Expression of Insulin Receptor-1 in Different Tissues of Nile Tilapia and Its Response to Glucose Injection Postprint

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Abstract

This study aimed to investigate the expression of insulin receptor-1 (IR-1) in different tissues of Nile tilapia and its response to glucose injection. The IR-1 cDNA fragment was cloned from Nile tilapia muscle using PCR amplification, and semi-quantitative PCR was employed to compare the expression differences of IR-1 among muscle, liver, and heart. One hundred sixty Nile tilapia with a body weight of approximately 100 g were selected and randomly divided into 2 groups, with 4 replicates per group and 20 fish per replicate. The experimental group received an intraperitoneal injection of glucose (30 mg per 100 g body weight), while the control group received an intraperitoneal injection of 0.7% sterile saline at the same dosage. Sampling was conducted at 0 h (before injection) and at 1, 3, 6, and 12 h post-injection to measure plasma glucose and insulin contents, and real-time quantitative PCR was used to detect the relative mRNA expression of IR-1 in muscle, heart, and liver. The results showed: 1) The cloned IR-1 cDNA fragment had a GenBank accession number of JN967750, a size of 1,979 bp, and encoded 548 amino acids. Sequence analysis revealed that Nile tilapia IR-1 exhibited high conservation compared with other species and possessed abundant characteristic tyrosine kinase sequences. 2) IR-1 was highly expressed in muscle, heart, and liver of Nile tilapia, with expression levels in liver and muscle being essentially consistent, while the expression in heart was relatively lower. 3) Plasma glucose content in the experimental group peaked at 1 h after glucose injection and was significantly higher than that in the control group ($P<0.05$), then began to decline and returned to normal levels after 3 h; plasma insulin content in the experimental group peaked at 3 h after glucose injection and was significantly higher than that in the control group ($P<0.05$), then began to decline and returned to normal levels after 12 h. The relative expression of IR-1 mRNA in muscle and liver of the experimental group peaked at 6 h after glucose injection, which was significantly higher than that in the

control group (P<0.05), and returned to normal levels at 12 h; the relative expression of IR-1 mRNA in heart of the experimental group showed no significant changes within 12 h after glucose injection (P>0.05). The results indicate that glucose injection immediately elevated plasma glucose content in Nile tilapia; compared with the increase in plasma glucose, the increase in plasma insulin was relatively delayed, while the elevation of IR-1 mRNA relative expression in muscle and liver was further delayed compared with the increase in plasma insulin, thereby exacerbating the metabolic burden of glucose in Nile tilapia.

Full Text

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Abstract

This experiment was conducted to investigate the expression of insulin receptor-1 (IR-1) in different tissues of Nile tilapia (*Oreochromis nilotica*) and its response to glucose injection. A partial cDNA fragment of IR-1 was cloned from Nile tilapia muscle using PCR amplification, and semi-quantitative PCR was employed to compare IR-1 expression differences among muscle, liver, and heart tissues. One hundred sixty Nile tilapia with an average body weight of approximately 100 g were randomly divided into two groups, each consisting of four replicates of 20 fish. The experimental group received an intraperitoneal glucose injection (30 mg per 100 g body weight), while the control group was injected with an equivalent volume of 0.7% sterile saline. Sampling was performed before injection (0 h) and at 1, 3, 6, and 12 h post-injection to determine plasma glucose and insulin concentrations and to measure IR-1 mRNA relative expression levels in muscle, heart, and liver via real-time quantitative PCR. The results revealed: 1) The cloned IR-1 cDNA fragment (GenBank accession No. JN967750) was 1,979 bp in length, encoding 548 amino acids. Sequence analysis demonstrated that Nile tilapia IR-1 exhibited high conservation compared with other species and contained characteristic sequences rich in tyrosine kinase motifs. 2) IR-1 was highly expressed in muscle, heart, and liver of Nile tilapia, with expression levels in liver and muscle being essentially equivalent and relatively lower in heart. 3) Plasma glucose concentration in the experimental group peaked at 1 h post-injection, significantly higher than the control group (P<0.05), then declined and returned to normal levels by 3 h. Plasma insulin concentration peaked at 3 h post-injection, significantly higher than the control group (P<0.05), then decreased and approached normal levels by 12 h. The relative expression levels of

IR-1 mRNA in muscle and liver reached maximum values at 6 h post-injection, significantly higher than the control group ($P<0.05$), and returned to normal levels by 12 h. In contrast, IR-1 mRNA expression in heart showed no significant changes within 12 h post-injection ($P>0.05$). These findings indicate that glucose injection acutely elevated plasma glucose concentration in Nile tilapia, while the increase in plasma insulin was relatively delayed compared to glucose, and the upregulation of IR-1 mRNA expression in muscle and liver was further delayed relative to insulin, thereby exacerbating the metabolic burden of glucose in Nile tilapia.

Key words: Nile tilapia (*Oreochromis nilotica*); glucose; insulin; insulin receptor-1

Introduction

Insulin is a central hormone in glucose metabolism that strictly regulates glucose utilization. Early studies suggested that the inefficient utilization of high dietary carbohydrates by fish resulted from low endogenous insulin levels in serum. However, with the development of radioimmunoassay techniques for insulin, accumulating evidence has demonstrated that insulin concentrations in fish serum are similar to or even higher than those in mammals. Research has shown that insulin mRNA expression in tilapia pancreatic β-cells is 3.7 times higher than the corresponding expression in mammals. Insulin exerts its effects by binding to specific receptors on cell surfaces, forming disulfide bonds that maximize insulin efficacy. In birds and mammals, insulin receptors mediate insulin' s actions on fat synthesis and glucose uptake, prompting investigations into insulin receptor quantity and receptor-insulin affinity. While insulin receptor research has focused primarily on mammals, few studies have examined fish insulin receptors. Insulin receptor expression has been detected in various fish tissues, including liver, skeletal muscle, heart, and adipose tissue. Tilapia peripheral tissues such as skeletal muscle also possess functional insulin receptors. However, studies on fish insulin receptor numbers indicate that fish have far fewer insulin receptors than mammals. Ablett et al. proposed that high glucose promotes increased insulin receptor numbers, meaning low dietary carbohydrate levels result in fewer activated insulin receptors. Insufficient insulin receptor quantity may represent one reason for the low capacity for carbohydrate utilization in fish.

Mommsen et al. inferred from previous research that low glucose tolerance in fish might stem from differences in insulin-receptor interactions compared to mammalian systems. Ablett et al. introduced an alternative approach to study insulin secretion by measuring labeled insulin binding to receptors on hepatocyte and myocyte membranes, finding a correlation between insulin receptor number and insulin content in rainbow trout. Studies revealed that insulin receptor number in rainbow trout myocardium was inversely proportional to

hormone content, whereas the opposite relationship was observed in adipose tissue, skeletal muscle, and liver. Planas et al. suggested that the relationship between insulin receptor number and hormone content might be related to tissue functional characteristics. This study selected Nile tilapia as the experimental model to measure changes in plasma glucose and insulin concentrations following glucose injection and to investigate IR-1 expression in different tissues and its response to glucose injection, providing a theoretical basis for understanding glucose metabolism mechanisms in tilapia.

Materials and Methods

Experimental Animals and Reagents

Experimental Nile tilapia were purchased from a local farm and acclimated at the aquaculture facility of Shandong Agricultural University for two weeks. Healthy fish of uniform size (approximately 100 g average body weight) were selected for the experiment. Glucose was purchased from Shanghai Solarbio Bioscience & Technology Co., Ltd., dissolved in sterile distilled water to prepare a 30 mg/mL injection solution, and stored at 4°C until use.

Experimental Design

One hundred sixty Nile tilapia were randomly distributed into eight aquaria (water capacity 0.4 m^3 each), with four replicates per group and 20 fish per replicate. Fish were fed a conventional basal diet (composition and nutrient levels shown in Table 1). At the start of the experiment, both groups were fasted for 24 h. After anesthesia with MS-222 (20 g/m^3 water), the experimental group received an intraperitoneal glucose injection (30 mg per 100 g body weight), while the control group received an equivalent volume of 0.7% sterile saline. Sampling was conducted before injection (0 h) and at 1, 3, 6, and 12 h post-injection. Four fish were sampled from each aquarium at each time point. After anesthesia with MS-222, blood was collected from the caudal vein using heparinized tubes. Plasma was prepared by centrifugation at 3,000 r/min for 10 min at 4°C and stored in liquid nitrogen. Following blood collection, hepatopancreas, heart, and dorsal muscle were collected and stored in liquid nitrogen.

Plasma Glucose and Insulin Assays

Plasma glucose concentration was measured using a Beckman Cx-4 automatic biochemical analyzer. Insulin concentration was determined using a fish insulin ELISA kit from R&D Systems based on solid-phase sandwich enzyme-linked immunosorbent assay. A standard curve regression equation was derived from the relationship between insulin concentration and optical density (OD) values. Sample insulin OD values were substituted into this equation to calculate insulin content, with dilution factors applied as necessary.

IR-1 cDNA Cloning and Sequence Analysis

Total RNA was extracted from 50–100 mg of Nile tilapia muscle tissue ground to powder in liquid nitrogen using Trizol Reagent (Invitrogen) according to the manufacturer' s protocol, followed by purification with DNase I (Invitrogen). Purified RNA was reverse-transcribed using M-MLV Reverse Transcriptase (TaKaRa). Primers 1 and 2 (Table 2) were designed based on gene sequences of rainbow trout (GenBank accession No. AF062496.1), flounder (AB065096.1), goldfish (AF218355.1), and coho salmon (AF021040.1) obtained from NCBI. A partial IR-1 sequence was cloned using a PCR kit (2×Taq PCR) from Tiangen under the following conditions: 94°C for 5 min, 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. The amplified fragment was ligated into the pTZ-57R/T vector using a ligation kit from MBI, transformed into competent *E. coli* DH5α, and sequenced by Beijing Tiangen Biotech Co., Ltd. Based on the obtained fragment, primer 3 and anchor primer BRL-A2 were designed for 3' RACE, while primer 6 and anchor primer SmartP1 were designed for 5' RACE, both under PCR conditions of 94°C for 5 min, 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. These fragments were similarly ligated, cloned, and sequenced. Sequence alignment and analysis were performed using DNAMAN software, and phylogenetic trees were constructed using MEGA 5.0.

Semi-Quantitative PCR Detection of IR-1 Expression in Tissues

Muscle, liver, and heart were collected from Nile tilapia fed a conventional diet. Total RNA was extracted from each tissue using Trizol Reagent (Invitrogen) and reverse-transcribed. Semi-quantitative PCR was performed using 2×Taq PCR reaction mix (Tiangen) to detect IR-1 expression. Primers 7 and 8 (Table 2) were designed based on the cloned IR-1 sequence (GenBank accession No. JN967750) under the following conditions: 94°C for 5 min, 36 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. Primers 11 and 12 (Table 2) were designed based on tilapia β-actin sequence (GenBank accession No. EF026001.1) as an internal control under conditions of 94°C for 5 min, 32 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. Relative expression levels were determined by 0.8% agarose gel electrophoresis and photographed using a Bio-Rad automated gel imaging system.

Real-Time Quantitative PCR Detection of IR-1 Expression

One microgram of total RNA was reverse-transcribed using M-MLV Reverse Transcriptase (TaKaRa) according to the manufacturer' s instructions, and the resulting cDNA was stored at -20°C. Real-time PCR amplification was performed using SYBR Green I fluorescent dye on a 7500 Real-Time PCR System (Bio-Rad) to detect IR-1 mRNA relative expression levels in muscle, heart, and liver at 1, 3, 6, and 12 h post-injection.

The reaction mixture (20 µL total volume) contained 10 µL 2×SYBR® Premix Ex Taq™ (TaKaRa), 0.4 µL 50×ROX Reference Dye II (TaKaRa), 6.8 µL dH_2O, 0.4 µL each of forward and reverse primers (10 µmol/mL), and 2 µL template cDNA. Negative controls used RNase-free water instead of template. Primers 9 and 10 (Table 2) were designed based on the cloned IR-1 sequence (GenBank accession No. JN967750) under the following conditions: 94°C for 30 s, 40 cycles of 94°C for 5 s and 60°C for 34 s, followed by melting curve analysis from 55°C to 95°C. Results were analyzed using ABI 7500 System software.

Statistical Analysis

Data are expressed as means ± standard deviation. Relative mRNA expression levels were calculated using the 2^(-ΔΔCt) method with β-actin as the internal reference and control group expression as the baseline. Statistical analysis was performed using SPSS 13.0 software. Data were first analyzed by one-way ANOVA, and Duncan' s multiple comparison test was applied when significant differences were detected. Significance was set at $P<0.05$.

Results

Changes in Plasma Glucose and Insulin Concentrations After Glucose Injection

As shown in Figure 1 [Figure 1: see original paper], plasma glucose concentration in the experimental group peaked at 1 h post-injection, significantly higher than the control group ($P<0.05$), then declined and returned to normal levels by 6 h. Figure 2 [Figure 2: see original paper] shows that plasma insulin concentration peaked at 3 h post-injection, significantly higher than the control group ($P<0.05$), then decreased and approached normal levels by 12 h.

Cloning of Nile Tilapia IR-1 Sequence Fragment

A partial cDNA sequence of Nile tilapia IR-1 (GenBank accession No. JN967750) was obtained through PCR amplification and cloning. Agarose gel electrophoresis revealed a fragment of 1,979 bp encoding 548 amino acids (Figure 3 [Figure 3: see original paper]), consistent with the predicted size. The sequenced fragment was aligned with homologous sequences from other animals using DNAMAN software, confirming it as the target gene. The fragment showed high conservation with other fish species including flounder, tilapia, trout, and turbot. Amino acid gaps were indicated with dashes (-), identical residues with asterisks (*), and positions with 2-3 identical amino acids with dots (.). Predicted transmembrane helices were underlined, and highly conserved IR-1 residues were highlighted in gray.

Amino Acid Sequence Analysis of Nile Tilapia IR-1

Comparative analysis with other fish species revealed that Nile tilapia IR-1 amino acid sequence is highly conserved, containing characteristic insulin receptor sequences (Figure 3) and abundant tyrosine kinase motifs. Phylogenetic analysis with insulin receptors from other vertebrates (Figure 4 [Figure 4: see original paper]) showed that Nile tilapia IR-1 clusters with other vertebrate insulin receptors, including flounder and turbot IR-1, but separately from non-IR-1 insulin receptor types. Fish insulin receptors formed one cluster, while mammalian insulin receptors formed another.

At the amino acid level, Nile tilapia IR-1 showed very high homology with flounder (89.9%) and turbot (89.1%) IR-1, 73.0% homology with Nile tilapia insulin receptor-2 (IR-2), and 66.7%–68.8% homology with mammalian insulin receptors (Table 3). These comparisons confirm that tilapia IR-1 possesses conserved sequences characteristic of class I insulin receptors along with signature insulin receptor motifs.

Tissue Expression of IR-1 in Nile Tilapia

Semi-quantitative PCR analysis of muscle, liver, and heart from Nile tilapia maintained at 23–25°C and fed a conventional diet revealed high IR-1 expression in all three tissues (Figure 5 [Figure 5: see original paper]), indicating the presence of insulin receptors in tilapia muscle, heart, and liver. Real-time quantitative PCR confirmed that IR-1 expression levels in liver and muscle were essentially equivalent, while expression in heart was relatively lower (Figure 6 [Figure 6: see original paper]).

Changes in IR-1 mRNA Relative Expression in Different Tissues After Glucose Injection

Real-time quantitative PCR analysis of IR-1 mRNA expression within 12 h post-injection showed that IR-1 mRNA relative expression in muscle increased at 3 h and peaked at 6 h, significantly higher than the control group ($P<0.05$). In heart, IR-1 mRNA expression showed no significant changes within 12 h ($P>0.05$) but exhibited a trend of initial increase followed by decrease, peaking at 6 h. Liver IR-1 mRNA expression pattern was similar to muscle, increasing at 3 h, peaking at 6 h with significant difference from the control group ($P<0.05$).

Discussion

Gene Sequence Analysis of Nile Tilapia IR-1

Based on sequence, phylogenetic, and tissue distribution analyses, the cloned gene fragment belongs to the insulin receptor family as IR-1. Nile tilapia IR-1 shows remarkable similarity in sequence and structure to other fish IR-1, with 66.7%–68.8% homology to mammalian insulin receptors and 45.3%–89.9% homology to other fish insulin receptors. Protein sequence analysis revealed high

conservation and abundant tyrosine kinase sites. The alignment results demonstrate that the sequence cloned from tilapia muscle possesses universal IR-1 characteristics except for minor amino acid substitutions. This structural conservation of fish insulin receptors under strong selective pressure likely reflects the requirement to maintain general receptor features and signal transduction functions.

Using primers from Chan et al. for cloning salmon insulin receptors, we obtained two insulin receptor fragments. Sequence alignment revealed one fragment identical to our cloned sequence and another matching a segment cloned from Mozambique tilapia by Cheng et al. (GenBank accession No. AF493794). The low homology (73.0%) between Nile tilapia IR-1 and IR-2 suggests that tilapia insulin receptors are encoded by two distinct genes, confirming the existence of two receptor subtypes. Comparative analysis showed IR-1 and IR-2 share 68.8% and 66.1% homology with human insulin receptors, respectively, indicating high evolutionary conservation. The structural and functional similarities between fish and human insulin receptors suggest that findings from fish studies may be directly applicable to mammals.

Tissue Distribution Characteristics of IR-1 in Nile Tilapia

The insulin receptor is a transmembrane glycoprotein with typical allosteric enzyme properties, widely distributed on cell membranes throughout the body. In mammals, insulin receptors in peripheral target cells for blood glucose regulation are primarily located in muscle and adipose tissue, though limited evidence exists for similar distribution in fish. Nevertheless, fish muscle and heart, including those of Nile tilapia, possess functional insulin receptors. Mounier et al. reported high insulin receptor expression in mammalian liver and adipose tissue, where insulin-receptor interactions maintain glucose homeostasis. Insulin receptors are expressed in various fish tissues including muscle, liver, gill, and brain. Our results demonstrate high IR-1 expression in Nile tilapia muscle, heart, and liver, with comparable expression levels in liver and muscle and relatively lower expression in heart.

Effects of Glucose Injection on IR-1 Expression in Nile Tilapia

As the only hypoglycemic hormone, insulin secretion is regulated by blood glucose levels. Elevated glucose stimulates pancreatic β-cells to release insulin into circulation, which acts primarily on target organs—liver, muscle, and adipose tissue. Although insulin-specific receptors have been identified in fish liver and muscle, their numbers vary among species. Baños et al. fed rainbow trout diets containing easily digestible extruded wheat and found that both high-carbohydrate (37% extruded wheat) and low-carbohydrate (21% extruded wheat) diets supported high growth rates, increased plasma glucose and insulin concentrations, and elevated muscle insulin receptor numbers. Studies indicate that long-term consumption of different dietary carbohydrate levels affects fish sensitivity to exogenous insulin, possibly due to differential activation of insulin

receptors. Factors influencing insulin receptor number may also affect receptor affinity, and reductions in either parameter can decrease insulin binding and elevate glucose levels. Cai et al. demonstrated that exogenous insulin significantly lowered blood glucose in gibel carp, which showed strong sensitivity to exogenous insulin modulated by dietary carbohydrate levels, with sensitivity differences potentially related to insulin receptor quantity. Insulin-receptor binding affinity varies among fish species and tissues, with higher binding affinity reported in carp red muscle compared to rainbow trout, and higher myocardial binding affinity than other tissues. Our results showed that IR-1 mRNA expression in heart remained unchanged within 12 h post-injection, while expression in muscle and liver peaked at 6 h and returned to normal by 12 h. Collectively, plasma glucose peaked at 1 h, plasma insulin peaked at 3 h under hyperglycemic stimulation, and IR-1 expression in muscle and liver increased further, peaking at 6 h. This delayed response of insulin content and IR-1 expression relative to plasma glucose suggests that IR-1 may not play a role in regulating plasma glucose levels in Nile tilapia. The function of the other insulin receptor subtype (IR-2) requires further investigation.

Conclusions

1. The cloned Nile tilapia IR-1 gene sequence is highly conserved with abundant characteristic tyrosine kinase sequences.
2. IR-1 is highly expressed in Nile tilapia muscle, heart, and liver, with comparable expression in liver and muscle and relatively lower expression in heart.
3. Following glucose injection, IR-1 mRNA relative expression in Nile tilapia muscle and liver peaked at 6 h and returned to normal levels by 12 h. The increase in plasma insulin concentration was delayed relative to plasma glucose, and the elevation of IR-1 mRNA expression in muscle and liver was further delayed relative to insulin, thereby increasing the metabolic burden of glucose in tilapia.

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