Effects of Cortisol on Glucose Metabolism in Primary Cultured Hepatocytes of Epinephelus coicoides: Postprint

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Date: 2017-10-11T00:00:00+00:00

Abstract

The present study was designed to investigate the effects of cortisol on glucose metabolism in primary cultured hepatocytes of orange-spotted grouper (Epinephelus coioides). Hepatocytes were isolated from orange-spotted grouper. Using two incubation times (24 and 36 h) and three cortisol concentrations [0 (control), 100 and 1,000 nmol/L], the glucose content in culture supernatant (i.e., hepatocyte glucose release), hepatocyte glycogen and pyruvate contents, and activities of key enzymes in hepatocyte glucose metabolism [phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), glycogen synthase (GSase), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICD) and pyruvate kinase (PK)] were measured. The results showed: At 24 and 36 h incubation, compared with the control group, 100 and 1,000 nmol/L cortisol groups significantly increased hepatocyte glucose release ($P<0.05$) and significantly decreased hepatocyte pyruvate content ($P<0.05$); cortisol incubation time had no significant effect on hepatocyte glucose release or pyruvate content ($P>0.05$). At 24 h incubation, cortisol concentration had no significant effect on hepatocyte glycogen content ($P>0.05$), while at 36 h incubation, hepatocyte glycogen content significantly decreased with increasing cortisol concentration ($P<0.05$); with prolonged cortisol incubation time, hepatocyte glycogen content significantly decreased ($P<0.05$). At 24 and 36 h incubation, 100 and 1,000 nmol/L cortisol significantly increased activities of hepatocyte PEPCK and G6Pase ($P<0.05$), significantly decreased activities of hepatocyte MDH and ICD ($P<0.05$), but had no significant effect on hepatocyte GSase activity ($P>0.05$); at 24 h incubation, cortisol concentration had no significant effect on hepatocyte PK activity ($P>0.05$), but at 36 h incubation, 100 and 1,000 nmol/L cortisol significantly increased hepatocyte PK activity ($P<0.05$). With prolonged incubation time, hepatocyte G6Pase activity significantly decreased ($P<0.05$), PK activity significantly increased ($P<0.05$), while MDH and ICD

activities showed no significant changes (P>0.05). These results indicate that cortisol can promote gluconeogenesis and inhibit glucose catabolism in primary cultured hepatocytes of orange-spotted grouper, but has no regulatory effect on glycogen synthesis.

Full Text

Effects of Cortisol on Glycometabolism in Primary Cultured Hepatocytes from Epinephelus coioides

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Abstract

This experiment investigated the effects of cortisol on glycometabolism in primary cultured hepatocytes from Epinephelus coioides. Hepatocytes were isolated and incubated at two time points (24 and 36 h) with three cortisol concentrations [0 (control), 100, and 1,000 nmol/L]. We measured glucose content in culture supernatant (hepatocyte glucose production), hepatocyte glycogen and pyruvate contents, and activities of key glycometabolism enzymes including phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), glycogen synthase (GSase), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICD), and pyruvate kinase (PK). At both 24 and 36 h, 100 and 1,000 nmol/L cortisol significantly increased hepatocyte glucose production ($P < 0.05$) while decreasing pyruvate content ($P < 0.05$) compared with controls. Cortisol incubation time showed no significant effect on glucose production or pyruvate content ($P > 0.05$). At 24 h, cortisol concentration did not significantly affect glycogen content ($P > 0.05$), but at 36 h, glycogen content decreased significantly with increasing cortisol concentration ($P < 0.05$). Glycogen content also declined significantly with prolonged incubation time ($P < 0.05$). At both time points, 100 and 1,000 nmol/L cortisol significantly elevated PEPCK and G6Pase activities ($P < 0.05$) while reducing MDH and ICD activities ($P < 0.05$), but had no significant effect on GSase activity ($P > 0.05$). PK activity was unaffected by cortisol at 24 h ($P > 0.05$) but was significantly increased by 100 and 1,000 nmol/L cortisol at 36 h ($P < 0.05$). Prolonged incubation significantly decreased G6Pase activity ($P < 0.05$) and increased PK activity ($P < 0.05$), while MDH and ICD activities remained unchanged ($P > 0.05$). These results demonstrate that cortisol promotes gluconeogenesis and inhibits glucose catabolism in primary cultured hepatocytes from E. coioides, while exerting no regulatory effect on glycogen synthesis.

Keywords: Epinephelus coioides; primary culture; cortisol; glycometabolism; enzyme activity

Received: 2016-05-11
Funding: National Natural Science Foundation of China (31302198); National Public Welfare Industry (Agriculture) Special Project (201303053); Jimei University Undergraduate Innovation Experiment Program (201610390103)
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Introduction

Epinephelus coioides is an important marine aquaculture species worldwide. Stress responses triggered by adverse environmental factors adversely affect its growth and reproduction, activating the hypothalamic-pituitary-interrenal axis and altering various metabolic processes. Cortisol, a critical corticosteroid hormone in fish, acts extensively on tissues and organs and serves as a key regulator of stress responses, growth, metabolism, and immune function. When fish encounter environmental stressors, the hypothalamic-pituitary-interrenal axis is activated, releasing cortisol and elevating plasma cortisol concentrations, which precipitates dramatic changes in material and energy metabolism.

Fish glycometabolism encompasses glycolysis, gluconeogenesis, glycogen synthesis, glycogenolysis, the tricarboxylic acid cycle, and the pentose phosphate pathway, representing a vital component of overall metabolism. Similar to other vertebrates, fish glycometabolism is closely linked to endocrine activity, with hormones such as insulin and glucagon playing important regulatory roles. The liver is a crucial organ for glycometabolism and a target for endocrine system action. Cortisol, as a key metabolic hormone, is intimately involved in hepatic metabolic regulation. While most studies on cortisol' s hepatic effects have used whole-animal models—where interactions among multiple hormones complicate isolation of individual hormone effects—and have focused primarily on relationships between cortisol and key enzyme activities, primary hepatocyte cultures offer a powerful alternative. These cultures retain most hepatic physiological functions while preserving intact cellular morphology and metabolic activity, providing an excellent model for studying hormonal regulation of glycometabolism. However, few studies have examined cortisol' s effects on glycometabolism in isolated fish hepatocytes, and none have investigated its metabolic mechanisms in E. coioides hepatocytes. Therefore, this study employed a primary hepatocyte culture model from E. coioides to explore cortisol' s role in regulating glycometabolism.

Materials and Methods

1.1 Experimental Animals and Husbandry

Healthy E. coioides (approximately 50 g body weight) were purchased from a local aquaculture company in Xiamen and acclimated in 600 L recirculating tanks. Fish were fed to satiation twice daily at 08:30 and 18:30 with commercial feed. After 0.5 h, uneaten feed and feces were siphoned out, followed by a water exchange of 1/3 to 1/2 of the tank volume. Natural photoperiod was maintained throughout the experiment. Seawater salinity was 30‰, temperature (27 ± 2)°C, dissolved oxygen > 6.5 mg/L, ammonia < 0.02 mg/L, and pH 8.0–8.2.

1.2 Main Reagents

Cortisol (50 mol/L stock solution in saline, cell culture grade) was purchased from Sigma. L-15 medium, 0.25% trypsin, penicillin-streptomycin (10,000 IU/mL and 10,000 g/mL), amphotericin B, and fetal bovine serum (FBS) were from Gibco. Trypan blue and dimethyl sulfoxide (DMSO) were from Shanghai Jierui Biological Engineering. Assay kits for glucose, hepatic glycogen, pyruvate, PEPCK, G6Pase, GSase, MDH, ICD, and PK were obtained from Nanjing Jiancheng Bioengineering Institute.

1.3 Primary Hepatocyte Culture and Cortisol Incubation

Based on modified protocols from Segner et al. [14] and Qin et al. [15], hepatocytes were isolated by trypsin digestion. Freshly isolated hepatocytes were cultured in L-15 medium containing 20% FBS for 48 h. The medium was then discarded, and cells were washed three times with serum-free L-15 medium to remove other hormones. Fresh L-15 medium containing different cortisol concentrations (0 [control], 100, and 1,000 nmol/L) was added. For the control group, an equivalent volume of phosphate-buffered saline was added to maintain consistent treatment volumes. Cells were incubated for 24 or 36 h before collecting supernatant and hepatocytes for subsequent analyses. Each treatment had four replicates. Freshly isolated hepatocytes were seeded at 2×10^5 cells/mL in 12-well plates for most assays, with additional cells seeded in 25 cm^2 flasks for biochemical analyses.

1.4 Biochemical Analysis

Hepatocyte glucose production (measured as glucose content in culture supernatant), intracellular glycogen and triglyceride contents, and glycometabolism-related enzyme activities were determined using cells cultured in 25 cm^2 flasks. After incubation, cells were harvested by trypsinization and washed twice with PBS. Cell viability was assessed by trypan blue exclusion, and only viable cells were used for enzyme and metabolite assays.

Glucose production was measured following Kikuchi et al. [16]. For glycogen and triglyceride determination, harvested hepatocytes were ultrasonicated in PBS.

Glycogen content was quantified using the method of de Frutos et al. [17] and expressed as mg glycogen per mg cellular protein. GSase activity was determined according to Vijayan et al. [18], MDH and ICD activities by Sunny et al. [19], and G6Pase activity by Chan et al. [20]. For PK activity, hepatocytes were homogenized in buffer (100 mmol/L Tris-HCl, pH 7.5, 10°C) and assayed following Sullivan et al. [21]. PEPCK activity was measured using the method of Polakof et al. [22]: harvested hepatocytes were homogenized at 4°C in 10 volumes of buffer (as described in Polakof et al. [22]), centrifuged at 10,000 r/min for 30 min, and the supernatant was assayed at 30°C and 340 nm using a microplate reader. Intracellular protein concentration was determined by the Coomassie brilliant blue method [23] using bovine serum albumin as the standard. All enzyme activities were expressed as specific activity (U/mg protein).

1.5 Data Processing

Data are presented as mean ± standard error (SE). Statistical analysis was performed using SPSS 19.0 software. After testing for homogeneity of variance, data were analyzed by one-way ANOVA and independent samples t-test. When significant differences were detected among groups, Duncan' s multiple range test was applied. Statistical significance was set at $P < 0.05$.

Results

2.1 Effects of Cortisol on Glucose Production, Glycogen, and Pyruvate Content in Primary Cultured Hepatocytes

The effects of cortisol on glucose production and glycogen and pyruvate contents are shown in [Figure 1: see original paper]. After 24 or 36 h of incubation, hepatocyte glucose production increased with cortisol concentration. Both 100 and 1,000 nmol/L cortisol groups showed significantly higher glucose production than the control ($P < 0.05$), with the 1,000 nmol/L group exhibiting the highest value ($P < 0.05$). Glycogen content did not differ among groups at 24 h ($P > 0.05$), but decreased significantly with increasing cortisol concentration at 36 h ($P < 0.05$), with the lowest value in the 1,000 nmol/L group. Glycogen content at 36 h was significantly lower than at 24 h ($P < 0.05$). Pyruvate content was significantly lower in both cortisol-treated groups than in controls after 24 or 36 h ($P < 0.05$), with no difference between the two cortisol concentrations ($P > 0.05$). Incubation time had no significant effect on glucose production or pyruvate content ($P > 0.05$).

2.2 Effects of Cortisol on Glycometabolism-Related Enzyme Activities

Cortisol' s effects on enzyme activities are presented in [Figure 2: see original paper]. At both 24 and 36 h, PEPCK activity increased progressively with cortisol

concentration, with significant differences among all groups ($P < 0.05$). At 100 nmol/L cortisol, PEPCK activity rose significantly with longer incubation ($P < 0.05$). G6Pase activity was significantly higher in both cortisol-treated groups than in controls at 24 and 36 h ($P < 0.05$), with no difference between the two cortisol concentrations ($P > 0.05$). However, G6Pase activity decreased significantly with prolonged incubation at both cortisol concentrations ($P < 0.05$). MDH and ICD activities declined significantly with increasing cortisol concentration at both time points ($P < 0.05$), reaching minimum values in the 1,000 nmol/L group. Incubation time did not affect MDH or ICD activities at any cortisol concentration ($P > 0.05$). GSase activity was not significantly influenced by either cortisol concentration or incubation time ($P > 0.05$). PK activity showed no inter-group differences at 24 h ($P > 0.05$), but increased with cortisol concentration at 36 h, with both cortisol groups significantly higher than controls ($P < 0.05$). PK activity also increased significantly with incubation time in the 100 and 1,000 nmol/L groups ($P < 0.05$).

Discussion

Cortisol' s regulatory role in fish metabolism has been extensively studied, primarily examining effects of exogenous cortisol on plasma and hepatic metabolic levels and enzyme activities. However, the complex hormonal milieu in vivo makes it difficult to isolate the specific actions of individual hormones on glycometabolism. Using primary cultured hepatocytes from E. coioides, we investigated cortisol' s direct effects on glycometabolic regulation.

Our results show that cortisol treatment significantly altered hepatocyte metabolism. Cortisol dose-dependently increased glucose production, while incubation duration did not affect glucose output. However, incubation time did influence glycogen content: at 24 h, cortisol did not affect glycogen levels, but at 36 h, higher cortisol concentrations reduced glycogen content. This suggests that at 24 h, increased glucose production does not derive from glycogenolysis but likely from gluconeogenesis using amino acids from the culture medium. By 36 h, glucose production may involve both glycogen breakdown and other synthetic pathways. Previous studies have shown that cortisol stimulates hepatic gluconeogenesis from amino acids and triglycerides in fish, consistent with our findings and with reports in Hemitripterus americanus and Oncorhynchus keta.

Glycolysis, gluconeogenesis, and the tricarboxylic acid cycle are essential for glucose homeostasis. In our study, cortisol significantly elevated PEPCK and G6Pase activities in E. coioides hepatocytes, demonstrating dose- and time-dependent relationships. As key gluconeogenic enzymes, their increased activity indicates enhanced gluconeogenic capacity and glucose production, explaining the elevated glucose release. This supports the conclusion that cortisol stimulates hepatic gluconeogenesis to meet stress-induced glucose demands. While

Jones et al. reported that dexamethasone stimulates gluconeogenesis from pyruvate in mouse hepatocytes, we found that cortisol significantly reduced MDH and ICD activities—rate-limiting enzymes in the tricarboxylic acid cycle—indicating suppressed aerobic glucose catabolism and increased glucose availability for other tissues. Sunny et al. similarly reported cortisol-induced reductions in MDH and ICD activities in tilapia hepatocytes, corroborating our results.

PK activity, which catalyzes conversion of phosphoenolpyruvate to pyruvate in glycolysis, increased with incubation time in our study. However, pyruvate content decreased significantly with cortisol treatment, suggesting that despite enhanced glycolytic flux to pyruvate, cortisol inhibits glucose catabolism overall. Cortisol is known to regulate hepatic glycogen metabolism in fish, but our hepatocyte model revealed no significant effect of either cortisol concentration or incubation time on GSase activity, indicating that glycogen synthesis in primary cultured hepatocytes is not directly regulated by cortisol. This aligns with findings in Oncorhynchus kisutch but contrasts with reports of cortisol-stimulated glycogen synthesis in Sparus aurata and upregulated GSase transcription in sea bream hepatocytes. These discrepancies suggest that cortisol' s effects on glycogen synthesis depend on species, concentration, and incubation duration.

In conclusion, cortisol enhances gluconeogenic capacity and glucose production while inhibiting glucose catabolism in primary cultured hepatocytes from E. coioides, without exerting regulatory effects on glycogen synthesis.

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Note: Figure translations are in progress. See original paper for figures.