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Modulation of the steroidogenic activity of luteinizing hormone by insulin and insulin-like growth factor-I through interaction with the cAMP-dependent protein kinase signaling pathway in the trout ovary

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Abstract

In the salmonid ovary, luteinizing hormone (LH) is the major gonadotropic hormone stimulating the production of steroids during the periovulatory period and its effects are mediated by the cAMP-dependent protein kinase (PKA) signaling pathway. We have previously shown that the in vitro steroidogenic activity of LH in the salmonid ovary is inhibited by insulin-like growth factor I (IGF-I) which, like insulin, has specific receptors in both theca and granulosa layers. In the present study, we have investigated the modulatory effects of insulin on salmon LH (sLH)-stimulated steroid production in preovulatory theca layers of brown trout (*Salmo trutta*) and the effects of both insulin and IGF-I on the sLH-stimulated cAMP/PKA signaling pathway. Our results show that insulin, like IGF-I, blocked the stimulatory effects of sLH, dibutyryl cAMP and IBMX on testosterone (T) production but not those of sLH on cAMP production. Furthermore, insulin and IGF-I blocked the activation of PKA induced by sLH and these effects were correlated with changes in the total protein content of the catalytic (C) and type II regulatory (RII) subunits of PKA. Interestingly, insulin and IGF-I had different effects on total PKA subunit content since insulin potentiated the sLH-stimulated increase in RII subunit content whereas IGF-I blocked the sLH-stimulated increase in total C subunit content. The effects of insulin and IGF-I in trout theca layers appeared to be mediated by the mitogen-activated protein kinase (MAPK) signaling pathway because inhibition of extracellular signal-regulated kinase 1/2(ERK1/2) activity completely blocked the inhibitory effects of insulin and IGF-I on the sLH-stimulated production of T and because insulin and IGF-I increased the total protein content of ERK1/2 in trout theca layers. Therefore, our results suggest that insulin and IGF-I, probably through the MAPK pathway, block the action of sLH in trout theca layers by modulating the cAMP/PKA signaling pathway.
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Keywords: LH; Insulin; IGF-I; Testosterone; cAmp; PKA; Theca; Ovary; Trout

1. Introduction

In fish, like in other vertebrate species, the production of ovarian steroids is primarily under the control of pituitary gonadotropins (FSH and LH). Due to the availability of purified FSH and LH from salmon pituitaries, the steroidogenic and maturational activities of FSH and LH in the ovary have been relatively well described in salmonid fish: salmon and trout. In theca cells from salmonid fish, LH has been shown to stimulate the production of testosterone (T) and 17α-hydroxyprogesterone (Maestro et al., 1997; Mendez et al., 2003; Planas et al., 2000), which are converted to 17β-estradiol and 17α-20β-dihydroxyprogesterone, the maturation-inducing steroid (Nagahama, 1997), in granulosa cells during vitellogenesis and final oocyte maturation, respectively. Therefore, theca cells are fundamental in providing steroid precursors to the granulosa cells for the synthesis of 17β-estradiol and 17α-20β-dihydroxyprogesterone in the salmonid ovary (Kagawa et al., 1982). Furthermore, it is well known that the steroidogenic effects of LH take place through its interaction with specific receptors on theca cells (Miwa et

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al., 1994; Yan et al., 1992) and that these effects are mediated by the cAMP-dependent protein kinase (PKA) signaling pathway, as recently demonstrated in our laboratory (Mendez et al., 2003; Planas et al., 1997). Therefore, all the evidence obtained to date strongly suggests that the ovarian LH receptor in salmonid fish is coupled to the PKA signaling pathway through inhibitory and stimulatory G-proteins (Mita et al., 1994), like in mammals.

In addition to pituitary gonadotropins, a number of other factors are known to contribute to the regulation of the production of ovarian steroids. In mammals, intraovarian factors such as members of the insulin-like growth factor family have been shown to modulate the biological activity of LH on theca cells (Magoffin and Weitsman, 1993a,b, 1994). In particular, insulin and insulin-like growth factor I (IGF-I) have been shown to potentiate the stimulatory effects of LH on androgen production and expression of steroidogenic enzymes in mammalian theca cells (Magoffin et al., 1990; Magoffin and Weitsman, 1993b; Zhang et al., 2000). In fish, the presence of an intraovarian IGF system has also been described (Maestro et al., 1995), complete with ligand (Kagawa et al., 1995; Perrot et al., 2000; Schmid et al., 1999) and receptors (Maestro et al., 1997,1999). Specific high-affinity insulin and IGF-I receptors have been detected in preovulatory theca layers of salmonid fish (Maestro et al., 1997,1999) and, in accordance with these findings, IGF-I has been shown to inhibit LH-stimulated steroid production by salmon preovulatory theca layers (Maestro et al., 1997). However, to date, there is no information on the direct effects of insulin on the production of steroids or, more importantly, on the intracellular mechanism(s) by which insulin and IGF-I exert their effects in fish theca layers. Therefore, the objectives of this study were to investigate the modulatory effects of insulin on LH-stimulated steroid production in preovulatory theca layers of brown trout (*Salmo trutta*) and to determine the site of action of insulin and IGF-I on the LH-stimulated cAMP/PKA signaling pathway.

2. Materials and methods

2.1. Animals

Preovulatory brown trout (*Salmo trutta*) from a cultured stock at the Piscifactoria de Bagà (Generalitat de Catalunya) were kept under natural conditions of temperature and photoperiod. Fish at a preovulatory stage were anesthesized in 3-aminobenzoic acid ethyl ester (0.1 g/l; Sigma, St. Louis, MO, USA) dissolved in fresh water and sacrificed by decapitation prior to the collection of the ovaries.

2.2. Hormones and reagents

Coho salmon LH (sLH) was a kind gift from Dr. Penny Swanson (National Marine Fisheries Service, Seattle, WA) (Swanson et al., 1991) and was dissolved directly in incubation medium. Human recombinant insulin was purchased from Sigma–Aldrich Química, S. A. (Alcobendas, Madrid, Spain) and human recombinant IGF-I was a kind gift of Chiron Corporation (Emmeryville, CA) and both were dissolved in 0.01N HCl and diluted with incubation medium. Testosterone-, cAMP- and PKA-assay kits were purchased from Schering-CIS (Madrid, Spain), DPC-Dipesa S.A. (Madrid, Spain) and Gibco-Life Technologies (Barcelona, Spain), respectively. 3-isobutyl-1-methylxanthine (IBMX; Calbiochem, San Diego, CA) was initially dissolved in ethanol and then in incubation medium. Dibutyryl cAMP (dbcAMP; Sigma) was dissolved directly in incubation medium. [$^{32}\gamma$P] ATP (specific activity of 5000 Ci/mmol) was purchased from Amersham Life Sciences (Little Chalfont, Buckinghamshire, England). Polyclonal antibodies to human catalytic (Cγ) and mouse type II regulatory (RII) subunits of PKA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Upstate Biotechnology (Lake Placid, NY), respectively. The polyclonal antibody to mouse ERK-2 was purchased from Transduction Laboratories (Lexington, KY). PD98059 was purchased from Calbiochem.

2.3. Theca layer incubations

Individual ovarian follicles were separated from the ovary on ice and theca layers were manually separated from the rest of the follicle as described previously (Kagawa et al., 1982; Planas et al., 2000). Isolated theca layers (5 layers/well/0.5 ml for steroid and cAMP determinations and 20 layers/well/ml for PKA assays and Western analysis) were incubated in Hanks' balanced salt solution (HBSS; Maestro et al., 1997) containing 0.2% BSA (fraction V; Sigma) in the absence or presence of human recombinant insulin or IGF-I for 24 h at 15 °C in an air atmosphere with gentle shaking (100 rpm). Subsequently, the medium was discarded and theca layers were incubated with fresh medium containing insulin or IGF-I in the absence or presence of sLH (100 ng/ml; dissolved directly in HBSS–BSA) for an additional 18-h period. In the experiments in which the production of cAMP was measured, the second incubation was carried out for 4 h in the presence of 0.25 mM IBMX. At the termination of the second incubation, the medium and theca layers were removed and stored at −20 and −80 °C, respectively, until assayed.

2.4. Testosterone and cAMP RIAs

The production of testosterone by theca layers was measured directly in the incubation medium using a commercial radioimmunoassay (Schering-CIS, Madrid, Spain). For the measurement of cAMP, a 0.3 ml aliquot of the incubation medium was air dried at 60 °C, reconstituted with 0.3 ml of Tris–EDTA buffer and used directly in the cAMP assay (DPC-Dipesa S.A., Madrid, Spain), as described previously (Planas et al., 1997).

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