indirectly, would facilitate glucose transfer from liver to muscle—aspects that need future research.

The action of glucocorticoids in higher vertebrates is well established: they increase gluconeogenesis and glycogenolysis; but in fish, the responses to these hormones are sometimes different. Most fish increase blood glucose in response to glucocorticoids administration (Patent, 1970), hyperglycaemia being the consequence of an activation of the gluconeogenic process. In fish, enhanced gluconeogenesis can be associated with glycogensynthesis (Lewander, 1976; Epple and Lewis, 1977). In most cases, liver glycogen levels remain unaltered by glucocorticoids administration (Storer, 1967; Patent, 1970). The glucocorticoids effects obtained here are in agreement with those for mammals, nevertheless, the gluconeogenesis rate obtained was not significantly increased to explain higher blood glucose levels. In this respect it has to be pointed out that gluconeogenesis was evaluated by measuring glutamate incorporation into blood glucose, leaving other gluconeogenic precursors available whose levels may be increased by cortisol action on peripheral tissue proteins. As was discussed for insulin, cortisol effects could be related to substrate availabilities and the increased gluconeogenesis would be a consequence of increased amino acid concentrations from protein breakdown. Peret and Chanez (1976) have shown that in the rat the enzyme phospho-enol-pyruvate-carboxy-kinase was not affected by cortisol when rats were fed a high protein diet (50% protein), but was when the diet contained 10% protein, which is a substrate saturation effect.

The regulation of gluconeogenesis by hormones has been well established in omnivorous mammals. The metabolic role of insulin, glucagon and cortisol on gluconeogenesis is somewhat similar in fish although the site and mechanism of hormonal action remains uncertain and needs future work to be done in order to get a better understanding of the endocrine regulation of fish metabolism.

REFERENCES


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