ARTICLE IN PRESS

General and Comparative Endocrinology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Research paper

Reproductive stage- and sex-dependant effects of neurohypophyseal nonapeptides on gonadotropin subunit mRNA expression in the catfish *Heteropneustes fossilis*: An *in vitro* study

A. Acharjee [a], R. Chaube [a], K.P. Joy [b,*]

[a] Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221005, India
[b] Department of Biotechnology, Cochin University of Science and Technology, Kochi 682022, India

ARTICLE INFO

Article history:
Received 5 August 2017
Revised 11 December 2017
Accepted 1 January 2018
Available online xxxx

Keywords:
Catfish
Gonadotropin
Isotocin a
Isotocin b
Season and sex differences
Vasotocin

ABSTRACT

In the present study, *in vitro* effects of synthetic vasotocin (VT), isotocin (4Ser, 8Ile- oxytocin; ITb) and the recently cloned IT gene paralog product (8Val-Isotocin, ITa) were studied on the expression of pituitary gonadotropin (GtH) subunit mRNA levels. In male pituitaries of early (preparatory phase) and late (prespawning phase) recrudescing catfish, *Heteropneustes fossilis*, VT (10 nM, 100 nM and 1000 nM) stimulated *fshβ* expression dose-dependently. But in females, the dose-dependent effect was found only in the preparatory phase. In males, VT stimulated *lhβ* expression only at higher doses. In females, VT produced a significant dose-dependent increase of the *lhβ* expression only in the prespawning phase. VT stimulated the expression of *gpα*, dose-dependently in the preparatory phase in males and in the prespawning phase in females. The incubation of the pituitaries with ITb did not alter the *fshβ* expression in either sex in both preparatory and prespawning phases. In males, ITb stimulated the expression of *lhβ* and *gpα* only at the highest concentration (1000 nM) in both phases. In females, ITb stimulated both *lhβ* and *gpα* expression only at 1000 nM in the preparatory phase and dose-dependently in the prespawning phase. The incubation of the pituitaries with ITa produced effects similar to ITb on the expression of *fshβ*, *lhβ*, and *gpα*. The results show that the basic peptide VT modulates both *fsh*β and *lh*β expressions, which are influenced by the sex and reproductive stage. The neutral peptide ITA/ITb exerts an insignificant effect on the *fsh*β expression regardless of sex or season. Both VT and ITa/ITb elicit a significant effect on the *lh*β expression in late recrudescent phase especially in females.

© 2018 Published by Elsevier Inc.

1. Introduction

In teleosts, the basic peptide vasotocin (VT) and neutral peptide isotocin (IT) are the orthologs of mammalian vasopressin (VP) and oxytocin (OT), respectively (Acher, 1996; Gimpl and Farenholz, 2001). IT differs from VT in having serine at the 4th position and isoleucine at the 8th position. VT and IT are synthesized in discrete neurons of the nucleus preopticus (NPO) in the hypothalamus and are axonally transported to the neurohypophysis (NH) for release into the circulation (Urano et al., 1994). Though the VT and IT systems project mainly to the posterior neurohypophysis, they also innervate the adenohypophysis (Batten et al., 1986; Moons et al., 1989; Holmqvist and Ekstrom, 1995; Canosa et al., 2011; Singh et al., 2012). Fryer and Leung (1982) reported ACTH-releasing activity of VT and IT. An involvement of both VT and IT in gonadotropin (GtH) regulation has been reported in some teleosts (Groves and Batten, 1986; Ramallo et al., 2012; Singh et al., 2012).

VT and IT mediate the physiological effects by binding to cognate receptors, which are G-protein coupled receptors (Gimpl and Fahrenholz, 2001). Recently, cDNAs for 7 distinct VT receptor subtypes were isolated and characterized; two V1A sub-type (V1a1, V1a2) and 5 V2 subtypes (V2A1, V2A2, V2B1, V2B, V2C) (Lema, 2010; Lema et al., 2012; Ocampo-Daza et al., 2012). The VT receptor subtypes have been described in the pituitary of various vertebrates (Hasunuma et al., 2013). V1a-like immunoreactivity was detected in large cells of the PPD of rock hind (Kline et al., 2011). In the catfish *Heteropneustes fossilis*, V1a, V1a2 and V2A1 were characterized and their expressions varied seasonally and sexually (Rawat et al., 2015). The transcripts were localized differentially in the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). The V1a types were predominantly distributed while the V2 type is of lesser abundance. All of them

* Corresponding author.
E-mail addresses: arupacharjee@gmail.com (A. Acharjee), chauberadha@rediffmail.com (R. Chaube), kpjoybhu@gmail.com (K.P. Joy).

https://doi.org/10.1016/j.ygcen.2018.01.001
0016-6480/© 2018 Published by Elsevier Inc.

Please cite this article in press as: Acharjee, A., et al. Reproductive stage- and sex-dependant effects of neurohypophyseal nonapeptides on gonadotropin subunit mRNA expression in the catfish *Heteropneustes fossilis*: An *in vitro* study. Gen. Comp. Endocrinol. (2018), https://doi.org/10.1016/j.ygcen.2018.01.001

ARTICLE IN PRESS

showed intense labeling in RPD > PPD > PI (unpublished reports from our laboratory). There are also reports about the presence of IT receptor (ITR) in the pituitary of teleosts *Sparus aurata* (Martos-Sitcha et al., 2014). The ITR has been characterized in *Catostomus commersoni* (Hausmann et al., 1995). The teleost ITR can be activated by Arg-vasotocin, mesotocin, oxytocin and Arg-vasopressin, although these have lower potencies than IT. ITR displays only a threefold preference for IT compared to VT. The presence of VT and IT receptors further indicate that the pituitary is a locus for both VT and IT actions.

Recently in our laboratory, a new nonapeptide gene was cloned and characterized in the catfish *H. fossilis* (Accession No. JX669010.1) and *Clarias batrachus*. Thus, catfishes have one basic peptide (VT) and two neutral peptides. The novel peptide has a sequence CYISNCPVG and is distinct from the teleost IT in having a neutral amino acid valine at the 8th position, instead of isoleucine (4Ser, 8Val- isotocin or Sevatocin, ST). Such duplications have been reported in the cavefish *Astyanax mexicanus*, an ancestral teleost. In phylogenetic analysis, the catfish novel peptide and cavefish ITa clustered together and the catfish IT clustered with cavefish ITb. Therefore, the terms ITa and ITb are used for catfish ST and IT, respectively (P. Banerjee, R. Chaube and K. P. Joy, unpublished data). *In situ* hybridization study has shown that the ST neurons are identified in the NPO, along with VT and IT, and semi-quantitative/quantitative RT-PCR has shown its expression in the pituitary.

In vertebrates with the exception of cyclostomes, the duality and homology of GtHs (follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh) have been established (Li and Ford, 1998; Quérat et al., 2000; Levavi-Sivan, 2010). However, in catfishes only a single GtH of the Lh type has been characterized (Koide et al., 1992; Schulz et al., 1997), though the genes for both Fshβ and Lhβ subunits have been isolated and characterized (Liu et al., 2001; Vischer et al., 2003a; Wu et al., 2009). In the African catfish, Lh was the only GtH detected in circulation (Vischer et al., 2003b). Thus, the status of gonadotropin biology in case of catfishes is different from other teleosts (Chaube et al., 2015). The non-availability of native Fsh has been an impediment in investigating its role in catfishes. Recombinant GtHs have been used to study catfish GtH physiology (Vischer et al., 2003b; Zmora etal., 2007). Recently, we cloned and characterized the GtH subunit genes in another catfish *Heteropneustes fossilis* (Acharjee et al., 2015). This has enabled us to study the regulation of GtH function at the transcript levels.

The objective of the present study was to investigate the effects of synthetic VT, ITa and ITb on transcriptional activity of the GtH subunit genes *in vitro*. The *in vitro* system is clearly advantageous in that interference from outside (brain) regulatory molecules is minimal, if not null.

2. Material and methods

2.1. Animals

Adult *H. fossilis* (50–60 g) of the first sexual cycle were purchased from local fish markets in Chaukaghat area of Varanasi in early (March, preparatory phase) and late (May, prespawning phase) of the reproductive cycle. They were acclimatized in flow-through water tanks (cement tanks of $0.35 \times 0.35 \times 0.45\ m^3$ of 55 L capacity, the fish were maintained in 30 L water filling) under a photoperiod of 12.5 h light and 11.5 h darkness, and an ambient temperature of 22 ± 2 °C in March, and 14.5 h light and 9.5 h darkness and 28 ± 3 °C in May. During the maintenance, they were fed with boiled minced goat liver *ad libitum*.

The experiments were performed in accordance with the guidelines of Banaras Hindu University for experimentation in animals and all care was taken to prevent cruelty of any kind.

2.2. Chemicals and reagents

The following molecular biology kits and reagents were used: RNeasy lipid tissue mini kit (Qiagen GmBH, Germany), Revert-Aid H minus first strand cDNA synthesis kit (Fermentas, Hanover, MD, USA), DNase I RNase-free (Ambion Inc., Austin, TX, USA), RNA-later (Ambion Inc., Austin, TX, USA), 2X PCR master mix (Fermentas, Hanover, MD, USA), 2X VeriQuest SYBR Green qPCR Master Mix Ex (Cleveland, Ohio, USA). The primers used in the present study were synthesized by Integrated DNA Technologies (Coralville, IA, USA). Vasotocin (Arg8-oxytocin) acetate salt (VT) and 4Ser, 8Ile-oxytocin (isotocin, ITb) were purchased from Sigma Chemical Company, St. Louis, USA. 8-Val Isotocin (ITa; $HCYISNCPVG\text{-}NH_2$), a novel peptide deduced from cDNA cloning in the catfish *Heteropneustes fossilis,* cDNA precursor (Acc no: JX669010.1) was custom-synthesized by Bachem AG Bubendorf, Switzerland. Other chemicals were of molecular biology grade and purchased from E. Merck, Mumbai, India.

2.3. Experiments

2.3.1. Preparation of hormones

Stock solutions (1 mg/mL in triple distilled water) of VT, ITa and ITb were prepared and stored at −20 °C. Just before the incubation, the stock solutions were diluted with the incubation medium to make working concentrations of 10 nM, 100 nM and 1000 nM.

2.3.2. In vitro incubation of pituitary with nonapeptides

The acclimatized fish were anaesthetized by immersing in a 0.01% solution of tricaine methanesulfonate (MS222; Sigma St. Louis, USA), followed by rapid decapitation. The pituitaries were dissected out rapidly under aseptic conditions. They were washed with phosphate-buffered saline (PBS) several times to remove traces of blood and tissue debris. The pituitaries were dissected into two halves with a sterile and sharp razor blade. Subsequently, they were transferred to multi-well culture plates containing 2.5 mL of culture medium (Leibovitz's L-15, supplemented with 10% fetal bovine serum, 10 mM HEPES, 100 IU/ mL penicillin and 100 μg/mL streptomycin). The hemi-pituitaries were maintained in culture at 22 °C in an atmosphere of 5% CO_2 in a CO_2 incubator. After the first 24 h (day 0), the medium was removed in order to eliminate the effect of residual brain-derived factors, if any, in the neurohypophysial axonal terminals. On day 1, they were cultured without adding any nonapeptide. On day 2, the hemi-pituitaries were randomly divided into 4 groups of 15 pituitaries each. Group 1 was control. Group 2, 3 and 4 were incubated with 10 nM, 100 nM and 1000 nM, respectively, of each hormone preparations for 24 h. The experiment was replicated five times. For the qPCR assay, pituitaries were taken out and stored in RNAlater at −80 °C.

2.3.3. qPCR assay

The hemi-pituitaries from each group were homogenized using a T10 basic ULTRA-TURRAX homogeniser (IKA, Germany) in 1 mL QIAZOL (Qiagen) buffer and total RNA was isolated with RNeasy Lipid Tissue Mini Kit (Qiagen). Total RNA was treated with 2 U DNase I RNase free (Ambion) for 30 min at 37 °C, following the manufacturer's protocol to remove genomic DNA contamination. The quality of RNA was analyzed after separation on a 1% agarose gel containing 2.2 M formaldehyde. The total RNA yield was determined on a NanoDrop (ND-1000 Spectrophotometer, NanoDrop Technologies, Rockland, DE) and quality assessed by gel electrophoresis. The RNA samples with a A260/A280 ratio from 1.8 to

Please cite this article in press as: Acharjee, A., et al. Reproductive stage- and sex-dependant effects of neurohypophyseal nonapeptides on gonadotropin subunit mRNA expression in the catfish *Heteropneustes fossilis*: An *in vitro* study. Gen. Comp. Endocrinol. (2018), https://doi.org/10.1016/j.ygcen.2018.01.001

Download English Version:

https://daneshyari.com/en/article/8631057

Download Persian Version:

https://daneshyari.com/article/8631057

Daneshyari.com