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The inhibitory effect of NUCB2/nesfatin-1 on appetite regulation of Siberian sturgeon (*Acipenser baerii* Brandt)

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ABSTRACT

Since NUCB2 was discovered, the information about NUCB2/nesfatin-1 in appetite regulation in both mammals and teleost has been still limited. The present study aims to determine the effects of nesfatin-1 on food intake and to explore the appetite mechanism in Siberian sturgeon. In this study, *nucb2* cDNA sequence of 1571 bp was obtained, and the mRNA expression of *nucb2* was abundant in brain and liver. Levels of *nucb2* were appreciably increased in brain after feeding 1 and 3 h, while significantly decreased within fasting 15 days. Except for fasting 1 day, the expression pattern of *nucb2* in the liver was similar to the brain. Acute intraperitoneal (*i.p.*) injection of nesfatin-1 inhibited the food intake during 0–1 h in a dose-dependent manner and 50 or 100 ng/g BW nesfatin-1 significantly decreased the cumulative food intake during 3 h. The daily food intake and cumulative food intake were remarkably reduced post chronic (7 days) *i.p.* injection. Moreover, chronic *i.p.* injection of nesfatin-1 affected the expression of appetite factors including *cart*, *apelin* and *pyy* in the brain, stomach and liver with the consistent pattern of change, while the levels of *cck*, *ucn3* and *nucb2* in these have different patterns. This study demonstrates that nesfatin-1 acts as a satiety factor in reducing the short-term and long-term food intake of Siberian sturgeon. Therefore, the data suggesting nesfatin-1 inhibits the appetite through different signal pathways in the central and peripheral endocrine systems of Siberian sturgeon.

1. Introduction

Feeding, a complex behavior, is mainly influenced by the interaction of central nervous system and periphery tissues as well as environmental cues (Lenard and Berthoud, 2008; Matsuda, 2009; Volkoff, 2016). Many appetites factors (hunger factors and satiety factors) in central and peripheral signaling pathways play the important role in the regulation network of food intake (Hayes and Volkoff, 2014; Kristensen et al., 1998; Rønnestad et al., 2017; Tatemoto et al., 1982; Volkoff et al., 2010; Volkoff et al., 2016). Recently, several appetite factors have been identified in mammals. Nevertheless, it remains unclear whether conspecific feeding related factor has the similar function between teleost and mammals. Nesfatin-1 is an 82-amino acid protein derived from nucleobindin 2 (NUCB2), which has been reported the effects on inhibiting the food intake in Rat (Oh-I et al., 2006).

Since NUCB2 was discovered, it has been identified in many mammals, such as Humans (Yamada et al., 2010; Oh-I et al., 2006; Gaigé et al., 2013) and Dog (Nozawa et al., 2016), as well as Avers (Selvan et al., 2007) and Amphibians (Gen-Bank Accession No. NM_001015824). In teleost, *nucb2* was reported only in *Cyprinidae* including Goldfish (Gonzalez et al., 2010), Zebrafish (Hatef et al., 2014) and *Schizothorax prenanti* (Lin et al., 2014b). Several studies showed that Zebrafish has two genes of *nucb2* (*nucb2A* and *nucb2B*), while Goldfish and *Schizothorax prenanti* were reported only one *nucb2* gene (Gonzalez et al., 2010; Hatef et al., 2014; Lin et al., 2014b). It has been reported that *nucb2* was widely distributed in the central system and peripheral tissues of animals including Mice (Kim et al., 2014), Dog (Nozawa et al., 2016), Zebrafish (Hatef et al., 2014) and *Schizothorax prenanti* (Lin et al., 2014b). Unlike mammals, *nucb2* was abundant in hepatopancreas in *Cyprinidae*. Hitherto, the data of *nucb2* in teleost is very scarce.

Nesfatin-1 is the major lytic peptide of NUCB2 (Oh-I et al., 2006), which has a variety of biological functions (Aydin, 2013) including appetite regulation (Atsuchi et al., 2010; Shimizu et al., 2009; Stengel et al., 2012), cardiovascular function (Tanida and Mori, 2011; Yosten and Samson, 2009, 2010), glucostasis (Aslan et al., 2012; Gonzalez

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et al., 2011; Su et al., 2010) and sleeping (Shen et al., 2015; Vas et al., 2013). Several reports showed that intraperitoneal (*i.p.*) or intracerebroventricular injection of nesfatin-1 significantly reduced the food intake of rodents (Hui et al., 2017; Shimizu et al., 2009; Wernecke et al., 2014). Moreover, central or peripheral administration of nesfatin-1 dose dependently decreased the food intake in Japanese Quail and neonatal chicks (Heidarzadeh et al., 2018; Shousha et al., 2015). In teleost, the effects of central or peripheral injection of nesfatin-1 on food intake was only reported in Goldfish (Gonzalez et al., 2010; Kerbel and Unniappan, 2012). In addition, nutritional status (fasting) can alter the expression of *nucb2* mRNA in Zebrafish (Hatef et al., 2014) and *Schizothorax prenanti* (Lin et al., 2014b). However, the limited information on nesfatin-1 in teleost feeding regulation is available.

Siberian sturgeon (*Acipenser baerii* Brandt) is a kind of sub-cold water fish and widely farmed in the world (Bronzi and Rosenthal, 2014; Bronzi et al., 2011). However, there have been few studies on appetite regulation in Siberian sturgeon, such as Peptide YY (PYY) (Chen et al., 2015), urocortin-3 (UCN3) (Zhang et al., 2016), Apelin (Hao et al., 2017), Cholecystokinin (CCK) (Zhang et al., 2017) and Cocaine- and amphetamine-regulated transcript (CART) (Zhang et al., 2018). Recently, our laboratory took upon the task of improving understanding of the appetite controlling in Siberian sturgeon. For investigating the endocrine mechanism of nesfatin-1 in appetite regulation in *Acipenseridae* species, the cDNA sequence of *nucb2* was cloned for the first time and its tissue distribution was detected in Siberian sturgeon. The short-term and long-term feeding effects of NUCB2/nesfatin-1 were also detected by *periprandial* (pre- and post-feeding), fasting and re-feeding as well as acute and chronic *i.p.* injection experiments. To study the endocrine mechanism, the effects of chronic *i.p.* injection of nesfatin-1 on appetite factors mRNA levels in corresponding tissues were examined.

2. Material and methods

All the experiments were carried out in accordance with the Animal Care and Use Committee of Sichuan Agricultural University, following the guidelines of animal experiments of Sichuan Agricultural University, under permit No.DKY-S20150812.

2.1. Fish

A total number of two hundred and fifty eight juvenile Siberian sturgeons were obtained from Runzhao Fisheries Co., Ltd. (Sichuan, China). Fish were maintained at 20 ± 1 °C with a 12 h: 12 h light/dark cycle in 60.0 × 50.0 × 40.0 cm^3 indoor tanks in Sichuan Agricultural University Aquaculture Laboratory (Chengdu, China). All tanks were constantly aerated and supplied fresh water. Fish were fed to satiety with commercial sinking pellets (crude protein ≥40%, crude fat ≥12%, coarse fiber ≤6%, crude ash ≤18%; Tongyi, Suzhou, China) daily at 14:00. Before the experiments, fish were acclimated under these conditions for two weeks.

2.2. Tissues sampling, cloning and sequence analysis

Six juvenile Siberian sturgeons (12 months old, 438.77 ± 59.72 g) were randomly sampled for cloning and tissue distribution experiments. Eleven tissues were sampled including the whole brain, esophagus, stomach, pyloric caeca, duodenum, valvula intestine, rectum, liver, pancreas, spleen and trunk kidney for distribution analysis with the anatomical guide of Siberian sturgeon as basis (Daprà et al., 2009). The whole brain was used for cloning *nucb2*. All the eleven tissues were used to assess the tissue distribution of *nucb2*.

Total RNA from whole brain was isolated using the RNAiso Plus and cDNA synthesized by the PrimeScript™ RT Reagent Kit (Takara, Dalian, China). The quantity of RNA was determined using electrophoresis and the optical density absorption ratio at wavelengths of 260 nm and 280 nm were determined by a photometer (Bio-Rad, USA). The high-quantity RNA (A260/A280 > 1.8) was used for cDNA synthesis. Products were electrophoresed in 1.5% agarose gel by Universal DNA Purification Kit (TIANGEN, Beijing China), ligated into the pMD-19T vector (Takara, Dalian, China), and transformed into the competent cells *E.coli* DH5α (Takara, Dalian, China). Sequencing was performed at Beijing Genomics Institute (BGI, Chongqing, China). Cloning primers were designed with Primer premier 5.0 and listed in Table 1 (*nucb2*-R, *nucb2*-F). PCR procedures were used as previously described (Lin et al., 2014a).

Table 1
Primer sequences and function used in this study.

Primer name	Primer sequence (5′ to 3′)	Applications
nucb2-F	AGATAAACCCACCAGAGGAACA	*nucb2*-cloning
nucb2-R	TTGGTACAGTACCCCATTTGTGA	*nucb2*-cloning
nucb2-qF	TGGAGACAGACCAGCATTTCAG	*nucb2*- qPCR
nucb2-qR	GGCTCCGTAACCTGTTCACTTC	*nucb2*- qPCR
cck-qF	GAGGGTAGTCCTGTAGCATCTGA	*cck* - qPCR
cck-qR	TTCTACCAGACGAGCCTTTCC	*cck* - qPCR
ucn3-qF	CAGGGGAGGAGAGAGAAAAAA	*ucn3*- qPCR
ucn3-qR	CTGAGACATTAGGCGAGCGT	*ucn3*- qPCR
cart-qF	CGACTGTGGTTGAGAGCCG	*cart*- qPCR
cart-qR	GACAGTCACACAACTTGCCGAT	*cart*- qPCR
apelin-qF	CAGACACGCTGTTTTACACCAC	*apelin*- qPCR
apelin-qR	GCACAGCATGGACACCAAGAT	*apelin*- qPCR
pyy-qF	AGGCAGAGGTATGGCAAGCG	*pyy*- qPCR
pyy-qR	GGAGGGTCAGGAGACGGGAT	*pyy*- qPCR
npy-qF	GCTGGCTACCGTGGCTTTC	*npy*- qPCR
npy-qR	GACTGGACCTCTTCCCATACCT	*npy*- qPCR
β-actin-qF	GTTGGTATGGGACAGAAGGACA	*β-actin*- qPCR
β-actin-qR	CCAGTTGGTAACAATGCCGT	*β-actin*- qPCR
gapdh-qF	CATTTGATGTTGGCTGGGT	*gapdh*- qPCR
gapdh-qR	CTTTCTGGGAAGGTGGAGGT	*gapdh*- qPCR

The *nucb2* ORF was predicted with the Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The deduced NUCB2 amino acid sequence was analyzed using employing BLASTn and BLASTp (http://www.ncbi.nlm.nih.gov). The cleavage site of the signal peptide was predicted by using the SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Moreover, multiple alignments of amino acid sequences and phylogenetic analysis were performed by MEGA 7.0 software (http://www.megasoftware.net/index.html). The phylogenetic tree was constructed using the maximum likelihood method.

2.3. Periprandial, fasting and re-feeding experiments

Sixty three juvenile Siberian sturgeons (3 months old, 29.46 ± 3.56 g) were randomly reared among 7 tanks (9 fish per tank) for *periprandial* (pre- and post-feeding) experiments. Fishes were acclimated at least two weeks before the experiments. No remarkably altered feeding behavior was observed among these tanks prior to the experiment. During the experiment, 5 tanks were fed with normal feeding procedure, but the other 2 tanks were fasted at the feeding point (14:00). Six fish in each tank were randomly collected for sampling at sampled points. Based on the result of tissue distribution, the whole brain and liver of fish in 5 tanks were sampled at −3 h (11:00), −1 h (13:00), 0 h (14:00), +1 h (15:00) and +3 h (15:00), respectively. Similarly, the other 2 fasting tanks were sampled at +1 h and +3 h as the control of the feeding fish. These tissues were numbered, then flash frozen in liquid nitrogen and stored at −80 °C, respectively.

One hundred and seventeen juvenile Siberian sturgeons (3 months old, 29.45 ± 2.84 g) were randomly assigned to 13 tanks (9 fish per tank) for the fasting and re-feeding experiments. After the acclimation period, 6 tanks of fish were fasting, 5 tanks of fish were feeding and the remaining 2 tanks of fish were re-feeding after 15 days fasted. Six individuals were randomly sampled from each tank at the following time points: 1, 3, 6, 10, 15 days feeding; 1, 3, 6, 10, 15, 17 days fasting; 15th day (re-feeding 1 day) and 17th day (re-feeding 3 days) re-feeding. The whole brain and liver were collected as described above.