

# Structure of neurohypophysial hormone genes and changes in the levels of expression during spawning season in grass puffer (*Takifugu niphobles*)<sup>☆</sup>

Eiji Motohashi, Tomoko Hamabata, Hironori Ando \*

Laboratory of Advanced Animal and Marine Bioresources, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

Received 22 March 2007; revised 9 July 2007; accepted 28 July 2007  
Available online 11 August 2007

## Abstract

Vasotocin (VT) has been shown to influence various aspects of social and sexual behaviors in a broad range of vertebrate species, but less is known about the mechanisms through which this peptide modulates behavior. Additionally, much less is known about roles of isotocin (IT) in regulation of behavior. Grass puffer, *Takifugu niphobles*, has unique spawning behavior; spawning occurs on beach only for several days around the spring tide and is conducted by a group of 10–60 individuals, of which one is female. As a first step toward investigating the roles of VT and IT in this species' spawning behavior, we determined the structures of the VT and IT genes from grass puffer using the genome resources of the closely related tiger puffer and green puffer. We then used these sequences to develop real-time PCR assays and examined changes in expression of the VT and IT genes over the spawning season. The structures of VT and IT genes are well conserved among three puffer species. Particularly, the sequence similarities between grass and tiger puffers were very high not only in the coding region (85–99%), but also in the non-coding regions (92–98%) that include the 5'-upstream regions. The levels of expression of VT gene increased in the brain of pre-spawning females. The levels of VT mRNA in the spawning females tended to be higher than that in the spawning males. In contrast, the levels of IT mRNA did not show such variation. The present results suggest that VT gene expression augments in the brain of females during the spawning period. The unique spawning behavior of grass puffer provides a useful model for studying the molecular mechanism of sexual behavior utilizing the genome resources of tiger puffer.

© 2007 Elsevier Inc. All rights reserved.

**Keywords:** Vasotocin; Isotocin; Spawning behavior; Gene expression; Genome; Puffer

## 1. Introduction

The neurohypophysial hormones consist of nine amino acids including a disulfide bridge linking half-cystines in positions 1 and 6. They can be classified into two groups: the vasopressin (VP) and the oxytocin (OT) family. A given vertebrate species generally has one VP-like peptide and one OT-like peptide (Urano et al., 1992). Vasotocin (VT)

is the VP-like peptide found in all non-mammalian vertebrates, while isotocin (IT) is restricted to teleosts. VT has been found to participate in a variety of fish physiology including osmoregulation, cardiovascular activity, stress response, metabolism, reproduction, and circadian and seasonal biology (Balment et al., 2006).

VT and IT are also implicated in interneuronal communication in the central nervous system and modulate various aspects of social and sexual behaviors (Goodson and Bass, 2001; Rose and Moore, 2002). Goodson and Bass (2000) showed in the plainfin midshipman fish that VT and IT delivered into the preoptic area-anterior hypothalamus (POA-AH) differently modulated vocal-motor activity between males and females and also between two different

<sup>☆</sup> The nucleotide sequences reported in this paper have been submitted to the GenBank/EMBL/DDBJ with Accession Nos. AB297919 and AB297920.

\* Corresponding author. Fax: +81 92 642 4427.

E-mail address: [hando@brs.kyushu-u.ac.jp](mailto:hando@brs.kyushu-u.ac.jp) (H. Ando).

male morphs. VT inhibited vocal-motor response in type I males that vocally court and defend nests, but not in type II males that sneak/satellite spawn and females. In contrast, IT inhibited the response in the type II males and females. In the bluehead wrasse, the intraperitoneal injection of VT increased courtship behavior in territorial and non-territorial males (Semsar et al., 2001). Alteration of social and sexual behaviors in males by exogenous VT has also been reported in electric fish (Bastian et al., 2001), white perch (Salek et al., 2002), goldfish (Thompson and Walton, 2004), and pupfish (Lema and Nevitt, 2004). These observations indicate that VT and IT play important roles in regulation of social and sexual behaviors in fish. Particularly, VT is most likely important in male social and sexual behaviors.

Variation in anatomical characteristics of VT neurons is also associated with social and reproductive status in fish. In the plainfin midshipman fish, the size of VT-immunoreactive (ir) neurons in the type I males and females was larger than in the type II males (Foran and Brass, 1998). In the bluehead wrasse, which exhibits a female-to-male sex change, the number of magnocellular neurons expressing VT mRNA was greater in territorial males than females and males undergoing change from initial to terminal phase phenotype, and that the VT mRNA hybridization signal per cell was greater in these magnocellular neurons in the territorial phase males than in the initial phase males and females (Godwin et al., 2000; Semsar and Godwin, 2003). These results suggest that large dominant male morphs are expected to produce more VT in the magnocellular neurons. Moreover, sexually dimorphic expression of VT in the POA-AH is also apparent, basically with higher expression in males than females (Goodson and Bass, 2001).

In some fish species, however, VT expression levels have been shown to be higher in females than males. In the goby, which exhibits reversible sex change, the females had larger VT-containing neurons (Grober and Sunobe, 1996). In the peacock blenny, sneaker males and females had higher levels of VT mRNA in the POA than nest-holder males (Grober et al., 2002). In chum salmon, the amount of VT mRNA decreased during spawning migration in females (Ota et al., 1996; Hiraoka et al., 1997). These results indicate that VT may be involved in reproductive activity in the females of these species. Taken together, VT is considered to have an important role in intersexual variation of behavioral and physiological functions in fish.

In contrast to the large amount of information on VT, those of IT remain far less explored. In the female midshipman, local administration of IT inhibited the vocal-motor response elicited by POA-AH stimulation, whereas VT was ineffective (Goodson and Bass, 2000). In *Lythrypnus dalli*, which exhibits a female-to-male sex change, females had significantly greater number of IT-ir neurons than intact and sex-changed males (Black et al., 2004). Therefore, IT may be involved in the female social and sexual behavior, but available descriptions are not enough to delineate the roles of IT.

We intended to clarify molecular mechanisms of action of VT and IT in regulation of fish social and sexual behaviors. We chose grass puffer (*Takifugu niphobles*) as an experimental model. Grass puffer belongs to the family tetraodontidae, and its spawning behavior is unique and sexually dimorphic. Spawning occurs on beach several hours before high tide only for several days around the spring tide from April to August (Yamahira, 2004). Therefore, we are aware of time and place of spawning, and thus we can obtain spawning fish as well as non-spawning fish that usually subsist in the coastal area. Furthermore, the spawning of grass puffer is conducted by a group of 10–60 individuals, of which one is female (Nozaki et al., 1976). Therefore, grass puffer provides a good model to investigate neuronal regulation of intersexual variation of spawning behavior. In addition, grass puffer and tiger puffer (*Takifugu rubripes*) are closely related. The genome project of tiger puffer was completed in 2002 and the draft sequence is available on the Web. The whole genome of green puffer (*Tetoraodon nigroviridis*) is also available on the Web. Thus, the information of the puffer genome must be very useful to clarify molecular mechanism underlying regulation of the unique spawning behavior.

As the first step to examine roles of VT and IT in the spawning behavior of grass puffer, we cloned VT and IT genes and determined their structures based on the genomic sequences of tiger and green puffers. Furthermore, real-time polymerase chain reaction (PCR) assays for VT and IT mRNAs were established to examine changes in their levels in the brain of fish at different stages during spawning season. In addition, the levels of VT and IT mRNAs in the brain of spawning fish were compared between males and females.

## 2. Materials and methods

### 2.1. Fishes

Grass puffer of both sexes was obtained from Nokonoshima Island, Fukuoka, Japan, in September and December 2004 and April, May, and July 2005. The fish were caught by a settled net around the Nokonoshima Island. The depth of the water was five meters. They were transferred to the Fishery Research Laboratory station, Kyushu University, Fukutsu, Japan, and were kept in indoor tanks (120 L) with flow of seawater under natural photoperiod for 1–2 weeks. Water temperature varied seasonally: September, 25–26 °C; December, 14–16 °C; April, 15–16 °C; May, 19–20 °C; July, 25–27 °C. The spawning season of the puffer in this area began at the end of April and continued until the beginning of July.

Spawning fish were sampled at a spawning point in Tomioka Bay, Kumamoto, Japan at the end of June and July 2005. The fish of both sexes that appeared on beach for group spawning were caught by a dipnet or hand, and were immediately anesthetized with 0.03% tricaine methane sulfonate (MS222, Sigma–Aldrich, Tokyo, Japan).

### 2.2. Sample collection

Under anesthesia with MS222, fork length and body weight of fishes were measured. Gonads were removed immediately after decapitation, and weighed to calculate the gonadosomatic index (GSI = gonad weight/body weight × 100). To examine VT and IT mRNA levels, brains were removed after decapitation from the fish samples obtained in Nokon-

Download English Version:

<https://daneshyari.com/en/article/2802123>

Download Persian Version:

<https://daneshyari.com/article/2802123>

[Daneshyari.com](https://daneshyari.com)