The Control Mechanism of Gonadotropin-Releasing Hormone and Dopamine on Gonadotropin Release from Cultured Pituitary Cells of Rainbow Trout *Oncorhynchus mykiss* at Different Reproductive Stages

Dae-Jung Kim1*, Yuzuru Suzuki2 and Katsumi Aida2a

1New Strategy Research Center, National Fisheries Research and Development Institute, Busan 619-705, Republic of Korea
2Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo, 113, Japan

*Present address: Japan Society for the Promotion of Science, Chiyoda, Tokyo, 102-8471, Japan

Abstract

The mechanism by which gonadotropin-releasing hormone (GnRH) and dopamine (DA) control gonadotropin (GTH) release was studied in male and female rainbow trout using cultured pituitary cells obtained at different reproductive stages. The mechanisms of follicle-stimulating hormone (FSH) release by GnRH and DA could not be determined yet. However, basal and salmon-type GnRH (sGnRH)- or chicken-II-type GnRH (cGnRH-II)- induced luteinizing hormone (LH) release increased with gonadal maturation in both sexes. LH release activity was higher after sGnRH stimulation than cGnRH-II stimulation at maturing stages in both sexes. The GnRH antagonist ([Ac-3, 4-dehydro-Pro1, D-p-F-Phe2, D-Trp3,6] GnRH) suppressed LH release by sGnRH stimulation in a dose-dependent manner, although the effect was weak in maturing fish. The role of DA as a GTH-release inhibitory factor differs during the reproductive cycle: the inhibition of sGnRH-stimulated LH release by DA was stronger in immature fish than in maturing, ovulating, or spermiated fish. DA did not completely inhibit sGnRH-stimulated LH release, and DA alone did not alter basal LH release. Relatively high doses (10^-6 or 10^-5 M) of domperidone (DOM, a DA D2 antagonist) increased LH release, which did not change with reproductive stage in either sex. The potency of DOM to enhance sGnRH-stimulated LH release was higher in maturing and ovulated fish than in immature fish. These data suggest that LH release from the pituitary gland is controlled by dual neuroendocrine mechanisms by GnRH and DA in rainbow trout, as has been reported in other teleosts. The mechanism of control of FSH release, however, remains unknown.

Key words: Pituitary cell culture, Gonadotropin, Dopamine, GnRH, Fish

Introduction

The pituitary gland of teleost fish is innervated by peptidergic and aminergic hypothalamic neurons (Dufour et al. 2010), which potentially allows for direct control of pituitary hormone release by hypothalamic neurons, instead of indirect control via the hypothalamo-hypophysial portal blood system, as in mammals (Zohar et al. 2010). Gonadotropin (GTH) secretion is also regulated by at least two hypothalamic factors: stimulation by gonadotropin-releasing hormone (GnRH) and inhibition by dopamine (DA) (Saligaut et al. 1999; Zohar et al. 2010). Hence, investigating the function of GnRH and DA further throughout the reproductive cycle is important.

The brain of teleost fish contains at least two different
forms of GnRH (Peter et al. 1991; Chang et al. 2009; Zohar et al. 2010). In the goldfish, Carassius auratus, GTH release is regulated by both salmon-type GnRH (sGnRH) and chicken-II-type GnRH (cGnRH-II) via a specific type of hypothalamic neuron innervated to the pituitary (Chang et al. 1990a). In contrast, only sGnRH neurons are found in the pituitary of salmonid fishes, although both types of GnRH fibers are distributed in all areas of the brain (Okuzawa et al. 1990; Amano et al. 1991). Amano et al. (1991) proposed that only sGnRH regulates GTH secretion. Salmon GnRH regulates GTH secretion in salmonid fish, as reported by Vacher et al. (2002) and Kim et al. (2009). However, insufficient data exist to determine the role of cGnRH-II in the regulation of GTH secretion at different reproductive stages.

The involvement of DA in the neuroendocrine regulation of GTH release in teleost fish was demonstrated in goldfish (Chang and Peter, 1983). DA inhibits spontaneous GTH release, as well as GnRH-stimulated GTH release, particularly in sexually mature fish (Peter et al. 1986, 1991; Chang et al. 2009). In contrast, DA may be less important in the regulation of GTH release in mature salmonid fish (Billard et al. 1984; Van der Kraak et al. 1986; Dufour et al. 2010). Saligaut et al. (1992) reported that pituitary DA release was high during the vitellogenesis stage, but low in mature female rainbow trout. In addition, inhibitors of catecholamine synthesis caused an increase in plasma GTH levels in estoradiol-17β-implanted immature female fish and in vitellogenic female fish (Linard et al. 1995). These results lead to the assumption that the inhibitory potency of DA on GTH release changes during the reproductive cycle of rainbow trout. As such, correlating the role of DA in the regulation of GTH release with gonadal maturity is necessary.

In the reproductive endocrinology of teleost fish, only one type of GnRH is generally assumed to regulate gonadal development, unlike dual control by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in higher vertebrates. In salmonids, however, two pituitary GTHs (FSH and LH) were identified (Suzuki et al. 1988a; Swanson et al. 1991). Their biological activities and plasma profiles are distinct (Suzuki et al. 1988b; Prat et al. 1996); FSH stimulates gonadal development during early developmental phases such as vitellogenesis and spermatogenesis, while LH is involved in later developmental phases such as ovulation and spermatogenesis. Little is known about the mechanisms of control of FSH release, although LH release from the pituitary gland is known to be under the dual control of GnRH and DA, as reported by Chang et al. (2009) and Zohar et al. (2010). However, information regarding how GnRH and DA control mechanisms change with gonadal maturation is lacking.

In this study, we examined the effect of DA and two GnRH peptides on the regulation of FSH and LH release using dispersed rainbow trout pituitary cells at different gonadal maturation stages. In addition, antagonists of GnRH and DA were used to determine the physiological relevance of our in vitro data.

Materials and Methods

Fish

Rainbow trout (Oncorhynchus mykiss) at different stages of gonadal maturation were used in this study. Immature fish (average body weight, 300 g; gonadosomatic index [GSI; gonad weight × 100/body weight]: male 0.07 ± 0.0 2%, female 0.18 ± 0.02%), maturing fish (average body weight, 1300 g; GSI: male 3.5 ± 0.2%, female 4.6 ± 0.2%), ovulated females (average body weight, 1700 g; GSI: 19 ± 0.9%) and spermatizing males (average body weight, 500 g; GSI: 3.8 ± 0.17%) were obtained from the Ohizumi Fisheries Experimental Station at the Tokyo University of Marine Science and Technology. Fish were reared in a 3,400 L tank with thermoregulated (13°C) recirculating freshwater for at least 1 week before use. Fish were exposed to the natural photoperiods of Tokyo, Japan, using an electric timer. Fish were fed commercial pellets (Oriental Yeast Co., Tokyo, Japan) at 1% of body weight per day.

Test substances

sGnRH and cGnRH-II were purchased from Peninsula Laboratories (Belmont, CA, USA). Stock solutions (10⁻⁵ M) in 0.1 M acetic acid were stored at -80°C and diluted with test medium immediately prior to use. GnRH antagonist ([Ac-3,4-dehydro-Pro¹, D-Phe², D-Trp³]¹ GnRH) and DA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All test solutions were freshly prepared. Domperidone (DOM; a DA D2 antagonist) was purchased from Research Biochemicals International (Natick, MA, USA); the stock solution (10⁻² M) was prepared in dimethyl sulfoxide, stored at -80°C and diluted with test medium immediately prior to use. Final solvent concentrations were less than 0.1% and did not affect basal GTH release.

Dispersion of pituitary cells for cell culture

The fish were rapidly anesthetized with 2-phenoxycethanol (1 mL/2 L). Pituitary glands were removed and placed in ice-cold Hank’s balanced salt solution (HBSS; Gibco Laboratories, Grand Island, NY, USA), which was buffered with 25 mM HEPES and 4 mM NaHCO₃ containing 1% (v/v) antibiotic-antimycotic agent (pH 7.5; Gibco Laboratories). After washing three times with HBSS, the pituitaries were diced into fragments using a tissue slicer (Narishige Scientific Instrument Lab, Tokyo, Japan). Fragments (~0.15 g) of pituitaries were transferred to a siliconized culture flask (Wheaton Instruments, Millville, NJ, USA) and treated with 2 mg/mL collagenase (Type V; Sigma Chemical Co.) solution in HBSS for 75 min at 15°C. During this period, the cells were occasionally dissociated mechanically by aspirating the fragments with a siliconized Pasteur pipette. Then, 500 µL of 0.04% DNase I (w/v; Boehringer Mannheim-GmbH, Mannheim, Germany)
solution was added to 10 mL of the solution and incubated for 15 min. Dispersed cells were filtered through a 50-µm nylon mesh and harvested by centrifugation at 150 g for 5 min. The cells were then resuspended in RPMI-1640 medium (pH 7.5; Sigma Chemical Co.) containing 25 mM HEPES, 4 mM NaHCO₃, 10% fetal bovine serum (Gibco Laboratories) and 1% antibiotic-antimycotic agent. The cell viability rate was 91 ± 1%, as determined by the trypan blue test.

**Cell culture**

The cell suspension was plated on 48-well plates (Sumitomo Co., Tokyo, Japan) at a density of 2.5 × 10⁴ cells per well. The wells were pretreated with poly-L-lysine; 500 µL of 0.1% poly-L-lysine was added to the wells, and the plates were rinsed three times with distilled water and then dried on a clean bench before use. The cells were preincubated for 3 days at 18°C (Vacher et al. 2002; Kim et al. 2009). Before adding the test substances, the cells were washed twice with serum-free testing medium (RPMI-1640 with 25 mM HEPES, 4 mM NaHCO₃, 1% antibiotic-antimycotic agent and 0.1% bovine serum albumin, pH 7.5). The cells were incubated with sGnRH or cGnRH-II for 24 h to determine the amount of released hormone. In some experiments, cells were pretreated with the GnRH antagonist, DA, or DOM, 15 min before adding GnRH. After incubation, 400 µL of cell-free medium was removed and stored at -80°C until assayed for the GTH level.

**Radioimmunoassays**

The levels of chum salmon (*Oncorhynchus keta*) FSH and LH in culture media were measured by specific radioimmunoassays (RIAs). Two types of FSH (stable and unstable) are present in the chum salmon pituitary (Suzuki et al. 1988b); purified stable FSH was used as both the standard and radiolabeled hormone in this study. Hormones and antibodies were kindly provided by Prof. H Kawauchi, Kitasato University, Japan. Antibodies were raised in rabbits against the β-subunit of FSH (FSHβ). FSHβ was emulsified with an equal volume of Freund’s incomplete adjuvant. Rabbits were bled 1 week after the last injection. Iodination and assay procedures for FSH and LH were carried out according to the method of Kim et al. (2009). Displacement curves for culture media and dispersed pituitary cell extracts were run in parallel to the standard curves in each FSH and LH RIA system (Kim et al. 2009). Standard, radiolabeled hormone and antibody against LH were previously characterized by Kim et al. (2009). Cross-reactions of LH in the FSH RIA or FSH cross-reactivity in the LH RIA were ~9.2% and 2.1%, respectively. The intra- and inter-assay coefficients of variation in the FSH RIA were 7.0% (n = 5) and 11.5% (n = 5), respectively, at ~50% binding. The sensitivity of the assay, defined as twice the standard deviation at zero dose, was 0.39 mg/mL (n = 5).

**Experimental design**

Dispersed pituitary cells obtained from immature and maturing male and female fish were examined for the effects of sGnRH and cGnRH-II on LH and FSH release. Spermiated and ovulated fish were examined for the effect of sGnRH on LH and FSH levels. The effect of GnRH antagonist on sGnRH-induced LH and FSH release were examined using cultured pituitary cells obtained from immature and maturing male and female fish as well as ovulated fish. The effect of DA on sGnRH-induced LH and FSH release was examined using cultured pituitary cells obtained from immature, maturing and spermiated/ovulated fish of both sexes. The effect of DOM on LH and FSH release was investigated using cultured pituitary cells obtained from immature and maturing male and female fish, as well as ovulated fish.

**Statistical analysis**

Student’s t-test, Cochran-Cox test, and Duncan’s new multiple range test were used for statistical analyses.

**Results**

**The effects of GnRHs on FSH and LH release**

Dispersed pituitary cells obtained from immature, maturing and spermiated male fish were treated with increasing doses of sGnRH and/or cGnRH-II (Fig. 1). Neither GnRH type had an effect on FSH release in any of the cells from different gonadal maturational stages. However, basal and GnRHs-stimulated cells showed increased LH release with the advance of spermatogenesis. In immature fish, LH release was increased significantly by sGnRH and cGnRH-II although the levels were low. The LH response to both GnRHs was similar and reached a maximum at a GnRH concentration of 10⁻⁶ M. In maturing fish, both GnRH peptides were found to stimulate LH release even at the lowest concentration used (10⁻¹² M). The maximal level of sGnRH-stimulated and cGnRH-II-stimulated LH release was obtained at GnRH concentrations of 10⁻⁴ M and 10⁻⁵ M, respectively. In addition, the volume of LH released after sGnRH stimulation was consistently higher than that obtained by cGnRH-II stimulation (GnRH concentrations of 10⁻¹² to 10⁻⁶ M). In spermiated males, a significant increase in LH release was observed even at a concentration of 10⁻¹² M sGnRH, and the maximal level of LH release was obtained at a sGnRH dose of 10⁻⁹ M.

The release of FSH from dispersed pituitary cells was not affected by either sGnRH or cGnRH-II in female fish at various developmental stages (Fig. 2). In contrast, basal and GnRHs-stimulated release of LH increased in accordance with ovarian development. In immature female fish, both sGnRH and cGnRH-II stimulated LH release in a dose-dependent
Fig. 1. Effects of different concentrations of salmon-type GnRH (sGnRH) and chicken-II-type GnRH (cGnRH-II) on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from cultured pituitary cells of immature, maturing and spermiated male rainbow trout. Data are expressed as the mean±SEM (n=6). A significant difference (p<0.05) was observed between columns indicated by different letters. * and ** indicated the levels of significant differences at p<0.01 and p<0.001, respectively between sGnRH- and cGnRH-II-stimulated LH release in each group.

Fig. 2. Effects of different concentrations of salmon-type GnRH (sGnRH) and chicken-II-type GnRH (cGnRH-II) on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from cultured pituitary cells of immature, maturing and ovulated female rainbow trout. Data are expressed as the mean±SEM (n=6). Presentation of statistical significance is as in Fig. 1. * and ** indicated the levels of significant differences at p<0.01 and p<0.001, respectively between sGnRH- and cGnRH-II-stimulated LH release in each group.

http://dx.doi.org/10.5657/FAS.2011.0379
fish, respectively. In cells from maturing fish of both sexes, sGnRH-stimulated LH release decreased at a GnRH antago-
nist concentration of $10^{-7}$ M and was completely inhibited at $10^{-6}$ M. In cells from ovulated females, sGnRH-stimulated LH release began to decrease at a GnRH antagonist concentration of $10^{-6}$ M and was completely inhibited at $10^{-7}$ M.

**The effect of DA on FSH and LH release**

The effect of DA on sGnRH-stimulated LH and FSH release from cultured pituitary cells is shown Fig. 4. The presence of DA did not affect sGnRH-stimulated basal FSH release in cells from either sex at any gonadal maturation stage. Treatment with DA alone did not change LH release in cells from either sex, although sGnRH-stimulated LH release was reduced by DA. In cells from immature fish of both sexes, $10^{-6}$ M DA completely inhibited sGnRH-stimulated LH release at sGnRH concentrations of $10^{-10}$ and $10^{-9}$ M. Addition of $10^{-6}$ M DA decreased LH release at a sGnRH concentration of $10^{-8}$ M by ~50% in male cells and 35% in female cells. In cells from maturing fish of both sexes, the increase in LH release in response to sGnRH was partially inhibited by $10^{-6}$ M DA but was not completely eliminated. DA at a concentration of $10^{-6}$ M inhibited LH release by sGnRH ($10^{-8}$ M) by ~20% in male cells and 45% in female cells. In cells from spermiated males, LH release in response to sGnRH from $10^{-11}$ to $10^{-9}$ M was to-

**The effects of a GnRH antagonist on FSH and LH release**

The effects of a GnRH antagonist on sGnRH-induced LH and FSH release from cultured pituitary cells from male and female fish are shown in Fig. 3. The presence of a GnRH antagonist had no effect on sGnRH-induced FSH release. Treatment with the GnRH antagonist alone at the highest concentration used ($10^{-6}$ M) did not alter basal LH release in cells from any reproductive stage of either sex. However, in dispersed pituitary cells from immature fish of both sexes, sGnRH-stimulated LH release decreased after GnRH antagonist treatment at a concentration of $10^{-6}$ M, and LH release was completely inhibited at $10^{-7}$ M and $10^{-6}$ M in cells from female and male Fig. 3. Effects of the GnRH antagonist on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release by salmon-type GnRH (sGnRH, $10^{-7}$M) from cultured pituitary cells of fish at different reproductive stages. Data are expressed as the mean±SEM ($n=6$). A significant difference ($p<0.05$) was observed between columns indicated by different letters.
effectively stimulated LH release, its activity was consistently lower than that of sGnRH. However, our data did not indicate a regulatory mechanism of FSH.

In this study, basal LH levels increased in accordance with gonadal maturation in both sexes. Although pituitary LH responsiveness to sGnRH (ratio of LH release between the maximal level with and without sGnRH treatment) increased in accordance with gonadal maturation, pituitary sensitivity to sGnRH (the minimal effective dose of sGnRH on LH release) did not differ. In addition, differences in the effect of sGnRH versus cGnRH-II on LH release were observed in maturing fish: the efficacy of sGnRH was greater than that of cGnRH-II. Salmon-type GnRH-stimulated LH release in cultured pituitary cells obtained at different gonadal maturation stages from both sexes was dose-dependently suppressed by the addition of a GnRH antagonist. Furthermore, GnRH antagonist-mediated decrease in sGnRH-stimulated LH release was more pronounced in immature or ovulated fish than in maturing fish: the physiological effect of sGnRH in regulating LH release seems to be stronger in maturing fish.

Amano et al. (1991) reported involvement of sGnRH in gonadal maturation via regulation of GTH synthesis and release in masu salmon, whereas cGnRH-II had little or no involvement in reproduction. In addition, Weil and Marcuzzi (1990a, 1990b) provided evidence that pituitary LH responsiveness to sGnRH increased with the advancement of gametogenesis and
tally abolished by $10^{-6}$ M DA. In cells from ovulated females, however, LH release in response to sGnRH at $10^{-11}$ M was not completely eliminated by $10^{-6}$ M DA. DA at a concentration of $10^{-8}$ M inhibited LH release due to $10^{-6}$ M sGnRH by ~65% in spermiated males and 40% in ovulated females.

The effect of the D2 antagonist, DOM on FSH and LH release

The effect of DOM alone, as well as in combination with sGnRH, on LH and FSH release from cultured pituitary cells is shown in Fig. 5. At all gonadal maturation stages tested, neither DOM nor sGnRH altered FSH release. Stimulation of LH release by DOM was observed only at concentrations of $10^{-6}$ and $10^{-5}$ M. Moreover, addition of DOM ($10^{-5}$ M) significantly stimulated sGnRH-stimulated LH release from cultured pituitary cells in maturing fish of both sexes and ovulated females, but LH release was absent in cells from immature fish of either sex.

Discussion

Our data suggest the regulatory mechanisms of LH and FSH release during gonadal maturation in rainbow trout: stimulation by GnRH and inhibition by DA. While cGnRH-II effectively stimulated LH release, its activity was consistently lower than that of sGnRH. However, our data did not indicate a regulatory mechanism of FSH.

In this study, basal LH levels increased in accordance with gonadal maturation in both sexes. Although pituitary LH responsiveness to sGnRH (ratio of LH release between the maximal level with and without sGnRH treatment) increased in accordance with gonadal maturation, pituitary sensitivity to sGnRH (the minimal effective dose of sGnRH on LH release) did not differ. In addition, differences in the effect of sGnRH versus cGnRH-II on LH release were observed in maturing fish: the efficacy of sGnRH was greater than that of cGnRH-II. Salmon-type GnRH-stimulated LH release in cultured pituitary cells obtained at different gonadal maturation stages from both sexes was dose-dependently suppressed by the addition of a GnRH antagonist. Furthermore, GnRH antagonist-mediated decrease in sGnRH-stimulated LH release was more pronounced in immature or ovulated fish than in maturing fish: the physiological effect of sGnRH in regulating LH release seems to be stronger in maturing fish.

Amano et al. (1991) reported involvement of sGnRH in gonadal maturation via regulation of GTH synthesis and release in masu salmon, whereas cGnRH-II had little or no involvement in reproduction. In addition, Weil and Marcuzzi (1990a, 1990b) provided evidence that pituitary LH responsiveness to sGnRH increased with the advancement of gametogenesis and
reached a maximum during the spawning period in rainbow trout. These results suggest that sGnRH is the major form of GnRH in salmonids.

Although cGnRH-II was not detected in the pituitary of rainbow trout (Okuzawa et al. 1990), it stimulated LH release from cultured pituitary cells in the present study. This result may have been a pharmacological effect due to the similarity of the amino acid sequences of sGnRH and cGnRH-II. Whether both GnRHs function through the same receptor in rainbow trout has not yet been established.

The GnRH antagonist, [Ac-3,4-dehydro-Pro\(^1\), D-p-F-Phe\(^2\), D-Trp\(^3,6\)] mammalian-type GnRH, was used as a probe to investigate the inhibition of sGnRH-stimulated LH release in rainbow trout using cultured pituitary cells. Kim et al. (2000) demonstrated that this GnRH antagonist can block the increase in plasma LH secretion induced by treatment with a mammalian GnRH analog or sGnRH in precocious male rainbow trout. Flett et al. (1994) also reported that the [D-pGlu\(^1\), D-phe\(^2\), D-Trp\(^3,6\)] GnRH antagonist inhibited release of LH in response to the sGnRH analog from perifused pituitary glands of testosterone-primed immature rainbow trout. In goldfish, the GnRH antagonist used in this study was shown to block the sGnRH- and cGnRH-II-induced LH release from the pituitary fragments perifusion system (Murthy et al. 1993). However, whether the GnRH antagonist acts directly at the pituitary cell level is not known. Our results indicate that the sGnRH-stimulated LH release from cultured pituitary cells obtained at different sexual stages from both males and females was dose-dependently suppressed by the addition of a GnRH antagonist. Furthermore, this was more pronounced in cells from immature or ovulated fish than in cells from maturing fish: the physiological effect of sGnRH in regulating LH release seems to be stronger in maturing fish. Treatment with the GnRH antagonist alone did not alter the basal release of LH or FSH.

Studies on goldfish have shown that treatment with pimozide, a DA antagonist, has a variable influence on plasma LH levels, which can be influenced by both temperature and the reproductive condition of the fish (Chang and Peter, 1983; Sokolowska et al. 1985). In previous studies on salmonid fish, the DA inhibition of LH release was less pronounced than that observed in goldfish, and salmonids are capable of oocyte maturation and ovulation in response to a GnRH analog alone (Van der Kraak et al. 1986; Park et al. 2007). Therefore, the stronger DA inhibition in goldfish compared with rainbow trout could account for this difference, although differences in pituitary sensitivity to GnRH may also play a role. Our data suggest that the role of DA as a gonadotropin release-inhibiting factor (GRIF) in rainbow trout differed during the reproductive cycle: inhibition by DA was stronger in immature fish than maturing, ovulated or spermated fish. These results suggest marked inhibition by DA of LH release in immature fish.
presumably due to the low intensity of the sGnRH response. However, DA neither abolished GnRH-stimulated LH release nor altered the basal LH release.

No information regarding the modulation of LH release by DA during the different reproductive stages of salmonid fish is currently available. Saligaut et al. (1992, 1999) reported high pituitary DA turnover in rainbow trout during vitellogenesis that dropped at maturation. An inhibitor of catecholamine synthesis, α-methyl-p-tyrosine (MPT), caused an increase in plasma LH levels in 17β-estradiol-imprinted immature and vitellogenic female rainbow trout (Linard et al. 1995; Saligaut et al. 1998). Furthermore, the effect of DA on GnRH-induced plasma LH secretion was prolonged by exogenous testosterone treatment in immature and precocious male rainbow trout (Kim and Aida, 2000). These results suggest that sex steroids may influence modulation of DA synthesis, release, or turnover, which may be related to the regulation of LH release. Except for goldfish, however, basal LH release does not seem to be influenced by DA in other teleost species (de Leeuw et al. 1986; Levavi-Sivan et al. 1995). Therefore, DA as a GRIF in other teleost species may inhibit GnRH-stimulated LH release (de Leeuw et al. 1986; Levavi-Sivan et al. 1995).

In teleosts, the role of DA in the regulation of LH secretion remains controversial. Some data indicate an inhibitory role of DA on LH levels in vivo or in vitro in mature female rainbow trout (Vacher et al. 2000); however, other evidence suggests that DA has no effect on FH secretion (Saligaut et al. 1998). Our data show that FSH levels were not enhanced by sGnRH, and DA did not affect FSH release. Notably, in the Atlantic croaker Micropogonias undulatus, no evidence indicates that DA inhibits the regulation of LH secretion (Copeland and Thomas, 1989), which suggests that inhibition of GTH release by DA may not be a common phenomenon in teleost fish. The existence of a dual control mechanism of FSH release in rainbow trout is therefore a question that remains to be answered.

Our results indicate that relatively high doses (10^6 or 10^5 M) of DOM, a DA D2 antagonist, increased LH release, but LH release in response to DOM was similar in cultured pituitary cells from different reproductive stages in both sexes. Moreover, the effect of DOM on sGnRH-stimulated LH release was higher in cells from maturing and ovulated fish than in cells from immature fish. In goldfish, DA inhibition of LH release may be due to a direct effect on the pituitary (Chang et al. 1993) and may be mediated via D2-type receptors (Omeljanjuk et al. 1987; Chang et al. 1990b). DOM is unusual in that administration of DOM alone to goldfish induces a substantial increase in LH levels in vivo and in vitro without coadministration of a GnRH peptide (Omeljanjuk et al. 1987, 1989). Furthermore, Sokolowska et al. (1985) demonstrated that the magnitude of the plasma LH response to GnRHs, pimozide, or a combination of both was directly related to the stage of gonadal development in goldfish. In testosterone-treated immature rainbow trout, GnRHs combined with DOM resulted in a greater increase in plasma LH levels than GnRHs alone due to blocking of pituitary DA D2 receptors and potentially due to increased GnRH stimulation of the pituitary (Kim and Aida, 2000).

Swanson et al. (1989) reported that FSH release was increased by the action of GnRHs from organ-cultured pituitaries of juvenile Coho salmon. The discrepancy between this report and our data may be related to differences in culture systems between dispersed pituitary cells and the entire pituitary organ, differences in the biological activities of native GnRHs and GnRH analogs and/or differences in the RIA systems used. In this investigation, we used native forms of GnRH to determine the physiological response to FSH in cells; FSH release was not affected by either the native GnRH peptide concentration or the stage of sexual development. Therefore, we conclude that FSH release is not regulated by GnRH under physiological conditions.

This study demonstrates that in the rainbow trout, as in other teleosts, LH release from the pituitary gland is under the control of dual neuroendocrine mechanisms mediated by GnRH and DA. The existence of such dual mechanisms of control of FSH release, however, is a question that remains to be answered. Furthermore, pituitary LH release in response to GnRH and DA varies with gonadal stage.

Acknowledgments

This work was supported in part by grant (RP-2011-AQ-013) from the NFRDI of Korea.

References


Prat F, Sumpter JP and Tyler CR. 1996. Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (Oncorhynchus mykiss). Biol Reprod 54, 1375-1382.
Vacher C, Mañanos EL, Breton B, Marmignon MH and Saligaut C. 2000. Modulation of pituitary dopamine D1 or D2 receptors and secretion of follicle stimulating hormone and luteinizing hormone during the annual reproductive cycle of female rainbow trout. J Neuroendocrinol 12, 1219-1226.


