General and Comparative Endocrinology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Regulation and mechanism of leptin on lipid metabolism in ovarian follicle cells from yellow catfish *Pelteobagrus fulvidraco*

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ARTICLE INFO

Article history:
Received 6 April 2015
Revised 10 June 2015
Accepted 15 June 2015
Available online xxxx

Keywords:
Leptin
Lipid metabolism
Pelteobagrus fulvidraco
Primary culture
Ovarian follicles

ABSTRACT

The present study was conducted to determine the effect of leptin on lipid metabolism in ovarian follicle cells of yellow catfish *Pelteobagrus fulvidraco*. For that purpose, primary ovarian follicle cells were isolated from yellow catfish, cultured and subjected to different treatments (control, 0.1% DMSO, 500 ng/ml leptin, 500 ng/ml leptin plus 100 μM wortmannin, 500 ng/ml leptin plus 50 nM AG490, respectively) for 48 h. Intracellular triglyceride (TG) content, the activities (CPT I, FAS, G6PD, and 6PGD) and/or expression level of several enzymes (CPT I, FAS, G6PD, 6PGD, ACCa and ACCb), as well as the mRNA expression of transcription factors (PPARα, PPARγ and SREBP-1) involved in lipid metabolism were determined. Recombinant human leptin (rt-hLEP) incubation significantly reduced intracellular TG content, activities and mRNA levels of FAS, G6PD and 6PGD, SREBP-1 and PPARγ, but enhanced activity and mRNA level of CPT I, PPARα and ACCa. Specific inhibitors AG490 and wortmannin of JAK–STAT and IRS–PI3K signaling pathways prevented leptin-induced changes, indicating that JAK–STAT and IRS–PI3K signaling pathways were involved in the process of leptin-induced changes of lipid metabolism. Based on these observations above, for the first time, our study indicated that leptin reduced lipid deposition by activating lipolysis and suppressing lipogenesis in ovarian follicles of yellow catfish, and both JAK–STAT and IRS–PI3K signaling pathways were involved in the changes of leptin-induced lipid metabolism.

1. Introduction

In fish, gonadal development and reproductive strategy have widely been described in an effort to understand their developmental mechanisms and improve their reproductive performance (Cakici and Ucuncu, 2007). During ovarian development, lipid is one of the most important sources of nutrients and energy for reproduction and maintenance of ovarian tissue membrane (Sheridan and Kao, 1998). Ovary recrudescence and maturation are also associated with increases in egg size and lipid accumulation. During the process, the ovarian follicle cells play important roles in signal media, as well as material conveying and storing during gonadal development (Cecconi et al., 2004). When the oocyte starts with the growth phase, the follicle cells increase in number and height, which results from lipid accumulation in ovarian follicles. Storage and remobilization of lipid during and after oogenesis are key factors for reproductive investment and maternal output in fish. Thus, lipid metabolic characteristics changed during ovarian development, as an adaptation to meet the energy requirements of these unique follicles during reproduction. However, little is known about the lipid metabolism and its regulatory mechanism during the period.

Leptin, one of the best-characterized adipokines, has been implicated in many different functions, including reproduction. The physiological actions of leptin are mediated by membrane associated leptin receptor. The expression of leptin and its receptors has been demonstrated in ovary (follicle cells) from mammals to fish (Munoz-Gutierrez et al., 2005; Gong et al., 2013), which suggest that leptin may have a direct effect on ovarian development and folliculogenesis (Ryan et al., 2002; Srivastava and Krishna, 2011;

Abbreviations: ACC, acetyl-CoA carboxylase; CPT I, carnitine palmitoyl transferase-I; DMSO, dimethyl sulphoxide; FAS, Fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; IRS–PI3K, insulin receptor substrate (IRS)–phosphatidylinositol-3-OH-kinase; JAK–STAT, Janus kinase/signal transducer and activator of transcription; LepR, leptin receptor; PBS, phosphate buffered saline; PPAR, peroxisome proliferators-activated receptor; 6PGD, 6-phosphogluconate dehydrogenase; rt-hLEP, recombinant human leptin; SEM, standard error of mean; SREBP-1, sterol-regulator element-binding protein-1; TG, triglyceride.

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http://dx.doi.org/10.1016/j.ygcen.2015.06.008
0016-6480/

Batista et al., 2013). There is considerable evidence that leptin participates in regulation of ovarian folliculogenesis by influencing the proliferation of granulosa cells, steroidogenesis and apoptosis (Brannian and Hansen, 2002; Munoz-Gutierrez et al., 2005; Srivastava and Krishna, 2007). However, the regulatory role of leptin in lipid storage in follicle cells which is important for the development of ovarian follicles, remained unknown.

On the other hand, when acting its physiological function, the binding of leptin to leptin receptor activates several signaling pathways, including Janus kinase (JAK)–signal transducer and activator of transcription (STAT), and insulin receptor substrate (IRS)–phos phatidylinositol 3-kinase (PI3K) (Pai et al., 2005; Park and Ahima, 2015). Inhibitors of JAK–STAT and IRS–PI3K signaling pathway, such as AG490 and wortmannin, can block leptin activity *in vitro* (Halaas and Friedman, 1997; Harvey et al., 2000; Zhao et al., 2000). Thus, it can be hypothesized that these signaling pathways are involved in some of the biological effects elicited by leptin in ovary, such as their effects on lipid metabolism.

Yellow catfish *Pelteobagrus fulvidraco*, an omnivorous freshwater fish, is regarded as a potential model for studying the link between lipid metabolism and ovary development in fish. Recently, in our laboratory, many genes involved in lipid metabolism have been cloned in the fish species (Chen et al., 2013, 2014; Zheng et al., 2013b, 2015). We also cloned and characterized cDNA sequences of leptin and leptin receptor (Gong et al., 2013). Tissue expression profiles indicated the predominant mRNA expression of leptin and its receptor in ovary, inferring that leptin could directly act on ovary (Gong et al., 2013). In the present study, in order to rule out the possibility of an indirect effect of leptin and of possible interaction with other endocrine mediators on target gene expression in vivo, we used a primary yellow catfish follicles culture to analyze the transcriptional effects of leptin on lipid deposition and metabolism in this model. The effects of leptin are evaluated by determining TG level, and by investigating the activity of enzymes (CPT I, FAS, G6PD and 6PGD) and mRNA expression of genes (CPT I, FAS, G6PD, 6PGD, ACCa, ACCb, SREBP-1, PPARγ and PPARα). We also investigated the involvement of JAK–STAT and IRS–PI3K signaling pathways in the effects of leptin. To our best knowledge, this is the first to study the effects of leptin on lipogenesis and lipolysis at both transcriptional and enzymatic levels using primary ovarian follicle cells culture model in fish, which will give us new insight into the leptin role in lipid metabolism in fish.

2. Materials and methods

2.1. Chemicals

Medium 199 (M199), 0.25% sterile trypsin and fetal bovine serum (FBS) were obtained from Gibco/Invitrogen, UK. HPLC-purified recombinant human leptin (rt-hLEP), penicillin, streptomycin, glutamine, and other reagents were purchased from Sigma–Aldrich Chemical Co (St. Louis, USA). Mammalian-derived leptin orthologs were used in the present study since studies have indicated its role in yellow catfish in our recent study (Song et al., 2015).

2.2. Culture of yellow catfish

Before the experiment, uniform-sized yellow catfish (body weigh 23.8 ± 2.6 g) were obtained from a local farm, Wuhan, China. They were assigned to four indoors circular fiberglass tanks for 2-week acclimation. During the acclimatization, they were provided with a commercial feed at 2% of body weight daily. The water quality parameters were followed: dissolved oxygen ⩾ 6.3 mg/l, pH = 6.8–8.2, and total ammonia–nitrogen 0.02–0.041 mg/l, water temperature 24 ± 3 °C.

2.3. Isolation of ovarian follicles and its primary culture in yellow catfish

Follicle cells were isolated from yellow catfish according to the published protocols (Pang and Ge, 2002) with slight modification. Briefly, yellow catfish is cleared of blood by cutting off the branchial arch, and disinfected with 75% alcohol. After all the blood had been cleared, the ovaries were carefully excised from the abdominal cavity, transferred onto a plastic petri dish, and rinsed twice with phosphate buffered saline (PBS, pH 7.6, 4 °C) supplemented with amphotericin-B (25 μg/ml), streptomycin (100 μg/ml) and penicillin (100 IU/ml). The follicles from 5 to 10 females were aseptically separated with the aid of fine scalpel and scissors. The follicles were measured with an ocular micrometer in a microscope. Only the healthy vitellogenic follicles around 0.3 mm in diameter were selected. They were cultured in 100-mm plates for 4 days in the medium of M199 + 10% fetal bovine serum (FBS) under the condition of 28 °C and 5% CO_2. The culture medium was changed daily and discarded at the end of 4-day incubation. The cells in the dish were washed once with M199 + 10% FBS followed by digestion with 0.25% trypsin in PBS (2.68 mM KCl, 137 mM NaCl, 0.68 mM KH_2PO_4, 7.85 mM Na_2HPO_4) at 28 °C for 15 min. After the cells were washed three times with medium M199. They were centrifuged in 15-ml tubes at 1000 rpm for 2 min, filtered with a 40-μM Falcon cell strainer (Becton Dickinson Labware, NJ, USA) to remove the follicle debris, and then sub-cultured in 24-well plates at the density of 90,000 cells/1 ml per well for 24 h in M199 + 10% FBS before leptin and/or inhibitors treatment. We assured that experiments performed on cell culture followed the ethical guidelines of Huazhong Agricultural University for the care and use of animal cells.

2.4. Leptin treatment

For leptin treatment experiment, five groups were designed as follows: control, 0.1% DMSO, leptin (500 ng/ml), and leptin (500 ng/ml) plus wortmannin (100 nM), leptin (500 ng/ml) plus AG490 (50 μM), respectively. Each treatment was performed in triplicate. The inhibitors (AG490 or wortmannin) were added 2 h prior to the addition of leptin. The concentration of leptin and specific inhibitors was selected according to our preliminary experiment and to previous in *vitro* studies carried out in fish (Lu et al., 2012; Song et al., 2015). The cells were gathered for the following analysis at 48-h incubation. The maximal DMSO concentration applied to cells in culture did not exceed 0.1%, and had no major effect on cell viability and parameters.

2.5. TG accumulation and enzyme activity determination

For the assay of enzyme activities and intracellular TG content, the cells were collected from flasks with 0.25% (w/v) trypsin and washed with PBS. Then, cells were homogenized by sonication in different extraction buffers. Only follicle cells were used for the analysis of enzymatic activities.

For the intracellular triglyceride (TG) accumulation assay, the cells were homogenized in PBS. TG was determined by glycerol-3-phosphate oxidase p-aminophenol (GPO-PAP) methods, using a commercial kit from Nanjing Jian Cheng Bio-engineering Institute, Nanjing, China. The cellular TG content was expressed as μmol TG per mg cellular protein.

Selected enzymatic activities were assayed on cell homogenates using spectrophotometric procedures. For the determination of

Please cite this article in press as: Zhang, L.-H., et al. Regulation and mechanism of leptin on lipid metabolism in ovarian follicle cells from yellow catfish *Pelteobagrus fulvidraco*. Gen. Comp. Endocrinol. (2015), http://dx.doi.org/10.1016/j.ygcen.2015.06.008