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Comparative Biochemistry and Physiology, Part B 148 (2007) 55–64
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Molecular characterization of neuropeptide Y gene in Chinese perch, an acanthomorph fish

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Received 16 February 2007; received in revised form 21 April 2007; accepted 21 April 2007
Available online 3 May 2007

Abstract

The full-length neuropeptide Y (NPY) cDNA of Chinese perch *Siniperca chuatsi* was 704 bp in length, and contained a 300 bp open reading frame encoding a prepro-NPY with 99 amino acids. The predicted prepro-NPY peptide contained a putative signal peptide of 28 amino acids and a mature NPY of 36 amino acids, followed by the proteolytic processing site Gly–Lys–Arg and 35 amino acids that comprise the C-terminal peptide of NPY. Amino acid alignment and phylogenetic analysis indicate that the predicted Chinese perch prepro-NPY (composed of 99 amino acids) had high identities to the prepro-NPY of acanthomorph fishes (93–95%), whereas it had much lower identities to the prepro-NPY (composed of 96 or 97 amino acids) of cyprinid fishes (59–60%) or mammals (57–63%). Chinese perch NPY gene consists of four exons and three introns. The ratio of intron 2 to intron 3 was over 14 in Chinese perch NPY gene, whereas this ratio was below 4 in zebrafish and mammalian NPY gene. The total size of the Chinese perch NPY gene was 2223 bp, which was only about 28% of the size of NPY gene in higher vertebrate. Analysis of a 1622 bp promoter region of Chinese perch NPY gene, revealed a typical TATA box, a GC box and an untypical CAAT box, located at 84 bp, 101 bp and 303 bp upstream of the start codon respectively. Three STAT binding site-like elements (TCCAGTA) which were necessary for the leptin-induced transcriptional control of rat NPY gene were identified. In consistence to the effect of cortisol on fish brain NPY mRNA expression, four glucocorticoid-responsive elements were detected. Besides the highest expression in brain, substantial level of Chinese perch NPY mRNA expression was detected in the spleen and liver, and trace level of NPY mRNA expression was also detected in the adipose tissue, intestine and muscle. These results indicated that Chinese perch NPY might be involved in the food intake control by leptin and cortisol system, and diversification of NPY signaling should exist between acanthomorph fishes and cyprinid fishes as well as mammals.

Keywords: Neuropeptide Y; cDNA; Genomic structure; 5′ flanking region; Tissue expression; Food intake control; Chinese perch; Acanthomorph fish

1. Introduction

Neuropeptide Y (NPY) belongs to a family of 36-amino acid peptides, which also includes peptide YY (PYY) and tetrapod pancreatic polypeptide (PP) (Larhammar, 1996; Cerdá-Reverter et al., 2000a), and the fish pancreatic polypeptide Y (PY) has been included in PYY (Sundström et al., 2005). NPY has been implicated in various physiological processes (Dumont et al., 1992). It has, for instance, been established as an important regulator of food intake (Clark et al., 1984; Kalra, 1997; Silverstein et al., 1998; López-Patiño et al., 1999). Moreover, NPY has been linked to the regulation of circadian rhythms and sexual behavior in mammals (Dumont et al., 1992) as well as in fish. The importance of NPY in neural regulation is supported by its strong conservation across species (Larhammar, 1996) as well as profound similarities in anatomical distribution (Batten et al., 1990; Cepriano and Schreibman, 1993; Söderberg et al., 1994; Vecino et al., 1994).

In mammals, it is known that NPY plays an important role in the neural regulation of food intake by leptin at the transcriptional or posttranscriptional level (Schwartz et al., 2000). The leptin-responsive element which confers NPY gene transactivation by leptin was determined in a 221-bp region of rat NPY gene promoter (−553/−335), where two STAT3-binding site-like elements (TCCAGTA) exist (Muraoka et al., 2003; Higuchi et al., 2005). Recently, fish leptin cDNA has been cloned from

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doi:10.1016/j.cbpb.2007.04.016

acanthomorph fishes, e.g. pufferfish, freshwater pufferfish and medaka (Kurokawa et al., 2005), as well as cyprinid fishes, e.g. common carp (duplicate leptin genes) and zebrafish (Huising et al., 2006). Leptin mRNA expression in common carp changed acutely after food intake, but involvement of leptin in the long-term regulation of food intake and energy metabolism was not evident from fasting for days or weeks or long-term feeding to satiation (Huising et al., 2006).

In mammals, the stimulatory effect on food intake is mediated by the Y1 and Y5 receptors in the hypothalamus (Lecklin et al., 2002). Interestingly the naturally occurring truncated form of PYY, PYY 3–36 has recently been shown to have an inhibitory effect on food intake in rodents and humans via Y2 receptors in the arcuate nucleus (Batterham et al., 2002). But so far neither Y1 nor Y5 have been found in any of the three species whose genomes have been almost completely sequenced, i.e., zebrafish, pufferfish and freshwater pufferfish (Lundell et al., 1997; Ringvall et al., 1997; Starbäck et al., 1999).

Chinese perch *Siniperca chuatsi*, an acanthomorph fish, is a commercially important and popular cultured freshwater fish species in China. Acanthomorph fishes are the largest subgroup of teleost fish, and their sequences of NPY family reflect unique evolutionary diversification in this lineage (Larhammar, 1996; Cerdá-Reverter et al., 1999, 2000a,b, 2001; Kurokawa and Suzuki, 2002; Sundström et al., 2005). The long-term goal is to study the structure, regulation and signaling of Chinese perch NPY gene. This information will provide a better understanding of the control of feeding in a key aquaculture species and allow future development of tools for improving the culture of this species, and perhaps other species of acanthomorph fishes.

2. Materials and methods

2.1. Fish sampling

Female and male Chinese perch *S. chuatsi* (mass 400–500 g) used in this study were obtained from Guangdong Mandarin Fish Farm (Nanhai, Guangdong Province, China). Randomly selected fish were killed, and brain, intestine, liver, muscle, spleen and adipose tissues were dissected immediately for RNA isolation.

2.2. RNA isolation and reverse transcription

Total RNA was isolated from brain, intestine, liver, muscle, spleen and adipose tissues using SV Total RNA Isolation System (Promega, USA), according to instructions from the manufacturer. Reverse transcription was performed with oligo $(dT)_{18}$ primer using First Strand cDNA Synthesis Kit (Toyobo, Japan), according to instructions from the manufacturer.

2.3. PCR cloning and sequencing of partial NPY cDNA

Two degenerate primers (NPY01F and NPY02R) (Table 1) were designed to clone partial brain NPY cDNA sequences of Chinese perch by PCR. The PCR parameters were 30 cycles of 94 °C for 1 min, 40 °C for 1 min and 72 °C for 1 min, with an additional initial 3-min denaturation at 94 °C and a 5-min final extension at 72 °C. PCR products of the expected length were purified from agarose gel, and cloned into the pGEM-T Easy vector (Promega, USA). Inserts were sequenced using an ABI PrismTM 377 (Perkin Elmer, USA).

2.4. RACE

Gene-specific primers were designed in the cloned PCR fragments of Chinese perch NPY for 3′-RACE and 5′-RACE (Table 1). 3′-RACE was performed using a 3′-Full RACE Core Set (TaKaRa, Japan). Total liver RNA was reverse-transcribed to cDNA in the presence of oligo(dT)-3 site adaptor primer (provided in the kit) using a sequential program of 30 °C for 1 min, 50 °C for 30 min and 95 °C for 5 min. The prepared cDNA was firstly amplified by PCR with 0.2 μM primer NPY3′S1 and 0.2 μM 3 site adaptor primer (provided in the kit). The secondary PCR was performed using primer NPY3′S2 and 3 site adaptor primer. The PCR parameters were 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, with an additional initial 3-min denaturation at 94 °C and a 5-min final extension at 72 °C. The 3′-RACE-PCR products were purified from agarose gel, cloned into the pGEM-T Easy vector and sequenced.

5′-RACE was performed using SMART RACE cDNA Amplification Kit (Clontech, USA). One microgram total RNA was reversely transcribed with the 5′-RACE CDS Primer and SMART II A Oligonucleotide (provided in the kit). In the first PCR, the cDNA was amplified with two primer sets, NPY5′A1 and Universal Primer A Mix (UPM, provided in the kit). In the second PCR, primer sets NPY5′A2 and Nested Universal Primer A (NUP, provided in the kit) were used. The PCR

Table 1
Primer sequences for PCR

Name of primer	Sequence of primer
NPY01F	5′-ATAC(T)CCGGTGAA A(G)CCGGAG(A)AA-3′
NPY02R	5′-ATACCT(G)C(T)TGTCT(G)TGTGARGAG(A)-3′
NPY03F	5′-AGAGCAGAAGAGACGCCACAGGA-3′
NPY04F	5′-GGAGCTGGCCAAGTACTA-3′
NPY05R	5′-CCAACACTGATGACAGCA-3′
NPY06R	5′-GTTGTGACTGTAGCTGATGGGT-3′
NPY3′S1	5′-GGAGCTGGCCAAGTACTA-3′
NPY3′S2	5′-TCAGCCCTGAGACACTACA-3′
NPY5′A1	5′-TGTAGTGTCTCAGGGCTGA-3′
NPY5′A2	5′-TAGTACTTGGCCAGCTCC-3′
NPYGSP1	5′-GCATAACCAGCAGACAATCATC-3′
NPYGSP2	5′-CCAGATTGTCAGAGGGAGATTTGCG-3′
3sites adaptor primer	5′-CTGATCTAGAGGTACCGGATCC-3′
AP1	5′-GTAATACGACTCACTATAGGGC-3′
AP2	5′-ACTATAGGGCACGCGTGGT-3′
5′-RACE CDS primer	5′-$(T)_{25}$VN-3′ (N=A, C, G, or T; V=A, G, or C)
SMART II A Oligo	5′-AAGCAGTGGTATCAACGCAGAGTACGCGGG-3′
UPM	5′-CATATACGACTCACTATAGGGC-3′ (short) 5′-CTAATACGACTACTATAGGGCAAGCAGTGGT-ATCAACGCAGAGT-3′ (long)
NUP	5′-AAGCAGTGGTATCAACGCAGAGT-3′
ACT01F	5′-CGTGACATCAAGGAGAAGC-3′
ACT02R	5′-TCTGCTGGAAGGTGGACAG-3′