Response of enzyme activities and metabolic intermediate concentrations to glucagon administration in hepatopancreas and muscle of carp

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SUMMARY: An experiment was carried out to investigate the effect of an intraperitoneal glucagon administration (60 μg/kg) on enzyme activities and metabolic intermediate concentrations in the hepatopancreas and muscle of common carp Cyprinus carpio. In the hepatopancreas, glycogen phosphorylase activity together with cyclic adenosine-5′-monophosphate (AMP) concentration were significantly increased, and glycogen content was significantly decreased, at 1 h after the glucagon administration. Additionally, glucose-6-phosphatase and fructose-1,6-bisphosphatase activities as well as plasma glucose and free amino acid concentrations were increased after its administration. In the muscle, glucose and glycogen contents increased after the administration. Furthermore, the hormone administration also increased phosphofructokinase activity together with fructose-6-phosphate, AMP, and adenosine-5′-diphosphate concentrations and decreased adenosine-5′-triphosphate concentration. From 3 h after the administration, many parameter concentrations in both tissues showed a tendency to recover to the initial levels. These results suggest that glucagon administration enhanced glycogenolysis and gluconeogenesis in the hepatopancreas, and as a consequence, glucose was released into the bloodstream. The blood glucose seems to be metabolized through enhanced glycolysis and glycojenesis in the muscle.

KEY WORDS: cyclic AMP, Cyprinus carpio, glucagon, gluconeogenesis, glycogen phosphorylase, glycogenolysis, glycolysis, phosphofructokinase.

INTRODUCTION

Previous studies indicate that it takes more than 20 days to induce many carbohydrate-metabolizing enzymes in fish, which is much longer than in mammals. However, rapid changes in the hepatic enzyme activities after hormonal administration and refeeding could not be explained from the view of enzyme induction. Hence, the authors studied the rapid response of carbohydrate metabolism in fish, and reported that phosphofructokinase (PFK) activity, one of the rate-limiting enzymes in glycolysis, was influenced by substrates and effectors such as fructose-6-phosphate (F6P), citrate, adenosine-5′-monophosphate (AMP), adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP). In addition, activities of other enzymes such as glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatase (FBPase), and glucose-6-phosphate dehydrogenase (G6PDH) rapidly responded to an insulin and epinephrine administration and short-term exercise.

Even so, the rapid response of carbohydrate metabolism in fish is still not well understood. The present experiment was conducted to investigate the rapid response of enzyme activities and metabolic intermediate concentrations to a glucagon administration in the hepatopancreas and muscle of carp.

MATERIALS AND METHODS

Fish and rearing methods

Yearling common carp Cyprinus carpio were obtained from a fish farmer in Nankoku City,
RESULTS

Plasma components

Figure 1 shows the response of plasma components to the glucagon administration. In contrast to an insulin administration in the previous paper, glucose concentration significantly \((P<0.01)\) increased to about three times the initial value during 3 h after the hormone administration, then it showed a tendency to recover. Free amino acid concentration also increased from 6.76 to 11.2 mg N/100 mL after 1 h, and it recovered after 5 h, while lactate and protein concentrations did not change so markedly during the experimental period.

General components in hepatopancreas and muscle

Hepatopancreatic glycogen content rapidly and markedly decreased to about 70% of the pre-administration level during 1–3 h after glucagon administration, while muscular glycogen content rapidly increased. Thereafter, glycogen content in both tissues did not completely return to the initial level during the experimental period (Fig. 2). Glucose content in both tissues significantly \((P<0.05)\) increased during 1 h, and thereafter it quickly recovered in the hepatopancreas, whereas it stayed high in the muscle.

Experimental protocols

Glucagon (Sigma Co. Ltd, St. Louis, USA) was dissolved in a 0.75% NaCl solution. After 24 h from the last feeding, the solution was intraperitoneally administered to carp averaging 78.4 g at a dose of 60 \(\mu g/\text{kg bodyweight}\). Ten fish each were sampled at 0 (pre-administration), 1, 3, and 5 h after its administration.

Of the 10 fish sampled, five fish were used for the analysis of plasma components and enzyme activities of hepatopancreas and muscle. Blood was taken from the caudal vein by a heparinized syringe and centrifuged at 10 000 g for 5 min to separate plasma. Then, the fish were killed, and the hepatopancreas and muscle were removed for enzyme analysis. Another five fish were quickly killed, and the hepatopancreas and muscle were removed to measure metabolic intermediate concentration. The hepatopancreas and muscle of individual fish were immediately frozen by liquid nitrogen, and stored in a freezer at \(-80^\circ\text{C}\) until used.

Analytical methods

Glucose content in the hepatopancreas and muscle was determined with a Wako kit (Wako Chemical Co., Osaka, Japan). The concentrations of plasma glucose, lactate, free amino acid and protein, and the contents of glycogen, lactate, cyclic AMP (cAMP), glucose-6-phosphate (G6P), F6P, citrate, AMP, ADP, and ATP in both the tissues were determined as previously described.8,9 The activities of phosphofructokinase (PFK, EC 2.7.1.11), glycogen phosphorylase (GPase, EC 2.4.1.1), glucose-6-phosphatase (G6Pase, EC 3.1.3.9), fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), aspartate aminotransferase (GOT, EC 2.6.1.1), and alanine aminotransferase (GPT, EC 2.6.1.2) in both tissues were determined as previously described.8,9 All the enzyme activities were expressed as \(\mu\text{mol of substrate or coenzyme converted to per min per g of tissue.}\)

Statistical analysis of the results was conducted by the Student’s \(t\)-test \((P<0.05 \text{ or } P<0.01, n=5)\).
Muscular lactate content slowly but significantly \((P<0.05)\) increased from 1.79 to 2.18 mg/g tissue during 5 h. Also, hepatopancreatic lactate content tended to increase at first, then gradually decreased over 5 h. The hormone administration did not influence free amino acid content as much in both tissues.

**Metabolic intermediate concentration**

In the muscle, F6P and G6P concentrations continued to rise throughout the experimental period, and finally they increased to about 2.5 times (Fig. 3). The ATP concentration slightly but significantly \((P<0.01)\) fell with a corresponding rise of AMP and ADP concentrations after the glucagon administration; thereafter they showed a recovering tendency. Similar responses of these nucleotides were observed for short duration exercise and an epinephrine administration in previous papers.8,9 Citrate concentration did not change so markedly after the hormone administration.

In the hepatopancreas, when glucagon was administered, AMP concentration gradually but significantly \((P<0.01)\) rose while ADP concentration rapidly and significantly \((P<0.05)\) fell after 1–3 h. Then, ADP concentration recovered to the preadministration level, while AMP concentration stayed low even after 5 h. Other metabolic intermediate concentrations did not change so markedly after the administration.

**cAMP concentration and GPase activity**

As is evident from Fig. 4, hepatopancreatic cAMP concentration was 1.15 nmol/g tissue at preadministration, and it rapidly and significantly \((P<0.01)\) increased to 2.84 nmol during 1 h, then it quickly recovered to the initial level at 3 h. Although total GPase \((a+b)\) activities stayed similar \((4.70–5.61 \mu\text{mol/g tissue})\) throughout the experimental period, GPase a activity significantly \((P<0.01)\) increased from 1.06 to 2.40 \(\mu\text{mol/g tissue}\) during 3 h, and it recovered to the initial level at 5 h. Such responses of cAMP and GPase were observed with epinephrine administration in the previous paper.8 In contrast, in the muscle, the concentration of cAMP, and activities of both...
DISCUSSION

In mammals, it is well established that glucagon has actions that counterbalance and oppose the biological activities of insulin. In short, the hormone has hyperglycemic action by stimulated hepatic glycogenolysis and gluconeogenesis.\(^{10,11}\) In contrast, the regulation of carbohydrate metabolism by the hormone in fishes is poorly understood relative to the mammalian system.\(^{10,11}\) In the present study, we investigated the response to a glucagon administration of enzyme activities and metabolic intermediate concentrations such as PFK and FBPase with F6P and AMP relating to carbohydrate metabolism.

Enzyme activity

As observed for short duration exercise and an epinephrine administration in previous papers,\(^{8,9}\) muscular PFK activity markedly increased from 21.4 to 39.1 μmol/g tissue during 3 h after the glucagon administration and thereafter stayed high, whereas its activity in the hepatopancreas did not change so markedly (Fig. 5). Hepatopancreatic G6Pase activity significantly (\(P<0.05\)) increased by about one and a half times the initial value after 3 h. Also, hepatopancreatic FBPase activity increased, but it did not change as markedly, and its activity in the muscle rapidly and significantly (\(P<0.01\)) decreased. The hormone administration hardly influenced the activities of G6PDH in the hepatopancreas, and GOT and GPT in both tissues.

GPase (a+b) and GPase a did not change so markedly by the hormone administration.

**DISCUSSION**

In mammals, it is well established that glucagon has actions that counterbalance and oppose the biological activities of insulin. In short, the hormone has hyperglycemic action by stimulated hepatic glycogenolysis and gluconeogenesis.\(^{10,11}\) In contrast, the regulation of carbohydrate metabolism by the hormone in fishes is poorly understood relative to the mammalian system.\(^{10,11}\) In the present study, we investigated the response to a glucagon administration of enzyme activities and metabolic intermediate concentrations such as PFK and FBPase with F6P and AMP relating to carbohydrate metabolism.

In order to clarify the metabolic response to the glucagon administration in hepatopancreas and muscle, the ratios of activity and concentration in the glucagon-administered fish to those in pre-administered fish at 1 h after the administration are shown in Fig. 6. In hepatopancreas, when glucagon was administered, GPase a activity
together with cAMP concentration was markedly increased and glycogen content was decreased. Since it has been reported in rainbow trout that cAMP changes inactive form of GPase (GPase b) to active form of GPase (GPase a),\textsuperscript{12} it was suggested by the results of the present study that glucagon administration increased cAMP at first, then activated GPase, resulting in an increased glycogenolysis in the hepatopancreas. The activation mechanism of glycogenolysis by glucagon has been studied in detail on the mammalian liver,\textsuperscript{13} and similar results were obtained in the present study on carp. The activities of hepatopancreatic G6Pase and FBPase relating to gluconeogenesis increased after the hormone administration. The activation of hepatic glycogenolysis and gluconeogenesis by glucagon has been reported in the isolated hepatocytes of teleost fishes.\textsuperscript{14} Therefore, the hormone administration could be considered to promote both glycogenolysis and gluconeogenesis in the hepatopancreas, resulting in an increased blood glucose.

The glucagon administration increased PFK activity in the muscle as well as G6P, F6P, AMP, ADP, and lactate concentrations while it decreased ATP concentration (Figs 2, 3, 5). In the previous papers,\textsuperscript{5,6} we reported that these changes in the metabolic intermediate concentrations activate PFK in the muscle and hepatopancreas of carp. Moreover, Su and Storey\textsuperscript{15} reported the activation of PFK through change of the protein conformation. These investigations suggested that blood glucose was catabolized by an activated glycolysis through the activation of PFK by changes in metabolic intermediate concentrations and/or its protein conformation in the muscle, then it was decomposed to lactate. The resultant lactate seems to be transported to hepatopancreas through the bloodstream.

Other investigators reported that glucagon administration did not have a significant effect on the muscular glycogenolysis.\textsuperscript{16,17} Also, in this experiment, the hormone administration did not markedly affect the levels of both cAMP and GPase, but it increased glycogen content in the muscle. The cause of the difference in both experiments remains unclear, therefore further detailed studies are required.

The results of the present experiment mean that glucagon administration enhanced glycogenolysis and gluconeogenesis in the hepatopancreas, and it released glucose into the bloodstream to supply it to the muscle. In the muscle, the blood glucose seems to be accumulated as glycogen and metabolized to lactate through enhanced glycolysis as well. The resultant lactate may be transported to hepatopancreas through the bloodstream, where it may function as a substrate for gluconeogenesis. These findings might indicate the presence of an interdependence of carbohydrate metabolism between hepatopancreas and muscle ("Cori cycle")\textsuperscript{18} in fish under glucagon administration. Such a mutual metabolic cooperation was seen in fish after short duration exercised and epinephrine administration.\textsuperscript{8,9}

After an internal environmental change, fish must restore it to their steady state. In the present experiment, from 3 h after the glucagon administration, almost all the biochemical parameter concentrations in the hepatopancreas, muscle, and blood showed a tendency to recover to the pre-administered levels (Figs 1–5), although some of the parameters such as muscular PFK activity, G6P and F6P concentrations had not completely recovered by the end of the experiment.

As mentioned above, respective components responded quickly to the glucagon administration, then they recovered to the initial levels after several hours. This suggests that carbohydrate metabolism in carp can be regulated not only by the enzyme induction but also by changes of enzyme activity through its protein conformation and/or metabolic intermediate concentrations.
REFERENCES


