General and Comparative Endocrinology 220 (2015) 33–40

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

"Brain sex differentiation" in teleosts: Emerging concepts with potential biomarkers

Balasubramanian Senthilkumaran *, Cheni-Chery Sudhakumari, Sajwan-Khatri Mamta, Kavarthapu Raghuveer [1], Immani Swapna [2], Raju Murugananthkumar

Department of Animal Biology, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500046, Telangana, India

ARTICLE INFO

Article history:
Received 3 September 2014
Revised 5 June 2015
Accepted 8 June 2015
Available online 23 June 2015

Keywords:
Sexual dimorphism
GnRH
Monoamines
Brain aromatase
Tryptophan hydroxylase
Tyrosine hydroxylase

ABSTRACT

"Brain sex differentiation" in teleosts is a contentious topic of research as most of the earlier reports tend to suggest that gonadal sex differentiation drives brain sex differentiation. However, identification of sex-specific marker genes in the developing brain of teleosts signifies brain–gonadal interaction during early sexual development in lower vertebrates. In this context, the influence of gonadotropin-releasing hormone (GnRH)–gonadotropin (GTH) axis on gonadal sex differentiation, if any requires in depth analysis. Presence of seabream (sb) GnRH immunoreactivity (ir−) in the brain of XY Nile tilapia was found as early as 5 days post hatch (dph) followed by qualitative reduction in the preoptic area-hypothalamus region. In contrast, in the XX female brain a steady ir− of sbGnRH was evident from 15 dph. Earlier studies using sea bass already implied the importance of hypothalamic gonadotropic axis completion during sex differentiation period. Such biphasic pattern of localization was also seen in pituitary GTHs using heterologous antisera in tilapia. However, more recent analysis in the same species could not detect any sexually dimorphic pattern using homologous antisera for pituitary GTHs. Detailed studies on the development of hypothalamo–hypophyseal–gonadal axis in teleosts focusing on hypothalamic monoamines (MA) and MA-related enzymes demonstrated sex-specific differential expression of tryptophan hydroxylase (Tph) in the early stages of developing male and female brains of tilapia and catfish. The changes in Tph expression was in agreement with the levels of serotonin (5-HT) and 5-hydroxytryptophan in the preoptic area-hypothalamus. Considering the stimulatory influence of 5-HT on GnRH and GTH release, it is possible to propose a network association between these correlates during early development, which may bring about brain sex dimorphism in males. A recent study from our laboratory during female brain sex development demonstrated high expression of tyrosine hydroxylase in correlation with catecholamine levels, brain aromatase and its related transcription factors such as fushi tarazu factor 1, Ftz-f1 and fork head box protein L2, foxl2. Taken together, gender differences in the levels of various transcripts provide new perspectives on brain sex differentiation in lower vertebrates. Sexually dimorphic or differentially expressing genes may play an essential role at the level of brain in response to gonadal differentiation, which might consequentially or causatively respond to gonadal sex.

1. Introduction

"Brain sex differentiation" refers to the process wherein exposure of fetal or neonatal brain to sex hormones produces significant and irreversible differences of brain structure and function which correlate with adult reproductive behavior based on their gonadal sex (Phoenix et al., 1959). This phenomenon is well established in mammals, yet in fishes the brain is not irreversibly sexualized (Le Page et al., 2010). In mammals, suppression of the female behavioral and cyclic pattern of gonadotropin (GTH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) release regulated by gonadotropin-releasing hormone (GnRH) causes the defeminization. It is presumed that the default sex is female, while differentiation towards masculine patterns of GTH secretion and male behavior as a result of exposure to hormones of testicular origin during development (see Juraska et al., 2013). Genetic and

* Corresponding author.
E-mail addresses: bsksl@uohyd.ernet.in, senthilkumaranb@yahoo.com (B. Senthilkumaran).
[1] Present address: Section on Molecular Endocrinology, National Institutes of Health, Building 49, 6A36, Bethesda, MD 20892, USA.
[2] Present address: Waggoner Center for Alcohol and Addiction Research, Section of Neurobiology, University of Texas, Austin, TX 78712, USA.

http://dx.doi.org/10.1016/j.ygcen.2015.06.003
0016-6480/

hormonal factors other than gonadal origin during early development may contribute for sexual dimorphism in brain. Further, brain sex differences in higher mammals are also influenced by environmental factors (Swaab, 2007; Tobet et al., 2009). The process of brain sex differentiation is not well established in lower vertebrates including fishes though brain sex dimorphism was evident and in fact induction of a female phenotype by estradiol-17β (E_2) seems possible (Melo and Ramsdell, 2001). This review is intended to highlight major findings including from our laboratory on early sex differences in the expression of key hormonal and enzymatic regulators of sexual maturation, in various fish models including catfish to understand brain sex differentiation in lower vertebrates.

Teleosts are the largest group of vertebrates with over 30,000 species. A remarkable variety of reproductive strategies are present in teleosts ranging from gonochoristic to hermaphrodite conditions such as protandry and protogyny (Devlin and Nagahama, 2002). Sex determination and differentiation are the most important processes for species reproduction (Hughes, 2001). Interestingly, the vertebrate sex determination mechanism is not conserved and hence it is important to consider various animal models and mechanisms (Cutting et al., 2013). Sex determination is defined as the commitment of indifferent gonad to develop either into a testis or an ovary based on genetic programming in a time and gene dosage-dependent pattern (Hughes, 2001). Sex differentiation is described as the phenotypic growth of structures consequential to the action of hormones produced after gonadal sex determination (Hughes, 2001). During sexual differentiation, the nervous system becomes structurally and functionally dissimilar in females and males (Juraska et al., 2013) which may drive gender-specific sexual behavior in mammals. In teleosts, during sex differentiation or sex inversion, gender-specific sexual changes depend upon the chronological appearance of gonadal and/or brain sex differences under the influence of gonadal factors in response to hormones (Grober and Sunobe, 1996; Miranda et al., 2001; Devlin and Nagahama, 2002; Nagahama, 2005; Sudhakumari et al., 2005; Le Page et al., 2010; Raghuveer et al., 2011a,b) On the other hand, in mammals, the production of testosterone by the male gonad drives brain sex dimorphism (Hughes, 2001; Juraska et al., 2013). Further, the gender-specific sexual behavior pattern varies considerably among serial sex changers and sex reversing fish species. In general, the process of brain sex differences might bring about gender-specific sexual behavior which usually happens after gonadal development in natural or induced sex reversal in fishes. On the contrary, serially sex changing teleosts show profound changes in sexual behavior even before the gonadal transdifferentiation into a testis or an ovary indicating the influence of central nervous system (CNS) including brain (Grober and Sunobe, 1996; Kobayashi et al., 2013). Nevertheless, brain sex differences are often presumed as a consequence to gonadal sex rather than a cause for it. The brain sex differentiation in lower vertebrates is highly complicated yet gender-specific differences in the levels of various transcripts/factors such as tryptophan hydroxylase (Tph), tyrosine hydroxylase (Th), serotonin (5-HT), catecholamines (DA and NE), fushi tarazu factor 1 (Ftz-f1), cyp19a1b (brain aromatase) in brain provide important information to understand this phenomenon to some extent, which is being highlighted in this review.

The first section explains the brain sex differences in GnRH and its influence on gonadal differentiation in teleosts. The second section debates on "Do GTHs show any brain sex differences or association with gonadal differentiation in teleosts?" The third section deals about the gender differences in the levels of various transcripts and monoamines related to brain sex differentiation in teleosts with special reference to (i) hypothalamic monoamines and related enzymes, and (ii) brain aromatase and other related factors. Based on these viewpoints, future research perspectives are discussed along with a conclusion.

2. Brain sex differences in GnRH and its influence on gonadal differentiation in teleosts

GnRH, an important trophic decaneuropeptide, plays an important role in the pituitary–gonadal axis for the coordination of the various events of gametogenesis (Somoza et al., 2006; Zohar et al., 2010; Maruska and Fernald, 2011). The presence of multiple forms of GnRH in teleosts seems to be the result of genome duplication (Powell et al., 1994). The species-specific form of GnRH plays a pivotal role in hormonal regulation of the hypophyseal–gonadal axis (Senthilkumaran et al., 1999, Table 1). Inputs from the GnRH releasing cells in the preoptic area-hypothalamus (POA-H) innervate the pituitary directly to regulate GTHs which is correlative to gonado-somatic index (Senthilkumaran et al., 1999). Other forms of GnRH expressed in different regions of the brain seem to be involved in spawning migration and neuromodulation (Senthilkumaran et al., 1999; Zohar et al., 2010). In general, species-specific form of GnRH determines the release of FSH and LH vis-à-vis steroidogenesis in most of the teleosts (Powell et al., 1994; Senthilkumaran et al., 1999; Zohar et al., 2010). It also determines the timing and progression of reproductive events like spawning (Senthilkumaran et al., 1999; Zohar et al., 2010). In spite of these, neither GnRH nor GTH have been implicated in gonadal sex differentiation yet several studies tend to endorse the importance of feedback regulation of gonadal steroids to orchestrate GnRH–GTH release, which in turn regulate sex steroid production during gametogenesis, serial sex change and natural sex reversal (Peter et al., 1991; Grober and Sunobe, 1996; Senthilkumaran and Joy, 1996; Goos et al., 1999; Zohar et al., 2010). Considering this, it might be difficult to correlate the factors of gonad versus brain–pituitary axis at least during the period of sex differentiation or sex reversal with the presence of varying degree of sexual plasticity in protandrous and protogynous hermaphrodite fishes when compared to gonochoristic fish species. Tracking GnRH in a gonochoristic species such as the Nile tilapia having distinguished XX and XY populations revealed sexually dimorphic patterns of seabream (sb) GnRH localization (Swapna et al., 2008). In brief, this report from our laboratory demonstrated the presence of sbGnRH immunoreactivity (ir−) in the brain of XY Nile tilapia as early as 5 days post hatch (dph) until 10 dph wherein the testicular differentiation is yet to be completed. Thereafter, qualitative reduction in sbGnRHir− neuron/cell bodies in the preoptic area-hypothalamus region was evident from 20 dph till 30 dph and elevated thereafter, while in the XX female brain a steady ir− was evident only from 15 dph yet ovarian differentiation was apparent. The authors envisaged the differential temporal patterns of GnRH localization and expression between males and females could be driven by differences in the underlying genetic factors or be secondary to gonadal differentiation (Table 1). Subsequent studies in our laboratory further lend support to this perspective. First signs of gonadal sex differentiation are seen as early as 5–7 dph in female tilapia while it is comparatively late in males (Nagahama, 2005). Nevertheless, early appearance of sbGnRH in male brain even before testicular differentiation provides certain degree of importance to GnRH. However, this contention needs to be analyzed extensively with different animal models having genetic sex-specific populations. Earlier, Pandolfi et al. (2002) documented differential distribution and ontogeny of three distinct GnRH forms during the period of sex differentiation of South American cichlid fish in mixed sex population (Table 1). Similarly, Soga et al. (2005) also reported coincidence of appearance of GnRHir− neurons in POA-H during gonadal differentiation