Journal of Scientific Research
Banaras Hindu University, Varanasi

Vol. 60, 2016 : 37-41
ISSN : 0447-9483

INTRA-GONADAL FACTORS IN FISH GONAD

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Introduction

In common to other vertebrates, fish hypothalamus produces releasing and release-inhibiting factors which control the secretory activity of the hormone producing cells of the pituitary. These hypothalamic hormones include GnRH, GRH, PACAP, CRH, TRH, SRIH, Urotensins, Neuropeptide-Y, CCK, Tachykinins, and others many other peptidergic as well as aminergic factors. GnRH among them is the pivotal and primarily controls all the reproductive events. GnRH acts on anterior pituitary to regulate the secretion of two gonadotropins; GTH-I (FSH) and GTH-II (LH). The FSH and LH bind to their respective receptors in gonads and signal the secretion of sex steroids like progestogens, androgens and estrogens which thereby control vitellogenesis, follicular growth & maturation and ovulation in females, and spermatogenesis spermiogenesis, spermiation and capacitation of spermatozoa.

In recent decades, studies in mammals suggest that gonad produces many other factors too, besides sex steroids, which play effective role in accomplishing the variety of reproductive events. These factors act on germ cells as well as somatic cells in autocrine and paracrine manner. The intra-gonadal factors not only control the reproductive episodes directly, but also modulate the action of endocrine hormones, delivered to gonad from hypothalamo- hypophysial axis (Knight and Glister, 2006). In recent past, varieties of intragonadal factor have been identified and characterized in mammals. These intragonadal factors include transforming growth factors-TGFs, bone morphogenetic proteins BMPs, epidermal growth factor- EGFs, fibroblast growth factor- FGFs, insulin like growth factors- IGFs, growth and differentiation factors-GDFs, etc and reactive oxygen and nitrogen species (sulphurdioxide-SO_2, hydrogen peroxide- H_2O_2, nitric oxide- NO). Nevertheless such studies in fishes are highly scanty. In this review we will discuss about some intragonadal growth factors (TGF-β, BMP-15 and EGF) and reactive nitrogen species (NO), which play significant role in regulation of gametogenesis and steroidogenesis in fishes.

Transforming Growth Factor- β (or TGF-β)

The TGF-β superfamily consists of several subfamilies. TGF- β subfamily, BMP subfamily, the growth and differentiation factor (GDF) subfamily, activin/inhibin subfamily (activins A, AB, B, inhibins A, B), the glial cell-derived neurotrophic factor (GDNF) subfamily and some other as anti-Mullerian hormone (AMH) and nodal (Knight and Glister, 2006). A common feature of most of the

members of the TGF-β superfamily is the presence of seven conserved cysteine residues, six of which form a characteristic "cysteine knot" structure and one of which forms a disulfide bridge between two subunits, making the molecule a covalently linked dimer.

TGF-β is synthesized as an inactive latent form, which is converted to its active form enzymatically. In mammals, three biologically active isoforms of TGF-β are known; TGF-β1, TGF-β2 and TGF-β3, while TGF-β4 and TGF-β5 are expressed in bird and *Xenopus laevis*, respectively (Lal, 2013). TGF-βs are 25 kDa homodimers linked by disulfide bonds, sharing a high level of sequence similarity and almost identical tertiary structure. All three isoforms of TGF-β are detected in the theca and granulosa cells of the mammalian ovary. TGF-β is involved in the bidirectional communication between granulosa and thecal cells and also between granulosa cells and the oocyte.

However, only few studies are available on the existence of TGF-β in fish gonad. TGF- β1 and its receptor type II have been cloned and mRNA expression has been observed in the early vitellogenic and pre-maturational full grown follicles in goldfish ovary (Calp et al., 2003). Studies have shown that TGF- β1 inhibits gonadotropin and 17α, 20β-dihydroxyprogesterone (DHP) - induced oocyte maturation in zebrafish (Kohli et al., 2005, 2003).

Bone Morphogenetic Protein-15 (or BMP-15)

The BMP subfamily, which includes over thirty members, constitutes the largest subfamily in the TGF-β superfamily. Bone morphogenetic proteins (BMPs) were first identified nearly 20 years ago as components of a protein extract derived from bone that could direct cartilage and bone formation. The BMPs have since been shown to play roles in multiple other developmental processes (Felin et al., 2010). Like other members of TGF-β superfamily, BMPs are synthesized as preproprotein of about 400–500 amino acids consisting of an N-terminal signal peptide which direct it to rough endoplasmic reticulum for proper folding, and a C-terminal mature peptide. C-terminal mature BMPs are to be proteolytically cleaved upon dimerization. Most of biologically active BMPs are composed of 50–100 amino acids with seven cysteines. But member like BMP-3, GDF-9, and BMP-15 lack the seventh cysteine. Other than these three (BMP-3, GDF-9, and BMP-15) all C-terminal peptide of BMPs form either homodimer or heterodimer which are mature and biologically active secreted signaling molecules (Bragdon et al., 2011). BMP-15, a member of BMP subfamily, plays key role in early follicle growth in mammalian ovary (Knight and Glister, 2006). Within the ovary, mRNA and protein for BMP-15 is found exclusively in the oocyte of most species suggesting that the oocyte is the primary source of BMP-15 in the ovary of sheep (Juengel et al., 2004). BMP-15 regulates granulosa cell (GC) proliferation and differentiation. BMP-15 promotes GC mitosis, suppresses follicle-stimulating hormone (FSH) receptor expression, and stimulates kit ligand expression (Moore, 2002). The role of oocyte factor, BMP-15 has been well established for normal fertility in female

mammals. The biological functions of recombinant BMP-15 demonstrates its capacity to promote granulosa cell processes involved in early follicle growth in sheep (Moore and Shimasaki, 2005).

Some studies have suggested the presence of BMP-15 mRNA and protein in fishes also. Presence of BMP-15 has been demonstrated in the ovaries of zebrafish (Clelland et al., 2006). However, its role is yet not clear, though it has been suggested that BMP-15 may prevent premature oocyte maturation (Lubzens et al., 2010)

Epidermal Growth Factor (EGF)

The epidermal growth factor (EGF) is a polypeptide which has 53 amino acids with three disulfide bonds. Members of this family are EGF, transforming growth factor- α (TGF-α), Schwannoma-derived growth factor (SDGF), etc. EGF promotes the proliferation of different cell types such as epidermal cells, fibroblasts, crystalline cells, glial cells, and vascular endothelial cells in mammals. EGF receptor is distinctly shown in thecal cells, granulosa cells and corpus luteum (Lal, 2013).

EGF expression is also reported in immature ovary of zebrafish fish and expression declines with the ovarian development. Previtellogenic and early vitellogenic follicles express relatively high levels of EGF than the more advanced ovarian follicles (Tse and Ge, 2010, 2009). EGFR expresses, however, very low in immature ovary, but increases with initiation of vitellogenesis till late stages of ovarian development. In ovarian follicles, EGF expresses in both follicular cells as well as oocytes; more in oocytes than follicular cells. But, EGFR expresses exclusively in the follicular cells and only in traces in oocytes. and (Tse and Ge, 2010, 2009) have cloned the full length of cDNA of EGF, TGFα, betacellulin (BTC) and heparin binding EGF and their receptors. In fish ovary, EGF is reported to play role in regulating follicle survival and prostaglandin synthesis (Janz and Van Der Kraak, 1997; Srivastava and Van Der Kraak, 1995). Final oocyte maturation is stimulated by EGF in goldfish (Pati et al., 1996) and TGF-α in zabrafish ovary. Their oocyte maturation stimulating activitiesc are reported to be mediated through activin, member of TGF-β superfamily (Pang and Ge, 2002; Yefei and Wei, 2002). EGF signaling network in zebrafish ovary is self-regulated by its own members (Tse and Ge, 2009).

Nitric oxide (NO)

Nitric oxide (NO), a free radical gaseous molecule, is produced during the conversion of arginine to citrulline and the reaction is catalyzed by nitric oxide synthase (NOS). NOS has three isoforms endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS). The two isoforms of nitric oxide synthase, iNOS and nNOS, and all the three isoforms eNOS, iNOS and nNOS have been reported in the testis and ovary of freshwater catfish *Clarias batrachus* (nee Pathak and Lal, 2010, 2008; Singh and Lal, 2014) and *Heteropneustes fossilis* (Tripathi and Krishna, 2008). Fish gonad is capable of producing NO. In the catfish testis, NO is produced by germ cells, Leydig cells, and macrophages. Production of testicular NO is under endocrine

inhibitory control. Expression of NOSs exhibits seasonality and that depends on the reproductive status of fish. Leydig cells are highly sensitive to chemical as well as biological NO. NO inhibits testosterone production by the testis in vivo as well as by the isolated Leydig cells in vitro.

In *C. batrachus,* all three isoforms of NOS express only in the thecal and granulosa cells in growing and fully grown follicle. Whereas, nNOS expresses also in the nucleus and cytoplasm of perinuclear stage oocytes and oocyte-I in the ovary of quiescence phase. These findings suggest that nitric oxide plays important role in folliculogensis and steroidogenesis in fish (Singh and Lal, 2014).

Conclusion

Existence of intra-gonadal peptides and reactive oxygen species particularly, nitrogen species in the gonad of fish, and changes therein with advancing gonadal activities suggest their significant role in regulation of reproduction in lower vertebrates also in common with the higher vertebrates, however, studies are very scanty. These finding further suggest that the involvement of such intra-gonadal factors, besides sex steroids, have some evolutionary significant too. There is need to extend such studies in many lower vertebrates with detailed experimental designs to answer several unanswered questions.

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