Gene xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Gene

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

# Molecular cloning, expression analysis, and appetite regulatory effect of *peptide YY* in Siberian sturgeon (*Acipenser baerii*) ☆

Hu Chen [1], Xin Zhang [1], Jin Hao, Defang Chen, Ju Liu, Yundi Gao, Jieyao Zhu, Hongwei Wu, Fangjun Lin, Yundan Pu, Dengyue Yuan, Rongbin Wei, Chaowei Zhou, Tao Wang, Zhiqiong Li *

*Department of Aquaculture, College of Animal Science and Technology, Sichuan Agricultural University, 46# Xinkang Road, Ya'an, China*

ARTICLE INFO

*Article history:*
Received 16 October 2014
Received in revised form 11 March 2015
Accepted 13 March 2015
Available online xxxx

*Keywords:*
*Acipenser baerii*
*Peptide YY*
Food intake
Intraperitoneal injection
Food deprivation

ABSTRACT

Peptide YY (PYY) is an anorectic brain–gut peptide involved in feeding regulation and well characterized in mammals. However, the functional role of *PYY* in the appetite regulatory of fish is not clear. In this study, we characterized a high conservation of *PYY* cDNA and found high expression levels of *PYY* mRNA in the brain and digestive tract of Siberian sturgeon. Then, we examined preprandial (pre- and post-feeding) changes of *PYY* mRNA expression in the brain that showed a significantly increased in 3 h post-feeding, suggesting an anorectic possible function of PYY in Siberian sturgeon. Next, we examined the expression of PYY mRNA during 15 days fasting and refeed after fasting. The Ss*PYY* mRNA expression of unfed fish had a significant 2.4, 1.7, 2.0, 2.2, and 2.1-fold decrease compared to 1-, 3-, 6-, 10- and 15-day ad libitum fed animals, respectively. After refeed, Ss*PYY* mRNA significantly increased 1.9 and 4.1-fold above that of the 15-day fed and unfed fish control group ($P < 0.01$). Furthermore, a single intraperitoneal injection of 10, 100 and 200 ng/g BW SsPYY1–36 caused a reduction in the next feeding and no significant reduction in food intake was observed in fish injected with a 1 ng/g BW. Overall, PYY has a potentially role in food intake attenuation of Siberian sturgeon.



## 1. Introduction

Appetite regulation relies on elaborate mechanisms, which is generally considered to be associated with endocrine factors. The endocrine system contains numerous of hormones and neuropeptides with anorexigenic and orexigenic actions, senses and co-ordinate energy signals, which allows the precise regulation of food intake. The 36 amino acid brain–gut peptide YY (hereafter referred to as PYY) was first discovered by means of an assay to detect peptides with carboxyl-terminal amides in porcine intestinal extracts (Tatemoto and Mutt, 1980; Tatemoto, 1982). As a brain–gut hormone in vertebrates, PYY was produced in the distal digestive tract and high expression was found in the brain, especially the hypothalamus (Lundberg et al., 1982; Ekman et al., 1986; Cerdá-Reverter and Larhammar, 2000; Morimoto et al., 2008; Gonzalez and Unniappan, 2010; Chen et al., 2013; Yuan et al., 2014).

Furthermore, the serine protease, dipeptidyl peptidase IV (DPP-IV) cleaves PYY (1–36) at the proline–alanine region of the N-terminal in mammals, so that the truncated peptide, PYY (3–36) can be produced (Grandt et al., 1994; Ballantyne, 2006). In most studies, PYY (1–36) and PYY (3–36) have been shown to modulate feeding in mammals—with a predominant anorectic role being observed (Eberlein et al., 1989; Batterham et al., 2002; Chelikani et al., 2005, 2007; Degen et al., 2005; Moran et al., 2005; Sloth et al., 2007; Unniappan and Kieffer, 2008). However, some studies have shown that a central administration of PYY (1–36) in rats stimulates feeding (Morley et al., 1985; Hagan and Moss, 1995; Kanatani et al., 2000) and a number of research groups could not find any effect of PYY (3–36) in regulating feeding in rodents (Tschöp et al., 2004; Abbott et al., 2005). In addition, PYY has been shown to have effects on intestinal motility, gastric emptying and pancreatic secretion (Lundberg et al., 1982; Pappas et al., 1985; Savage et al., 1987).

Previous studies regarding the feeding regulatory effect of *PYY* have been mainly conducted in mammals. PYY's role in regulating feeding in non-mammalian vertebrates is unclear, such as fish. In some cases, the appetite regulatory effect of *PYY* in fish is controversial (Bloom, 2004). Nevertheless, the consensus deriving from a range of fish models has demonstrated that PYY reduces appetite (Kurokawa et al., 2004; Gonzalez and Unniappan, 2010; Chen et al., 2013). Besides, previous studies have shown that peripherally administrated PYY (1–36) reduces food intake in *Carassius auratus* and *Ctenopharyngodon idellus* (Gonzalez and Unniappan, 2010; Chen et al., 2013). By demonstrating that PYY mRNA expression levels in fed fish were significantly higher when

*Abbreviations: PYY, peptide YY*; DPP-IV, dipeptidyl peptidase IV; SsPYY, Siberian sturgeon PYY; HPLC, High Performance Liquid Chromatography; CITES, Convention on International Trade in Endangered Species; RACE, rapid amplification of cDNA ends; BW, body weight.

☆ Disclosure summary: The authors have nothing to disclose.

* Corresponding author at: Department of Aquaculture, College of Animal Science and Technology, Sichuan Agricultural University, China.
*E-mail address:* lizhiqiong454@163.com (Z. Li).

[1] Hu Chen and Xin Zhang contributed equally to this work.

http://dx.doi.org/10.1016/j.gene.2015.03.028
0378-1119/

compared with unfed fish, a recent study in *Schizothorax prenanti* has provided another support for the anorexigenic actions of PYY

All sturgeon species are listed in the Convention on International Trade in Endangered Species (CITES), in which, Siberian sturgeon (*Acipenser baerii*) is a widely farmed important commercial fish in sturgeon breeding industry (Wei et al., 2004). In general, food intake is considered to be related to growth and reproduction in vertebrates (Volkoff et al., 2005; Yoneda and Wright, 2005). Understanding the mechanism of appetite regulation better might be beneficial for dealing with feeding, growth and reproduction issues in Siberian sturgeon.

In the present study, the *PYY* cDNA has been identified and characterized from Siberian sturgeon with its expression pattern being analyzed in various tissues of juvenile fish. Moreover, the postprandial and feeding status change of *PYY* mRNA expression in the brain have been discussed. In addition, the effect of exogenous PYY (1–36) on food intake in Siberian sturgeon was documented.

## 2. Material and methods

### 2.1. Materials

The peptide PYY (1–36) (FPPKPEHPGDDAPAEDVAKYYTALRHYINL ITRQRY-NH2) was synthesized with a purity of 90% purity at Shanghai Top-peptide Co., Ltd (Shanghai, China). The purity was confirmed through High Performance Liquid Chromatography (hereafter referred to as HPLC) analysis. Peptide was dissolved in fish physiological saline for 1, 10, 100, or 200 $ng \cdot \mu l^{-1}$ and stored at −20 °C until use.

### 2.2. Experimental animals and sampling

Siberian sturgeons with an average body weight of 94.5 ± 27.2 g were obtained from Runzhao fisheries (Sichuan Province, China). Before experimental use, fish were maintained at the Sichuan Agricultural University Farm in a 120 × 40 × 60 $cm^3$ aquarium (6 fish in each tank), which was supplied with a continuous flow of fresh water at 11–13 °C and under a natural photoperiod for at least 2 weeks. At the rate of 3% of the body weight (hereafter referred to as BW), fish were fed once daily at 16:00, on a commercial pellet diet (nutrient content: crude protein ≥ 40%, crude fat ≥ 12%, coarse fiber ≤ 6%, crude ash ≤ 18%, water ≤ 18% and total phosphorus ≥ 1.2%; Tongyi, Suzhou, China). The uneaten feed within an hour was removed by employing a dip net.

For cDNA cloning and tissue distribution analysis, six males and six females were killed with an overdose of tricaine methanesulfonate (MS-222), which had been fasted for 24 h and their tissues (brain, esophagus, ventriculus, duodenum, intestinum valula, rectum, liver, pyloric caeca, pancreas, spleen, kidney, gonad, heart, muscle, skin and eye) were rapidly dissected, snap-frozen in liquid nitrogen, and stored at −80 °C until RNA isolations were conducted.

To evaluate the preprandial and postprandial changes in *PYY* mRNA expression, pre- and post-feeding groups of Siberian sturgeon (n = 6/group) were sampled from 9 glass aquariums. With regard to the feeding behavior, no differences were found between tanks. Before the testing, all test fish have undergone a 21-day adaptation period and the fish were fed once daily at 16:00. Fish were respectively sampled 3 h prior to feeding (−3 h), 1 h prior to feeding (−1 h), upon commencement of feeding (0 h), 1 h after feeding (+1 h), 3 h after feeding (+3 h) and 6 h after feeding (+6 h). Three unfed groups were sampled at +1 h, +3 h, +6 h and served as unfed control groups. At each time point, a total of 6 fish (all of a tank) were captured. The whole brain and duodenum were dissected out, snap-frozen in liquid nitrogen, and stored at −80 °C.

For fasting experiment, fish were stripped of food for 15 d and then re-fed. Eleven groups of Siberian sturgeon (n = 6/group) were fed daily at 16:00 and acclimated for 3 weeks in the conditions mentioned above prior to the study. Upon commencement of the study, five groups of fish were continued to be fed daily for days 1, 3, 6, 10 and 15 respectively, while the remaining six groups were not fed. A total of 6 fish (all of a tank) were sampled at selected time points (1, 3, 6, 10 and 15 d). At the end of the 15-day fasting, one of the unfed group was re-fed. The whole brain and duodenum samples were collected 1 h after the feeding. As described previously, brain and duodenum extraction was conducted.

To examine the effect of exogenous PYY on food consumption, five groups of weight-matched fish (average body weight 95.42 ± 5.42 g) (n = 18/group) were daily fed and allowed to acclimate to conditions for 3 weeks. On the day of the study, fish were anesthetized by applying the pre-determined dose of MS-222 (35 $mg \cdot l^{-1}$) from a pilot study, which were respectively weighed and given an injection (1 $\mu l \cdot g^{-1}$ BW) of saline or 1, 10, 100, or 200 ng/g BW of peptide for each group. After injection, fish were returned to the aquarium and allowed to recover for 20 min. And then, an excess of pre-weighed diet was added to each tank and fish were permitted to be fed for 1 h. Uneaten food was collected 1 h after commencement of feeding to measure the total food intake. The experimental procedure was replicated in triplicate.

### 2.3. Cloning of full-length cDNA of SsPYY gene

According to the manufacturer's protocol, total RNA was isolated from the brain by the employment of Trizol® reagent (TaKaRa, Japan), so as to characterize the full-length cDNA of *SsPYY* gene. RNA concentrations were determined through the optical density absorption ratio (Bio-Rad) at wavelengths of 260 nm and 280 nm. Samples with an absorption ratio between 1.7 and 2.2 were adopted for cDNA synthesis. 2 μg of RNA was applied for the first strand cDNA synthesis by employing the Prime Script RT reagent Kit (TaKaRa, Dalian). Primers (*PYY*-F and *PYY*-R; Table 1) for partial *PYY* cDNA were designed according to highly conserved regions of other vertebrate *PYY*s with a partial fragment (425 bp) of *SsPYY* mRNA being obtained. With 5′ or 3′ adaptors being added, the first-strand cDNA of the whole brain was synthesized using SMART RACE cDNA Amplification Kit (Clontech, USA) for the rapid amplification of cDNA ends (RACE) PCR. For the full-length cDNA, 5′ and 3′ RACE PCR were performed. The RACE PCR conditions were described as previously (Lin et al., 2014). The RACE products were purified from agarose gel through Universal DNA Purification Kit (TIANGEN, China), and cloned into the pMD-19T vector (TaKaRa, Japan). The inserts were sequenced at Beijing Genomics Institute (Beijing, China).

### 2.4. Structural analysis

The nucleotide and deduced protein sequences were analyzed by employing BLASTn and BLASTp (http://www.ncbi.nlm.nih.gov). Besides, the ORF was predicted with the help of Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The cleavage site of the signal peptide was predicted by making use of SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Furthermore, through the neighbor-joining method, phylogenetic tree based on the amino acid

**Table 1**
Primer sequences and function used in this study.

| Primer name | Primer sequence (5–3′) | Applications |
|---|---|---|
| PYY-F | 5′GTCAAGAAGCAGCAGCGT3′ | PYY cloning |
| PYY-R | 5′CGTTAGGAGGAGGGTCAG3′ | PYY cloning |
| PYY-F1 | 5′GTTGGTGTGCCTGGGGACCTTTGT3′ | PYY 3′RACE outer |
| PYY-F2 | 5′CTCATCACGAGGCAGAGGTATGGCAA3′ | PYY 3′RACE inner |
| PYY-R1 | 5′CGCTTGCCATACCTCTGCCTCGTGA3′ | PYY 5′RACE outer |
| PYY-R2 | 5′CCAGGGCTTCAGGCAGGACACCAT3′ | PYY 5′RACE inner |
| PYY-F3 | 5′AGGCAGAGGTATGGCAAGCG3′ | PYY qPCR |
| PYY-R3 | 5′GGAGGGTCAGGAGACGGGAT3′ | PYY qPCR |
| β-Actin-F | 5′GTTGGTATGGGACAGAAGGACA3′ | β-Actin qPCR |
| β-Actin-R | 5′CCAGTTGGTAACAATGCCGT3′ | β-Actin qPCR |

β-Actin was used as housekeeping genes. Primers were designed using primer premier 5.0.

Please cite this article as: Chen, H., et al., Molecular cloning, expression analysis, and appetite regulatory effect of *peptide YY* in Siberian sturgeon (*Acipenser baerii*), Gene (2015), http://dx.doi.org/10.1016/j.gene.2015.03.028