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Characterization of proopiomelanocortin in the snakeskin gourami (*Trichopodus pectoralis*) and its expression in relation to food intake

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ABSTRACT

Proopiomelanocortin (POMC) is the precursor of several hormones involved in physiological systems including feed intake. The snakeskin gourami (*Trichopodus pectoralis*) *POMC* complementary DNA (*TpPOMC*) was cloned and characterized. Phylogenetic analysis showed that *TpPOMC* was clustered in a major *POMC* lineage in fish. Analysis of the K_a to K_s ratios for the entire POMC sequence and for each hormonal segment suggested that different POMC-derived peptide segments were subject to different evolutionary pressures. High expression level of *TpPOMC* was observed in all brain regions, with the highest levels in the diencephalon and pituitary gland. In situ hybridization also revealed that *TpPOMC*-expressing cells were distributed in discrete brain regions. The transcription level of *TpPOMC* was also found at moderate levels in several peripheral tissues, including gills, liver, head kidney, trunk kidney, stomach, intestine, spleen, ovary and testis, and at a low level in muscle. The expression level of *TpPOMC* was evaluated in each brain region (telencephalon, mesencephalon, metencephalon, and diencephalon together with the pituitary gland) at 1 h before the first and the last meals of the day and compared with expression levels at a time interval between the first and the last meals of the day. Low expression levels of *TpPOMC* were found at 1 h before the last meal of the day ($P < 0.05$). These finding suggest that decreased *POMC* expression level may lead to reduced melanocyte-stimulating hormones, which may in part be responsible for stimulating food intake. The effect of short-term fasting (24 h) on *TpPOMC* expression level in each brain region was also investigated. In telencephalon and diencephalon together with the pituitary gland, *TpPOMC* messenger RNA reached a nadir at 12 h of fasting, whereas *TpPOMC* transcript showed a nadir at 6 h of fasting in metencephalon and mesencephalon. A peak of *TpPOMC* level was observed at 18 h of fasting in metencephalon and diencephalon together with the pituitary gland. These findings suggest that *TpPOMC* expression is affected by nutritional status.

1. Introduction

Proopiomelanocortin (POMC)-derived peptides have been demonstrated to be associated with a wide range of physiological processes in vertebrates, including body pigmentation, steroidogenesis, reproduction, immune response, food intake, and energy homeostasis [1–3]. POMC is a precursor of hormonal peptides such as adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormones (γ-MSHs, α-MSHs, β-MSHs, and δ-MSHs), β-lipotropic hormone (β-LPH), and β-endorphin (β-END) [4]. Active POMC-derived peptides are generated by a series of posttranslational processes involving serine protease prohormone convertases, which perform

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proteolytic cleavage at specific pairs of dibasic amino acid residues (Arg-Arg, Arg-Lys, Lys-Arg, or Lys-Lys) that flank the hormonal peptide segments within POMC [5,6]. In mammals, the series of posttranslational cleavages is cell-specific; for example, POMC in the pars distalis undergoes posttranslational processing to generate an N-POMC domain, joining peptide, ACTH and β-LPH, although in the pars intermedia, the N-POMC domain, ACTH, and β-LPH are cleaved to γ-, α-, and β-MSH, respectively, and corticotrophin-like intermediate lobe peptide (CLIP) and β-END are consequently also produced [7]. Posttranslational processing of POMC-derived peptides in fish appears to be similar to that in mammals [8–10].

Characterization of the *POMC* gene in several fish species has shown that it varies among fish taxa. The number of MSH domains differs between fish species; for example, 2 MSHs, including α-MSH and β-MSH, occur in ray-finned fish (actinopterygii) and the agnathan lamprey (cephalaspidomorphi) [10–17], but 3 MSHs have been identified (γ-MSH, α-MSH, and β-MSH) in lobed-finned fish (sarcopterygii) and primitive ray-finned fish [8,18,19]. Another MSH, named δ-MSH, was characterized in cartilaginous fish with 4 MSH domains (chondrichthyes) [20]. Furthermore, like mammals, several ray-finned fish and cartilaginous fish have been reported to contain a single copy of the *POMC* gene [13,15,19]. However, *POMC* gene duplication has resulted in the presence of multiple types of POMCs. Two nonallelic types of *POMC* exist in several fish [8,12,14,17,18], whereas 3 distinct types of *POMC* mRNAs have been classified in the barfin flounder [10]. In addition, variable splicing of the *POMC* gene results in 4 different *POMC* mRNAs generated by 3 *POMC* genes in rainbow trout. Moreover, 2 different types of POMC, proopiocortin and proopiomelanotropin are found in the lamprey [11]. The *POMC* gene thus exhibits divergent characteristics throughout fish evolution, which suggests that *POMC* may represent a useful gene marker for studying molecular evolution in fish, which exhibit considerable species diversity among vertebrates.

POMC-derived peptides such as ACTH and the family of MSH peptides, known as melanocortins, act as natural ligands for the melanocortin receptor. Food intake and energy balance are controlled by multiple orexigenic and anorexigenic pathways. Binding of α-MSH to the melanocortin 4 receptor (MC4R) was revealed to inhibit appetite and increase metabolic activity (reviewed in [21]). Neuropeptide Y (NPY) inhibited *POMC* expression, which would result in orexigenic effects [22]. Expression levels of MC4R and NPY were recently investigated in relation to feeding status in the snakeskin gourami (*Trichopodus pectoralis*) [23,24]. Reduction of MC4R expression was observed 1 h before the last meal of the day, whereas this reduction was not found 1 h before the first meal of the day [23]. The reduction in NPY expression was detected after a meal, whereas the peak of NPY expression was found 1 h before the schedule of the first meal of the day [24]. The snakeskin gourami is a member of the *Trichopodus* gouramis, which are of economic importance both for food and in the global aquarium trade. Although the biology of this species has been well investigated, there is limited information on the molecular endocrinology of this species. However, a better understanding of the characteristics of *POMC* and its expression level in relation to feeding status in this species will impact not only on evolutionary studies but also on aquaculture perspectives.

In this study, we compared the characteristics of snakeskin gourami *POMC* messenger RNA (mRNA) with that of other teleosts and investigated its differential expression level in the central and peripheral tissues. We also investigated the evolutionary pressures acting on POMC as a whole and on each POMC-derived peptide. We further considered *POMC* expression level in relation to feeding status and fasting to provide information on one of its major biological functions.

2. Materials and methods

2.1. Fish

Mixed-sex snakeskin gourami (*T. pectoralis*) at sizes of 60 to 65 g were obtained from commercial farms and reared at the Suranaree University of Technology Farm. Throughout the experiments, fish were cultured in a 5 m × 10 m × 1 m concrete pond with a natural light-dark cycle and fed a commercial diet (40% crude protein and 6% fat) to satiety, twice a day at 10 AM and 4 PM.

2.2. Molecular cloning of POMC

The full-length complementary DNA (cDNA) of *POMC* was cloned from snakeskin gourami (*TpPOMC*) as follows. Total RNA was extracted from the brain of snakeskin gourami and treated with RNase-free DNase I, according to the manufacturer's instructions (Promega, Madison, WI). Rapid amplification of cDNA ends (RACE), 3′ and 5′, was conducted using the SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA). Two gene-specific degenerate primers, POMC-F1 (primary) and POMC-F2 (nested), were designed according to 2 highly conserved regions of teleost *POMC* mRNAs (Fig. 1). The 3′ end of *TpPOMC* was cloned by nested polymerase chain reaction (PCR) using POMC-F1 (5′-GTGTCCTGYGTGGYTWTTN-3′) and POMC-F2 (5′-CCGGCTCTGTCGTGCTGACCTTACC-3′) as the forward and reverse primers provided in the SMART RACE kit. PCR was carried out in a total volume of 15 μL, containing 200 μM of each deoxynucleotide, 1 pmol of each primer, 1X Ex Taq buffer, and 0.375 U Ex Taq (Takara Shuzo, Shiga, Japan). PCR was performed at 95°C for 3 min, followed by 40 reaction cycles consisting of 45 s at 95°C, 45 s at 60°C, and 90 s at 72°C. The final elongation step was conducted at 72°C for 5 min. PCR products of the expected size were isolated and purified using a Gelpure DNA Purification Kit (GeneMate, Kaysville, UT). The PCR-amplified DNA fragment was then cloned into pGEM T-Easy plasmid (Promega) and sequenced. The plasmid containing the 3′RACE-DNA fragment, p3-POMC, was kept for further use. Two gene-specific primers, POMC-R1 (primary; 5′-TCACTTCTGCTGCTGTCCD-3′) and POMC-R2 (nested; 5′-GGGCCTTCTCCTCCTCTACTGCTGC-3′), were designed based on the DNA sequences of the 3′ end of *TpPOMC* (Fig. 1). The 5′ end of *TpPOMC* was cloned by nested PCR using the forward primers provided in the SMART RACE kit and the reverse primers POMC-R1 and POMC-R2. The PCR reaction and