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Membrane receptor cross talk in gonadotropin-, IGF-I-, and insulin-mediated steroidogenesis in fish ovary: An overview

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ABSTRACT

Gonadal steroidogenesis is critical for survival and reproduction of all animals. The pathways that regulate gonadal steroidogenesis are therefore conserved among animals from the steroidogenic enzymes to the intracellular signaling molecules and G protein-coupled receptors (GPCRs) that mediate the activity of these enzymes. Regulation of fish ovarian steroidogenesis *in vitro* by gonadotropin (GtH) and GPCRs revealed interaction between adenylate cyclase and calcium/calmodulin-dependent protein kinases (CaMKs) and also MAP kinase pathway. Recent studies revealed another important pathway in GtH-induced fish ovarian steroidogenesis: cross talk between GPCRs and membrane receptor tyrosine kinases. Gonadotropin binding to Gαs-coupled membrane receptor in fish ovary leads to production of cAMP which in turn trans-activate the membrane-bound epidermal growth factor receptor (EGFR). This is followed by activation of ERK1/2 signaling that promotes steroid production. Interestingly, GtH-induced trans-activation of EGFR in the fish ovary uniquely requires matrix-metalloproteinase-mediated release of EGF. Inhibition of these proteases blocks GtH-induced steroidogenesis. Increased cAMP production in fish ovarian follicle upregulate follicular cyp19a1a mRNA expression and aromatase activity leading to increased biosynthesis of 17β-estradiol (E2). Evidence for involvement of SF-1 protein in inducing cyp19a1a mRNA and aromatase activity has also been demonstrated. In addition to GtH, insulin-like growth factor (IGF-I) and bovine insulin can alone induced steroidogenesis in fish ovary. In intact follicles and isolated theca cells, IGF-I and insulin had no effect on GtH-induced testosterone and 17a,hydroxysprogeaterone production. GtH-stimulated E2 and 17,20bdihydroxy-4-pregnane 3-one production in granulosa cells however, was significantly increased by IGF-I and insulin. Both IGF-I and insulin mediates their signaling via receptor tyrosine kinases leading to activation of PI3 kinase/Akt and MAP kinase. These kinase signals then activates steroidogenic enzymes which promotes steroid production. PI3 kinase, therefore considered to be an initial component of the signal transduction pathways which precedes MAP kinase in IGF-1 and insulininduced steroidogenesis in fish ovary. Thus, investigation on the mechanism of signal transduction regulating fish ovarian steroidogenesis have shown that multiple, apparently independent signal transduction pathways are needed to convey the message of single hormone or growth factor.

1. Introduction

Steroid production in the vertebrate ovary begins with gonadotropin releasing hormone (GnRH) secretion from the hypothalamus. GnRH stimulates pulsatile release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary, followed by their binding to specific G protein-coupled membrane receptors (GPCRs) on ovarian follicular cells to promote steroidogenesis. These GPCRs in follicular cells are primarily linked to $G\alpha_s$ signaling leading to activation of multiple signal transduction pathways, including the adenylate cyclase-/cAMP-dependent protein kinase A (PKA) and calcium-/calmodulin-dependent pathways (see review Leung and Steele, 1992; Van Der Kraak and Wade, 1994; Nagahama, 1987; Kanamori and Nagahama, 1988). Cross talk among these signal transduction systems has also been well

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documented in many cell types and in response to a variety of receptor agonists (Bygrave and Roberts, 1995; Richards, 2001; Benninghoff and Thomas, 2006a; Paul et al., 2010a). PKA activation by gonadotropins (GtHs) promotes two important signals in mammalian ovarian follicle cells: long term up-regulation of steroidogenic acute regulatory (StAR) gene expression over the course of hours and rapid trans-activation of the epidermal growth factor receptor (EGFR) over the course of minutes, which leads to immediate activation of StAR protein without altering its gene expression (Light and Hammes, 2013). StAR protein is required to bring cholesterol into mitochondria for its conversion to steroid. This event is generally believed to be the rate limiting step in steroid production.

Available information also reported for involvement of mitogen-activated protein kinase (MAP kinase) signaling in the regulation of GtH-induced ovarian steroidogenesis. Occurrence of a cross talk among various signal transduction system including adenylate cyclase-, calcium-/calmodulin-, and MAP kinase-dependent signaling pathways has been well documented in ovarian steroidogenesis. In addition to pituitary GtHs, insulin and insulin-like growth factors (IGFs) have also been shown to act as potent regulators of ovarian steroidogenesis. Both are reported to stimulate basal as well as GtH-stimulated steroid production in ovarian follicle cells through activation of receptor tyrosine kinases (RTKs). Insulin signaling is carried out predominantly through insulin receptors (IRs) and IGF-I through IGF-1 receptor (IGFR-1). Activation of such receptor leads to phosphorylation of insulin receptor substrate (IRS) which subsequently activates downstream signaling molecules namely PI3 kinase and MAP kinase.

Here, we provide a brief overview describing the cross talk among various signal transduction systems, including adenylate cyclase-, calcium-/calmodulin-, and MAP kinase-dependent signaling pathways as well as the mechanism of GPCR/EGFR cross talk in GtH-induced ovarian steroidogenesis in few teleosts. We will also describe the signal transduction pathways activated downstream of RTKs that are necessary for IGF-1- and insulin-stimulated ovarian steroidogenesis in fish.

2. GPCRs and activation of adenylate cyclase-, and calcium-dependent signaling cascade

Fish ovary is a dynamic structure in which like mammals, steroidogenesis and other processes occur through the combined effects of two cell types: theca and granulosa cells. The ovary consists of enormous number of follicles, each containing an oocytes surrounded by granulosa and theca cell layers. Ovarian follicles at different developmental stages engage in growth, differentiation, steroid production, oocyte maturation, ovulation and atresia, which are in large part influenced by pituitary GtHs: GtH-I, homologous to tetrapod FSH and GtH-II, homologous to tetrapod LH (Yoshiura et al., 1999; Basu and Bhattacharya, 2002; Swanson et al., 2003). Despite differences in steroidogenic potency, fish FSH and LH stimulated steroid production *in vitro* by the ovarian follicles. In salmonids, FSH is elevated during vitellogenesis and regulates E2 production and early phases of gametogenesis, whereas LH appears during final oocyte maturation and ovulation (Kagawa et al., 1982; Tanaka et al., 1992; Gomez et al., 1999; Yaron et al., 2003; Kobayashi et al., 2006). FSH in salmonids regulates E2 production through stimulation of P450arom gene expression and aromatase activity during vitellogenesis (Kagawa et al., 1982; Tanaka et al., 1992). However, studies with red seabream, common carp and a major carp *Labeo rohita*, show that LH, not FSH, stimulates E2 production in the ovarian follicles through stimulation of aromatase gene expression and aromatase activity (Gen et al., 2001; Kagawa et al., 2003; Paul et al., 2008, 2010b; Roy Moulik et al., 2016).

How then do GtH signals activated in outer theca and granulosa cells of fish ovary promotes steroidogenesis? The answer is (a) GPCR activation of adenylate cyclase/cAMP, calcium/calmodulin and MAP kinase pathways and their cross talk and (b) GPCR/EGFR cross talk. GPCRs contain seven membrane-spanning regions and undergo a conformational change upon agonist stimulation to activate heterotrimeric G protein, which elicits various signaling pathways depending on the type of G protein coupled by the receptor. These are $G\alpha_s$, $G\alpha_q$, and $G\alpha_i$. Evidences for GtH-receptor complex to bind to $G\alpha_s$ and activation of adenylate cyclase-/cAMP-dependent pathway in fish ovarian follicular steroidogenesis have been well documented (Nagahama, 1987; Kanamori and Nagahama, 1988). Involvement of $G\alpha_q$ and activation of phospholipase C has also been reported in GtH-stimulated ovarian steroidogenesis in brook trout and goldfish (Planas et al., 1997; Pati and Habibi, 2002). Regulatory roles of adenylate cyclase and PKA in primary cultured Atlantic croaker ovarian follicle cells in the production of testosterone (T) in presence of forskolin and dbcAMP, modulators of adenylyl cyclase and PKA respectively, and a rapid, transient increase in cytosolic cAMP concentrations induced by HCG has been well documented (Benninghoff and Thomas, 2006a). Increased T and 17β-estradiol (E2) production by co-incubated carp (*Cyprinus carpio*) ovarian follicle cells in presence of forskolin and dbcAMP, and inhibition of HCG-stimulated steroid production in presence of specific adenylate cyclase inhibitor, SQ225368 also demonstrated the regulatory role of adenylate cyclase and PKA in GtH-induced ovarian steroidogenesis in such fish (Paul et al., 2010b). LH-induced cAMP production by the intact follicles of carp ovary has also been reported (Das and Mukherjee, 2013).

Calcium-mediated cell signaling has been shown to be important in regulating GtH-induced steroid production in whole ovarian follicles of goldfish and other vertebrates (Van Der Kraak, 1991; Van Der Kraak and Wade, 1994), and in isolated follicular cells of croaker and carp ovary (Benninghoff and Thomas, 2005, 2006b; Paul et al., 2010a). Using L-type calcium channel blocker verapamil, it has been shown that calcium influx from extracellular source is required for GtH-stimulated steroid production in carp ovarian follicles. Furthermore, inhibition of steroid production in presence of calmodulin inhibitor W5 and W7 indicate that calcium binding protein is also involved in GtH-induced steroid production in goldfish, Atlantic croaker and carp ovarian follicles (Van Der Kraak, 1991; Mukherjee et al., 2001; Benninghoff and Thomas, 2005, 2006b; Paul et al., 2010a). Involvement of calcium-/calmodulin-dependent protein kinase (CaMKs) in mediating GtH-induced ovarian steroidogenesis has also been reported in croaker follicles (Benninghoff and Thomas, 2006b). Previous studies have shown the role of CaMKs in the regulation of adrenal cell aldosterone or cortisol production (Ganguly et al., 1995; Pezzi et al., 1996; Nishikawa et al., 1997). Although there are mainly three types of CaMKs; CaMK I, CaMK II, and CaMK IV in follicular cells and all CaMK inhibitors block the activity of three CaMKs (Hidaka and Yokokura, 1996; Benninghoff and Thomas, 2006b), it is still not clear which specific enzyme is involved in mediating GtH-induced steroid production in fish ovarian follicles. Moreover, using calcium ionophore A23187, regulatory role of intracellular calcium ion in GtH-stimulated goldfish, Atlantic croaker and carp ovarian steroidogenesis has been documented (Srivastava and Van Der Kraak, 1994; Mukherjee et al., 2001; Benninghoff and Thomas, 2006b; Paul et al., 2010a).

Although conflicting reports are available on the requirement of calcium ion in GtH-induced cAMP production by rat and bovine granulosa cells (Tsang and Carnegie 1984; Davis et al., 1987), reports on other mammals indicate that HCG-induced increase in cAMP production requires the presence of calcium ion (Veldhuis