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Adaptive expression of uncoupling protein 1 in the carp liver and kidney in response to changes in ambient temperature

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ABSTRACT

The expression of uncoupling protein (UCP1) is up-regulated in mammalian brown adipocytes during cold exposure. However, a previous study revealed that UCP1 was highly expressed in the liver of common carps, and that the hepatic expression of UCP1 was down-regulated during cold exposure. The present study examined the effects of temperature on the recovery of UCP1 expression levels and the expression of genes involved in UCP1 transcription in the livers and kidneys of common carps. The hepatic and renal expressions of UCP1 were decreased by acclimation from 22 °C to 8 °C, and a subsequent increase in the water temperature from 8 °C to 28 °C recovered the renal, but not hepatic expression of UCP1. Changes in the expression of peroxisome proliferator-activator receptor (PPAR) γ, retinoid X receptor (RXR) α and PPARγ co-activator (PGC)-1α, genes that are involved in the expression of UCP1 in mammals, with ambient temperature indicated that the expressions of PPARγ and RXRα, but not expression of PGC-1α was decreased in response to cold exposure; the hepatic and renal expressions of PPARγ and RXRα recovered to basal levels with the cessation of cold exposure, although this was not complete for hepatic expression of PPARγ. The results of the present study indicate that a unique regulatory mechanism is responsible for the hepatic and renal expressions of carp UCP1 during cold exposure and subsequent reacclimation, and is distinct from that in murine brown adipocytes.

1. Introduction

Uncoupling protein (UCP) 1 increases proton leakage from the mitochondrial inner membrane, resulting in a decreased proton motive force, which leads to increased oxygen consumption and heat generation without the concomitant generation of ATP. The restricted expression of UCP1 in mammalian brown adipocytes located in brown and white fat renders these cells responsible for non-shivering heat production (Cannon and Nedergaard, 2004).

The expression of UCP1 and its orthologs has been detected not only in placental mammals, but also in ectothermic vertebrates (Gesta et al., 2007). UCP1 was identified in the common carp (*Cyprinus carpio*), an ectothermic vertebrate, based on the conserved synteny within the mammalian lineage (Jastroch et al., 2005). It was found to be highly expressed in the liver and to a lesser extent in the kidney. The expression of UCP1 could not be detected in carp adipose tissues, which suggested that the role of UCP1 in energy expenditure in fish differed from that in placental mammals (Jastroch et al., 2005).

The expression of UCP1 in murine brown fat was previously shown to be up-regulated in response to cold exposure (Puigserver et al., 1998; Barbatelli et al., 2010), whereas hepatic UCP1 transcript levels were lower in the common carp acclimated at 8 °C for 4 weeks than in those kept at 20 °C (Jastroch et al., 2005). Similar responses were observed in gilthead sea bream (*Sparus aurata*); expression levels of hepatic UCP1 were lower in winter than in summer and fall (Bermejo-Nogales et al., 2010). The mechanism underlying the regulation of fish UCP1 expression currently remains unknown. In addition, changes in UCP1 expression upon recovery from cold exposure have not experimentally been examined. Furthermore, studies are needed to elucidate the relationship between the expression of UCP1 and non-shivering heat production in ectothermic vertebrates.

In murine brown fat, the transcription of UCP1 is regulated by several key molecules including the tissue-restricted transcription factors peroxisome proliferator-activator receptor (PPAR) α, β/δ and γ, thyroid hormone receptor (TR) α, and the transcriptional co-activator PPARγ co-activator (PGC)-1α (Puigserver et al., 1998; Barbera et al., 2001;

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Cannon and Nedergaard, 2004; Cao et al., 2004; Komatsu et al., 2010; Seebacher and Glanville, 2010). In addition, PGC-1β, a molecule structurally related to PGC-1α, is known to be highly expressed in murine brown fat (Lin et al., 2002; Seale et al., 2007). PPAR and TR heterodimerize with retinoid X receptor (RXR), and the formed complex regulates transcription of target genes including UCP1 and PGC-1 (Cannon and Nedergaard, 2004; Evans and Mangelsdorf, 2014). Furthermore, a transcription factor, cAMP responsive element binding protein (CREB) is phosphorylated and activated by protein kinase A, which leads to stimulation of UCP1 transcription (Cannon and Nedergaard, 2004). The expression levels of nuclear respiratory factor 1 (NRF1), which stimulates mitochondrial biogenesis by interacting with PGC-1α (Scarpulla, 2008), have been closely related to those of UCP1, PPARδ, and PGC-1α during cold exposure in murine brown fat (Seebacher and Glanville, 2010).

We hypothesized that regulation of carp UCP1 expression is similar to that established in murine UCP1 expression, although response to cold exposure is different between common carps and mice. As the first step to clarify regulation of fish UCP1 expression, we isolated not only 5′ flanking region of carp UCP1 gene but also coding region of carp mRNA for several molecules involved in regulation of murine UCP1 expression partially, because the information has not yet been available yet. By use of the information, we examined the effects of ambient temperature on expression levels of UCP1 and the candidates to regulate UCP1 expression in common carps.

2. Materials and methods

2.1. Animals

A total of 30 common carps aged 7 months were used. These carps were fed with commercial pellets *ad libitum* with a 12-h light/12-h dark cycle. They were kept in a temperature-controlled recirculating water system maintained at 22 °C for at least 2 weeks (day 0). The water temperature was gradually lowered to 8 °C over 3 days. After 14 days at 8 °C (days 3–17), the water temperature was gradually returned to 28 °C over 3–5 days and kept at 28 °C for 14 days (days 21–35). Carps were sacrificed on days 0, 17, and 35, and the livers and kidneys were removed.

2.2. RNA isolation and RT-quantitative real-time PCR

RNA isolation and reverse transcription (RT)-quantitative real-time PCR (qPCR) analyses were performed as described previously (Murakami et al., 2008; Asai et al., 2014). Total RNA was isolated from the livers and kidneys of carps using QuickGene RNA tissue kit S (Wako, Osaka, Japan) in QuickGene-810 (Wako, Osaka, Japan), an automatic nucleic acid extraction system, according to the manufacturer's protocol. The concentration of RNA was determined from absorbance at 260 nm. In RT-qPCR analyses, cDNA was synthesized using the high capacity cDNA reverse transcription kit with an RNase inhibitor (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's protocol. The cDNA corresponding to 5 ng of total RNA was used as a template of qPCR; the qPCR was performed using KAPA SYBR FAST Universal qPCR Master Mix (Kapa Biosystems, Boston, MA, USA) in Thermal Cycler Dice Real Time System TP800 (Takara, Otsu, Japan), according to the manufacturer's protocol. The qPCR profile was as follows: after denaturing for 30 s at 95 °C, 40 cycles consisted of 5 s at 95 °C and 30 s at 60 °C. The oligonucleotide primers for qPCR are shown in Table 1. After 40 cycles of RT-qPCR, the dissociation (melting) curve of the products was examined by changes in the ramp temperature from 60 °C to 95 °C. Each sample showed a single peak, suggesting the expected PCR products. The mRNA levels were expressed relative to EF1α mRNA levels, and the expression level in the liver of control carps was set at 100.

2.3. Isolation of 5′ flanking region of UCP1 gene from carps

The 5′ flanking region of carp UCP1 gene was isolated by two rounds of PCR; we designed 4 PCR primers. Because of lack of information on 5′ flanking region of carp UCP1 gene, forward primers were designed on the basis of information of zebrafish UCP1 gene; 5′-gttgtagttttgg tttattacacaagg-3′ named as primer A that corresponds to nt −4500 to nt −4474 of zebrafish UCP1 gene (GenBank accession number NC_007112) and 5′-taaagtcctgctgcagtggaaacag-3′ as primer B that is nt −3000 to nt −2976 of zebrafish UCP1 gene. The reverse primers were designed based on the carp UCP1 gene (AY461434), *i.e.*, 5′-gatggagtgacaggatgatgcctcgtg-3′ (nt +45 to nt +19) and 5′-ctgacagctgtgattgagttcctctg-3′ (nt +74 to nt +49) as primer C and primer D, respectively. The first round PCR was performed using genomic DNA, which was isolated from carp muscle cells, as a template DNA as well as primers A and D; PCR was conducted in a total volume of 50 μL by use of PrimeSTAR GXL DNA Polymerase that is a high fidelity polymerase (TaKaRa). The PCR profile consisted of 35 cycles of denature for 10 s at 98 °C, annealing for 15 s at 55 °C and extension for 4 min at 68 °C. The second round of PCR re-amplified using one-fiftieth of the PCR products as the template, and primers B and C with the same PCR profile. The products of the nested-PCR were electrophoresed in agarose gels, followed by ethidium bromide staining and visualization under ultraviolet light. A significant band was detected at ~2800 bp, which was within the range of expected size. The band was excised from the gels, and the nucleotide sequence was determined by direct sequencing and deposited in GenBank (LC003596). To examine the validity of sequence, PCR was performed by use of genomic DNA as a template DNA and PCR primers spanning nt −434 to nt −412 (5′-tttctgagctc ctttaatgcatc-3′) and nt +930 to nt +908 (5′-gcggtgtccagaggg aaggtgac-3′), and the nucleotide sequence of PCR product was confirmed by direct sequencing. The 5′ flanking region of carp, human, mouse, rat and zebrafish UCP gene was compared by use of Pairwise Sequence Alignment of EMBOSS (http://emboss.open-bio.org/).

Table 1
Nucleotide sequence of primers used in RT-qPCR analyses.

Gene	Forward primer	Reverse primer	GenBank accession number
CREB	5′-ctcagcagattgccaccttgg-3′	5′-gggcagctgaactaaggtcac-3′	LC000680[1]
EF1α	5′-atgcggtggaatcgacaa-3′	5′-cagagagcaatgtcaatggtg-3′	AF485331
NRF1	5′-aagccctgaggactattgtt-3′	5′-gctcctgtgccaacctgtat-3′	AB924641[1]
PGC-1α	5′-tgcctgagcttgacctctct-3′	5′-cgtcttcatccactgggatac-3′	AB767302[1]
PGC-1β	5′-tggggaagaggaggtctgc-3′	5′-ccgtccaggctgtctgtg-3′	AB767303[1]
PPARα	5′-gggaaagagcagcacgagtcc-3′	5′-ggtaggcttcatgcatctgtc-3′	FJ849065
PPARβ/δ	5′-tggctttgtggatctcttcc-3′	5′-gatctcgccgaaaggtttgc-3′	LC000683[1]
PPARγ	5′-aggcaactctacgagtcctatct-3′	5′-agttgatcatctgctcgccttcc-3′	FJ849064
RXRα	5′-cacccaatgatccagtcacaaaca-3′	5′-agctcattccatcctgctcgtaga-3′	LC000682[1]
TRα	5′-aatcacccgcaaccagtgccag-3′	5′-tcgatcagacgcctcttggcc-3′	LC000681[1]
UCP1	5′-cgccttctacaaaggtttcg-3′	5′-cgaatgacacgaacatcacc-3′	AY461434

[1] Nucleotide sequence was determined in this study.