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Interactions of Catecholamines and GnRH in Regulation of Gonadotropin Secretion in Teleost Fish

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I. Introduction

In mammals a key element in the neuroendocrine regulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion is the decapeptide neurohormone luteinizing hormone-releasing hormone (LHRH). The actions of LHRH, and various synthetic analogs on pituitary LH and FSH cells, have been extensively studied, using the laboratory rat as the primary experimental model (e.g., Bex and Corbin, 1984; Kalra and Kalra, 1985). In view of the central role of LHRH in regulation of secretion of LH and FSH in mammals, the factors that regulate activity of LHRH cells are clearly of fundamental importance in understanding the neuroendocrine regulation of reproduction. Studies in mammals, again primarily on the rat, indicate that a number of amine neurotransmitters are involved in regulation of LH secretion, presumably by influencing the activity of LHRH neurons (e.g., Barraclough *et al.*, 1984; Ramirez *et al.*, 1984); the current interpretation is that amines do not act directly at the level of the pituitary to influence LH or FSH secretion. In contrast to this, our recent studies on teleost fishes, primarily the goldfish, indicate that direct actions of the catecholamines dopamine (DA) and norepinephrine (NE) on the pituitary are of major importance in the regulation of gonadotropin (GtH) secretion. Most importantly we have found that DA acts as a gonadotropin release inhibitory factor (GRIF) to modulate the action of gonadotropin-releasing hormone (GnRH), as well as spontaneous release of GtH. In this review we shall discuss supporting evidence for this, and draw parallels with situations in mammals, primarily the human, in which a similar interaction of DA and LHRH may occur.

II. Nature of Teleost Gonadotropins

The purpose of this section is to provide some information on the nature of teleost GtH, to enable workers in other fields to better relate the discussion to the neuroendocrine regulation of LH and FSH secretion.

It was thought for some time that in teleost fishes only one GtH was responsible for the full range of gonadal actions ascribed to LH and FSH (for review, Peter and Crim, 1979). However, it is now apparent that there are two pituitary hormones that act on the gonads in teleost fishes (for review, Idler and Ng, 1983). One of these, isolated from pituitary glands from carp, salmonids, plaice, flounder, and pike eel, is a classical GtH in that it has a high carbohydrate content, and is made up of α - and β -subunits (for review, Burzawa-Gerard, 1982; Idler and Ng, 1983). The β -subunit from carp GtH will associate with the α -subunit of bovine LH, restoring biological activity to a level similar to that of intact carp GtH in a cyclic AMP bioassay on eel ovary (Burzawa-Gerard, 1982). These and other data suggest a high level of homology between this form of teleost GtH, and LH and FSH from other vertebrates. The teleost GtHs with high carbohydrate content have a broad spectrum of biological activity, including stimulation of cyclic AMP production in gonadal tissue, stimulation of steroidogenesis, spermatogenesis, spermiation, some aspects of oocyte growth, and oocyte maturation and ovulation (for review, Idler and Ng, 1983; Ng and Idler, 1983). Specific radioimmunoassays have been developed for "high carbohydrate" GtH in cyprinid teleosts such as goldfish and carp, various salmonid species (for review, Peter, 1981), and the African catfish (de Leeuw *et al.*, 1985b); the β -subunit of carp GtH has been used to develop a radioimmunoassay for GtH in the European eel (Dufour *et al.*, 1983). A second GtH, isolated from pituitaries from common carp, chum salmon, plaice, and flounder, has a low carbohydrate content, and acts to stimulate incorporation of yolk protein into developing oocytes and to stimulate steroidogenesis, to a greater or lesser degree depending on the species (for review, Idler and Ng, 1983; Ng and Idler, 1983). A subunit structure for this "low carbohydrate" GtH has not yet been demonstrated. Immunohistochemical studies have demonstrated that both the "low" and "high" carbohydrate GtHs originate from basophils, in some cases the same basophils, in the pars distalis of the pituitary (for review, Van Oordt and Peute, 1983), which suggests some relatedness of the two hormones. Unfortunately, radioimmunoassays for the "low carbohydrate" GtHs have not yet been developed. Thus, for the remainder of this review where measurements of GtH are reported, we refer to only the "high carbohydrate" GtH with the broad spectrum of actions on the gonads.

III. Gonadotropin-Releasing Hormone (GnRH) in Teleosts

Several early studies on a number of teleost species demonstrated the presence of a factor in crude hypothalamic extracts that stimulated GtH release (for review, Peter, 1983a,b). Radioimmunoassay studies on crude hypothalamic extracts using antisera with characterized specificity against LHRH suggested similarity in the forms of GnRH present in the chicken, a tortoise, and a teleost (tilapia, *Sarotherodon mossambicus*) (King and Millar, 1979, 1980). The behavior of immunoreactive "LHRH" fractions on cation-exchange and affinity chromatography columns, and in high-pressure liquid chromatography (HPLC) further indicated that the GnRH in a teleost (*S. mossambicus*), an elasmobranch, reptiles, and birds is similar if not identical; the GnRH present in amphibians appeared to be identical to LHRH (King and Millar, 1979, 1980). Chromatography and immunological studies on hypothalamic extracts from cod, *Gadus morhua* (Barnett *et al.*, 1982), winter flounder, *Pseudopleuronectes americanus* (Idler and Crim, 1985), and chum salmon *Onchorhynchus keta* (Sherwood *et al.*, 1983) confirmed that GnRH in teleosts is similar but not identical to LHRH; furthermore, two forms of GnRH similar in size to LHRH were detected by Barnett *et al.* (1982) and Sherwood *et al.* (1983). Some results in these studies also suggested the presence of an immunoreactive high-molecular-weight form of GnRH, which may represent a precursor of the authentic GnRH. On the basis of immunoreactive properties, King and Millar (1980) and Barnett *et al.* (1982) suggested that the GnRH present in tilapia and cod, respectively, differed from LHRH by substitutions at positions seven and/or eight of the decapeptide. Sherwood *et al.* (1983) carried out amino acid analysis of the major chum salmon GnRH immunoreactive fraction isolated by HPLC, and by comparison of the chromatographic behavior of various synthetic peptides with native GnRH determined that the structure of the major peptide was [Trp⁷,Leu⁸]-LHRH (tGnRH). More recently Sherwood *et al.* (1984) and Breton *et al.* (1984) have demonstrated that this form of GnRH is present in the brain of a wide range of teleost species.

A number of studies have shown that injection of LHRH or superactive analogs of LHRH into teleosts stimulates an increase in the circulating levels of GtH (for review, Peter, 1983a,b; Peter and Crim, 1979). In our *in vivo* studies on goldfish the response to a single intraperitoneal injection of LHRH or [D-Ala⁶,Pro⁹-NH₂]-LHRH (LHRH-A) was poor; however, a relatively large GtH release-response was found following a pair of injections of LHRH (compare Fig. 1A to Fig. 1B) or LHRH-A, indicating that self-potential of the action can occur (Peter, 1980; Lin *et al.*, 1985b; Peter *et al.*, 1985; Sokolowska *et al.*, 1984, 1985b). Notably, a clear dose-

FIG. 1. Serum gonadotropin levels (mean $\pm$ SE, $n = 6$ or 7) of mature male goldfish, held at 12–14°C, following a single intraperitoneal injection (A) or a pair of injections given 12 hours apart (B) of LHRH. Significant differences from controls at same sample time were determined by the U test (* $p < 0.05$; ** $p < 0.01$). Reproduced from Peter (1980).

dependent response to LHRH or LHRH-A was not evident; furthermore, the magnitude of the responses, in terms of the peak concentrations of serum GtH reached, were similar between LHRH and the superactive analog, although the responses to the analog had a longer duration. A clear dose-dependent response to LHRH or a superactive analog was not found in *in vivo* experiments with common carp (Weil *et al.*, 1975) and coho salmon (Van Der Kraak *et al.*, 1983); however, a dose-dependent

response has been reported in other investigations on common carp (Breton and Weil, 1973), brown trout (Crim *et al.*, 1981), and coho salmon (Van Der Kraak *et al.*, 1985).

Similar to our findings in the goldfish, Van Der Kraak *et al.* (1983) found in coho salmon that similar dosages of LHRH and LHRH-A caused increases in plasma GtH of similar magnitude, but that the duration of the response to the analog was longer. Results from *in vitro* studies reveal a different aspect of the responsiveness of teleost pituitaries to GnRH. Rainbow trout pituitaries in static culture display a significant dose-related response to LHRH (Crim and Evans, 1980; Crim *et al.*, 1981; Fahraeus-van Ree *et al.*, 1983); however, there was no indication of superactivity of analogs compared to LHRH (Crim *et al.*, 1981). Likewise, goldfish pituitary glands in perfusion culture show a dose-dependent response of similar magnitude and duration to LHRH, tGnRH, and LHRH-A (MacKenzie *et al.*, 1984). Several aspects of these results require further explanation. For example, why are dose-response relationships not readily apparent *in vivo* in teleosts? Why is the magnitude of the GtH release-response, in terms of the peak GtH levels accomplished, to LHRH and superactive analogs similar *in vivo* and *in vitro*? In latter sections evidence will be presented that DA acts as a gonadotropin release-inhibitory factor (GRIF) directly at the level of the gonadotrope in the pituitary. Furthermore, when the GRIF activity of endogenous DA is blocked, dose-response relationships and superactivity of various analogs become evident *in vivo*.

IV. Evidence for a Gonadotropin Release-Inhibitory Factor

Peter *et al.* (1978) reported that preovulatory female goldfish receiving large radiofrequency current lesions in the ventrobasal hypothalamus that destroyed the nucleus lateralis tuberis (NLT; homologous to arcuate nucleus) in the pituitary stalk region had a marked increase in serum GtH levels and ovulation; serum GtH levels were significantly elevated for at least 12 days postlesioning. These results were interpreted to indicate the presence of a gonadotropin release inhibitory factor (GRIF), and that blocking the action of GRIF on the pituitary allowed a prolonged bout of spontaneous secretion of GtH. In additional radiofrequency current brain lesioning studies on male and female goldfish, Peter and Paulencu (1980) mapped the location of brain lesions which were effective in causing a significant increase in serum GtH levels. On the basis of these studies, we suggested that GRIF originates from the anterior-ventral nucleus preopticus periventricularis, in the region of the preoptic recess, and that a pathway courses from this center through the lateral preoptic region, and

FIG. 2. Diagrammatic summary showing the proposed origin in the goldfish of the gonadotropin release-inhibitory factor in the anterior preoptic region (shaded area) and the proposed pathway of the factor (arrows) in the lateral preoptic region, lateral anterior hypothalamic region, and pituitary stalk. Distances between the cross-sections given in millimeters above the drawings. AC, Anterior commissure; HOC, horizontal commissure; NAH, nucleus anterioris hypothalami; NAPv, nucleus anterioris periventricularis; NE, nucleus entopeduncularis; NLT, nucleus lateralis tuberis; NPO, nucleus preopticus; NPP, nucleus preopticus periventricularis; OC, optic chiasma; OT, optic tract; P, pituitary; T, telencephalon. Reproduced, by permission of S. Karger AG, from Peter and Paulencu, *Neuroendocrinology* 31, 133-141 (1980).

lateral-anterior hypothalamic region to the pituitary (Fig. 2). In teleost fishes there is no median eminence per se, as neurosecretory fibers directly innervate the anterior pituitary (for review, Peter and Fryer, 1983). Lesions in the ventrobasal hypothalamus which destroy the pituitary stalk caused a marked increase in serum GtH levels (Peter and Paulencu, 1980). Immunohistochemical studies on goldfish brain using antisera against LHRH (Kah *et al.*, 1982) or native tGnRH (O. Kah, J. Dulka and R. E. Peter, unpublished results) demonstrate a major concentration of GnRH perikarya in the lateral preoptic region adjacent to the most lateral aspects of the nucleus preopticus periventricularis, nucleus preopticus, and the anterior part of the nucleus anterioris periventricularis. This distribution of GnRH perikarya does not overlap the location of the proposed GRIF center. However, large lesions in the anterior preoptic region which de-

stroy the GRIF center would also destroy a major number of GnRH cell bodies. In any case, it is highly unlikely that the prolonged increases in GtH secretion found following lesions of either the pituitary stalk, the proposed GRIF center, or the proposed GRIF pathway are due to release of GnRH from terminals of lesioned cells remaining in the pituitary, as such fibers are phagocytosed within a few days (R. E. Peter, unpublished observations). Furthermore, small lesions in the ventrobasal hypothalamus of goldfish which destroy the nucleus lateralis tuberis in the posterior pituitary stalk region cause a significant decrease in the gonadosomatic index, due to onset of ovarian atresia, and abolition of significant daily cycles in GtH secretion (Peter and Paulencu, 1980; Peter, 1982). These results are consistent with early evidence interpreted to indicate that this particular region of the ventrobasal hypothalamus is involved in stimulation of gonadal activity via GnRH (Peter, 1970).

Additional evidence for the presence of GRIF comes from studies in which the pars distalis of the pituitary was transplanted to various locations (Peter *et al.*, 1984). In these studies the pars distalis was transplanted from one goldfish to another goldfish, of matched size, sex, and stage of gonadal development, to either beside the brain, or into the third ventricle in the preoptic region, or into the brain ventricle underlying the optic tectum. Such a transplanted pars distalis releases GtH spontaneously, irrespective of location of the transplant, indicating that GtH secretion is normally under tonic inhibition. Pars distalis transplants beside the brain released more GtH than those transplanted into the brain ventricles, indicating the presence of GRIF in the brain. Pars distalis transplant studies done with male and female goldfish at different stages of seasonal gonadal development indicate that GtH secretion is under tonic inhibition at all stages of gonadal development, although pituitaries from fish that are in the latter stages of ovarian or testicular recrudescence prior to spawning have the greatest potential for spontaneous secretion of GtH.

Brain lesioning studies have been carried out on male and female goldfish at different stages of seasonal gonadal development to further explore the changes in ability of the pituitary to spontaneously secrete GtH (R. E. Peter and C. S. Nahorniak, unpublished results). Radiofrequency current lesions were stereotaxically placed in the preoptic region of goldfish to destroy the GRIF center, following which blood samples were taken for measurement of serum GtH levels by radioimmunoassay; the pituitary concentration of GtH was also measured in a number of experiments. Preoptic lesions resulted in increased serum GtH levels in male and female goldfish at all stages of seasonal gonadal development, indicating that GRIF is present continuously. Female goldfish had the greatest difference in potential to spontaneously secrete GtH, with sexually re-

gressed females having the least potential and females in latter stages of ovarian recrudescence having the greatest potential (Fig. 3). In fish held at 12°C, but not at 20°C, there was a decrease in the concentration of GtH in the pituitary following preoptic lesions that abolished GRIF (R. E. Peter and C. S. Nahorniak, unpublished results). In other studies, the ultrastructural appearance of the pituitary following brain lesions that abolish GRIF suggested that the GtH cells in the pituitary were very actively secreting and synthesizing protein-like material (Nagahama and Peter, 1982). The depletion of pituitary GtH following a preoptic lesion in fish held at 12°C suggests that at this temperature the rate of synthesis cannot keep pace with the secretion rate. Overall these data provide evidence that GRIF is of importance in regulating GtH secretion in goldfish at all stages of the seasonal sexual cycle.

FIG. 3. Effects on serum gonadotropin levels of radiofrequency current lesions of the preoptic region, to destroy the gonadotropin release-inhibitory factor, in sexually regressed and sexually mature (preovulatory) female goldfish held at 12°C. Significant differences from sham operated controls were determined by the *U* test (* $p < 0.05$; *** $p < 0.001$). R. E. Peter and C. S. Nahorniak, unpublished results.

V. Involvement of Catecholamines in Neuroendocrine Regulation of GtH Release in Goldfish

A. DOPAMINE

1. Dopamine (DA) as an Inhibitor of GtH Release

The involvement of catecholamines in the neuroendocrine regulation of GtH release in the goldfish was first demonstrated by Chang *et al.* (1983). Long-term treatment with 6-hydroxydopamine as well as a single intraperitoneal injection of reserpine increased circulating GtH levels. Intraperitoneal injections of α -methyl-*p*-tyrosine or carbidopa, drugs known to block L-dopa and DA synthesis, respectively, elevated serum GtH levels; on the other hand, injections of diethyl-dithiocarbamate, an agent capable of inhibiting the conversion of DA to NE, did not alter circulating GtH concentrations. These observations suggest that DA may have an inhibitory influence on GtH secretion in the goldfish.

The ability of DA to inhibit GtH release in the goldfish has since been confirmed. Intraperitoneal injections of DA and its agonist apomorphine decreased, whereas intraperitoneal injections of the DA antagonists pimozide and metoclopramide elevated serum GtH concentrations (Chang and Peter, 1983a,b; Chang *et al.*, 1984b; Sokolowska *et al.*, 1984). Although intraperitoneal injection of DA decreased serum GtH levels, injection of DA into the third cranial ventricle was without effect (Chang and Peter, 1983a). DA does not cross the blood-brain barrier in mammals and a blood-brain barrier for DA also seems to exist in the goldfish; systemically administered radiolabeled DA does not accumulate in the brain of goldfish (J. P. Chang and R. E. Peter, unpublished results, cited in Chang, 1983). The ability of peripherally, but not centrally administered DA to alter serum GtH levels suggests that it acts outside the blood-brain barrier to decrease GtH release in the goldfish. Since the pituitary lies outside of the blood-brain barrier, and the gonadotropes of goldfish are directly innervated by neurons that are morphologically similar to aminergic neurons (for review see Ball, 1981; Peter and Fryer, 1983), these results suggest that DA may act at the level of the pituitary to inhibit GtH secretion.

Evidence for the direct action of DA on the goldfish pituitary to inhibit spontaneous GtH release was provided by *in vitro* and *in vivo* studies. Goldfish pituitary fragments and dispersed cells, in a column perfusion system, have a high rate of spontaneous GtH release (Chang *et al.*, 1984c). Addition of DA to the perfusate reduced the level of GtH released spontaneously from dispersed goldfish pituitary cells as well as pituitary

FIG. 4. Changes in gonadotropin (GtH) released from perifused (A) dispersed pituitary cells obtained from sexually regressed female goldfish, and (B) pituitary fragment preparations from sexually mature (prespawning) female goldfish during and following exposure to dopamine (DA). Reprinted with permission from *Life Sciences* 35, 2027-2033, Chang *et al.* (1984c); copyright Pergamon Press, Ltd.

fragments (Fig. 4). Goldfish in which the anterior preoptic region has been lesioned have increased serum GtH levels, due to abolition of endogenous GRIF allowing a high rate of spontaneous GtH secretion (see Section IV); intraperitoneal injection of either DA or apomorphine significantly depressed the lesion-induced increase in serum GtH levels (Chang and Peter, 1983a). Pars distalis transplants have a high spontaneous GtH release rate, causing marked increases in circulating GtH levels (see Section IV); intraperitoneal injection of DA or apomorphine significantly reduced the highly elevated serum GtH values in fish bearing pars distalis transplants (Chang *et al.*, 1984a).

Notably, DA also inhibits the GnRH-stimulated GtH release in goldfish. Intraperitoneal injection of DA, or its agonist apomorphine, reduced the magnitude of the LHRH-A stimulated increase in serum GtH levels on a dose-dependent basis (Chang and Peter, 1983a; Chang *et al.*, 1984b; Fig. 5). Similarly, another DA agonist, bromocryptine also decreased the LHRH-A stimulated increase in serum GtH concentrations on a dose-dependent basis (Chang *et al.*, 1984a). Application of DA completely blocked the LHRH-A stimulation of GtH release *in vitro* (Chang *et al.*, 1984c; Fig. 6). Injections of the DA antagonists, pimozide (Fig. 7) or metoclopramide (Fig. 8), potentiated the LHRH-A-induced increase in serum GtH levels (Chang *et al.*, 1984b; Sokolowska *et al.*, 1984, 1985a,b; Peter *et al.*, 1985; R. E. Peter, R. Billard, and C. S. Nahorniak, unpub-

FIG. 5. Effects of intraperitoneal injections of apomorphine, administered simultaneously with both the first and second injections of LHRH-A given 12 hours apart, on serum gonadotropin (GtH) concentrations in female goldfish held at 12°C (mean $\pm$ SE are plotted). Significant differences determined by the *U* test. Reproduced, by permission of S. Karger AG, from Chang and Peter, *Neuroendocrinology* 36, 351–357 (1983a).

FIG. 6. Changes in gonadotropin (GtH) released from perifused (A) dispersed pituitary cells obtained from sexually regressed female goldfish in response to LHRH-A and dopamine (DA), or LHRH-A alone, and (B) pituitary fragments prepared from sexually mature (prespawning) female goldfish in response to a 2 minute pulse (arrow) of LHRH-A during perifusion with either albumin-supplemented Hepes-Hanks' solution alone, 500 nM DA, or 500 nM metoclopramide. Reprinted with permission from *Life Sciences* 35, 2027-2033, Chang *et al.* (1984c); copyright Pergamon Press, Ltd.

FIG. 7. Effects on serum gonadotropin (GtH) levels of intraperitoneal injection of pimo- zide (Pim: 1.0 $\mu\text{g/g}$ body weight) and different dosages of LHRH-A in sexually mature (prespawning) female goldfish held at 20°C. Dosages of LHRH-A given in $\mu\text{g/g}$ body weight on the figure. Values are mean $\pm$ SE. Number of animals ovulating in each group are given at the base of each column. Significant differences determined by the U test. Adapted from Sokolowska *et al.* (1985b).

FIG. 8. Effects of intraperitoneal injection of metoclopramide (Met: 100 $\mu\text{g/g}$ body weight) on the LHRH-A- (0.1 $\mu\text{g/g}$) induced increase in serum gonadotropin (GtH) levels in female goldfish undergoing ovarian recrudescence, held at 20°C. Values are mean $\pm$ SE ($n = 8$ or 9). Significant differences determined by the U test ($p < 0.05$; Δ , versus Veh + PS group; *, versus Veh + LHRH-A group). R. E. Peter, R. Billard, and C. S. Nahorniak, unpublished results.

lished results). Pimozide injection also potentiated the LHRH- and tGnRH-induced increase in serum GtH concentrations (Peter, 1983a; Peter *et al.*, 1985). Notably, the effects of pimozide (Chang *et al.*, 1984b; Peter *et al.*, 1985; Sokolowska *et al.*, 1985b) and metoclopramide (Chang *et al.*, 1984b; R. E. Peter, R. Billard, and C. S. Nahorniak, unpublished results) in potentiating the action of LHRH-A are dose dependent.

If DA acts as a GRIF, then drugs that block the synthesis of DA or cause depletion of DA from presynaptic terminals should also influence the responsiveness of goldfish to injected LHRH-A. Intraperitoneal injection of reserpine, 6-hydroxydopamine, α -methyl- p -tyrosine, and carbidopa all potentiated the GtH releasing activity of LHRH-A in goldfish (Fig. 9); however, injection of diethyl-dithiocarbamate did not influence the responsiveness to LHRH-A. This lends further support to the idea that DA serves as GRIF in goldfish.

By use of immunohistochemistry, Kah *et al.* (1984) demonstrated a dopaminergic group of perikarya in the anterior-ventral nucleus preopticus periventricularis, in the region of the preoptic recess, of the goldfish; these DA neurons were found to project to the pituitary via bilateral tracts in the lateral preoptic and anterior hypothalamus. The location of this dopaminergic nucleus and pathway coincides with the proposed origin of

FIG. 9. Effects of intraperitoneal injections of pimozone (Pim; 10 $\mu\text{g/g}$ body weight), reserpine (Res; 100 $\mu\text{g/g}$), 6-hydroxydopamine (6-OHDA; 50 $\mu\text{g/g}$), α -methyl-*p*-tyrosine (α -MPT; 100 $\mu\text{g/g}$), carbidopa (CBD; 50 $\mu\text{g/g}$), and diethyl-dithiocarbamate (DDC; 2.5 $\mu\text{g/g}$) on the LHRH-A- (0.1 $\mu\text{g/g}$) induced increase in serum gonadotropin levels in sexually mature (prespawning) female goldfish held at 20°C. Values are mean $\pm$ SE ($n = 8$ or 9). Significant differences by the *U* test. C. S. Nahorniak and R. E. Peter, unpublished results.

GRIF and the GRIF pathway to the pituitary (Peter and Paulencu, 1980). Radiofrequency lesions that interrupted the neural pathway carrying GRIF also caused degeneration of DA terminals adjacent to gonadotropes (O. Kah, J. Dulka, and R. E. Peter, unpublished results). Together these data lend additional strong support for the idea that DA serves as GRIF in goldfish.

2. Importance of DA as GRIF

Several lines of evidence indicate that the GRIF actions of DA play an integral role in the neuroendocrine regulation of GtH release in the goldfish. In teleosts, germinal vesicle migration is a preliminary step to ovulation (Yamazaki, 1965; Billard *et al.*, 1978) and final oocyte maturation and ovulation are induced by a preovulatory GtH surge (for review see Peter, 1981, 1983a,b). In sexually matured (prespawning) female goldfish, injection of the DA antagonist pimozide not only potentiates the actions of LHRH, tGnRH, and LHRH superactive analogs in stimulating GtH secretion, but treatment with the combination of pimozide and LHRH-A can stimulate increases in blood levels of GtH similar to or higher than those observed during spontaneous ovulation, and induce a high rate of ovulation (Chang and Peter, 1983b; Chang *et al.*, 1984b; Sokolowska *et al.*, 1984, 1985a,b). The ovulatory response in the goldfish is probably dependent on both the magnitude of the serum GtH concentration as well as the rate of increase in circulating GtH levels, since a large but slow increase in serum GtH levels, as by injection of a high dosage of LHRH-A alone (Chang and Peter, 1983b; Sokolowska *et al.*, 1984, 1985a,b; Fig. 7) or by implants of pellets containing a large dosage of LHRH-agonist (Sokolowska *et al.*, 1984), were not effective in inducing ovulation in the goldfish. Thus, in the normal *in vivo* situation, removal of the DA inhibition and an increase in GnRH stimulation of GtH secretion are probably both required to allow for the rapid increase in circulating GtH levels that trigger ovulation.

By following the effects of intraperitoneal injection of pimozide on basal, as well as LHRH-A stimulated, serum GtH levels throughout the reproductive cycle of the goldfish, it is evident that the inhibitory DA tone on GtH release is present continuously. The magnitude of the increase in basal and LHRH-A-stimulated serum GtH levels following pimozide injection can be correlated with the degree of ovarian development in the goldfish (Sokolowska *et al.*, 1985a), suggesting that the inhibitory DA tone increases in intensity with advancement in the degree of reproductive readiness. Additional support for an increase in the degree of DA inhibition with ovarian maturation in the female goldfish comes from the observations that the preoptic lesion-induced elevations in serum GtH become greater as the fish progress through the various stages of ovarian recrudescence (see Section IV). This increase in the intensity of DA inhibition is probably responsible for the maintenance of relatively low circulating GtH levels in the face of increases in the readily releasable GtH pool as the goldfish enters the reproductive season. The seasonal changes in DA inhibitory tone may be due to influences of sex steroids, as

treatment of goldfish with estradiol and testosterone results in a greater GtH release-response to pimozone and LHRH-A (R. E. Peter and C. S. Nahorniak, unpublished results).

As discussed in Section III, LHRH and its superactive agonists stimulate GtH release in goldfish, but dose-response relationships to injections of various concentrations of these peptides, as well as superactivity, cannot be readily demonstrated *in vivo*. However, when the DA inhibition of GtH release was blocked by injection of pimozone, *in vivo* applications of various concentrations of LHRH-A in sexually mature (prespawning) females (Sokolowska *et al.*, 1985b; Fig. 7), and LHRH-A and [D-Arg⁶, D-Trp⁷, D-Leu⁸, Pro⁹-NET]-LHRH in mature (prespawning) males (Peter *et al.*, 1985) increased serum GtH levels in a more clearly defined dose-dependent manner. When combined with pimozone, several LHRH analogs that were superactive in mammals were also shown to be superactive in increasing serum GtH in goldfish; the magnitudes of the increase in GtH levels were up to 8-fold greater than that resulting from treatment with a similar dose of LHRH plus pimozone (Peter *et al.*, 1985). These observations imply that not only is the dopaminergic GRIF system used to modulate stimulatory influences on GtH release, but the DA inhibitory tone is a powerful component in the neuroendocrine regulatory mechanisms controlling GtH secretion; only when this DA inhibition on GtH release is blocked, such as by the addition of pimozone, can dose relationships between GtH release and concentrations of releasing peptide, and superactivity of analogs, be demonstrated. It is interesting to note that although superactivity of LHRH-A in stimulating GtH release, relative to LHRH, can be demonstrated with the use of pimozone *in vivo*, LHRH-A, LHRH, and tGnRH are equipotent in inducing GtH secretion *in vitro* (MacKenzie *et al.*, 1984). It may be that the structure-activity requirements of the GnRH receptor of the goldfish are different from that in mammals, and that the *in vivo* superactivity of various LHRH analogs is due primarily to a difference in the degradation or metabolism of these peptides, rather than differences in binding affinity or receptor-ligand interactions.

3. Specificity of the DA Inhibition on GtH Release

Results from the available literature indicate that the DA inhibitory influence on GtH secretion is likely mediated by dopaminergic, but not α - or β -adrenergic receptors. As discussed previously, DA and its agonists, apomorphine and bromocryptine, inhibit, whereas the DA antagonists, pimozone and metoclopramide, increase spontaneous as well as GnRH-stimulated GtH release *in vivo*. Recent studies demonstrate that other DA

FIG. 10. Effects of intraperitoneal injection of pimozide (10 $\mu\text{g/g}$; PIM), haloperidol (10 $\mu\text{g/g}$; HALO), and spiperone (10 $\mu\text{g/g}$; SPIP) on serum gonadotropin (GtH) concentrations in LHRH-A-injected goldfish undergoing gonadal recrudescence and held at 12°C. LHRH-A (0.1 $\mu\text{g/g}$) was injected twice at an interval of 12 hours. PIM, HALO, and SPIP were injected at the time of the second LHRH-A injection. Control injections were acidified physiological saline (PS). Serum GtH levels are mean $\pm$ SE at 6 hours after the last set of injections. Significant differences were determined by the *U* test ($p < 0.05$; Δ , versus LHRH-A + Veh group). R. J. Omeljaniuk, S. H. Shih, M. Sokolowska, and R. E. Peter, unpublished results.

antagonists including haloperidol, spiperone (Fig. 10), and domperidone (R. J. Omeljaniuk, S. H. Shih, M. Sokolowska, and R. E. Peter, unpublished results) are also effective. However, injection of the α -receptor antagonist, phentolamine, and the β -receptor antagonist, propranolol, did not alter basal or LHRH-A-stimulated serum GtH concentrations (Chang *et al.*, 1984a, Fig. 11). The ability of the specific D-2 dopaminergic agonist bromocryptine (Kebabian and Calne, 1979) to inhibit, and the ability of DA antagonists with D-2 receptor specificity, pimozide, metoclopramide, spiperone, and domperidone (Creese *et al.*, 1981, 1983; Kebabian and Calne, 1979), to enhance the LHRH-A-induced GtH response suggest that DA acts at a receptor resembling the mammalian D-2 type to exert its GRIF activity. (For definitions and properties of D-2 dopaminergic receptor, see Cote *et al.*, 1982; Crease, 1982; Creese *et al.*, 1981, 1983; Kebabian *et al.*, 1983; Seeman, 1984; Grigoriadis and Seeman, 1984.)

Results from preliminary experiments on the properties of goldfish pituitary DA receptors demonstrate that these receptors have characteristics

FIG. 11. Effects of intraperitoneal injection of pimozide (10 μ g/g; PIM), phentolamine (10 μ g/g; PHEN), or propranolol (10 μ g/g; PROP) on serum gonadotropin (GtH) concentrations in LHRH-A-injected goldfish undergoing gonadal rescrudescence. LHRH-A (0.1 μ g/g) was injected twice at an interval of 12 hours. PIM, PROP, or PHEN was injected at the time of the second LHRH-A injection. Control injections were acidified physiological saline (PS). Serum GtH levels are mean $\pm$ SE. Significant differences were determined by one-way analysis of variance and Duncan's multiple range test. Serum GtH concentrations of groups underlined by the same line are not significantly different ($p > 0.05$). Reproduced from Chang *et al.* (1984b).

FIG. 12. Binding of [³H]spiperone to goldfish whole pituitary homogenate. Specific binding of [³H]spiperone was calculated as the difference in binding of [³H]spiperone in the absence (total binding) or presence (nonspecific binding) of 10 μM domperidone, a specific dopamine D-2 receptor blocker. Data were expressed as specifically bound [³H]spiperone (fmol) as a function of [³H]spiperone concentration. Inset: Scatchard analysis of these same data ($r = -0.996$). R. J. Omeljaniuk and R. E. Peter, unpublished results.

consistent with mammalian D-2 dopaminergic receptors (R. J. Omeljaniuk and R. E. Peter, unpublished results). In an *in vitro* radioreceptor assay, [³H]spiperone binds to a goldfish pituitary tissue preparation in a saturable fashion and with high affinity (Fig. 12). The specific binding of [³H]spiperone to goldfish pituitary tissue preparation is heat labile and is differentially displaceable by dopamine agonists and antagonists in a manner similar to that reported for mammalian D-2 dopaminergic receptors. In addition, the order of potency of DA antagonists, injected intraperitoneally, in elevating serum GtH levels is consistent with that of DA antagonists binding to mammalian D-2 receptor sites (e.g., domperidone > pimozide).

B. OTHER CATECHOLAMINES

There is relatively little information available on the possible involvement of NE and epinephrine (E) in the neuroendocrine regulation of GtH release in fish. Deery (1975) reported that the adenylate cyclase activity of

the pars distalis of goldfish, measured *in vitro*, was increased by NE and E. Chang *et al.* (1983) reported that intraperitoneal injection of the α -agonist clonidine increased serum GtH levels in goldfish, and proposed that NE may stimulate GtH release.

Confirmation of the stimulatory influence of NE on GtH secretion was subsequently provided by Chang and Peter (1984). Intraperitoneal injection of NE was found to increase serum GtH levels in female goldfish with regressed ovaries or ovaries undergoing early stages of recrudescence, but not in female fish at other stages of the reproductive cycle. Since systemically applied radiolabeled NE was not taken up by brain tissues (Chang, 1983), a blood-brain barrier for NE probably exists in the goldfish, similar to mammals. Thus, the results from the *in vivo* studies suggest that NE probably acts directly on the pituitary to stimulate GtH release. Chang *et al.* (1984c) found that NE stimulated GtH release from perfused pituitary fragments, as well as dispersed pituitary cells, obtained from goldfish with regressed gonads (Fig. 13), confirming that NE acts directly on GtH cells. NE has been detected in the goldfish pituitary (approximately 110 pg/pituitary; Chang, 1983). Although the pituitary NE is presumed to be of neural origin, the source of these NE neurons is still unknown.

Variations in the ability of intraperitoneal injections of clonidine to increase serum GtH concentrations in female goldfish at different stages of the reproductive cycle paralleled those of intraperitoneal injections of NE. Clonidine increased circulating GtH concentrations in fish with ovaries at early stages of recrudescence, but not in those having gonads at late stages of ovarian recrudescence (Chang *et al.*, 1983; Chang and Peter, 1984). The similarity in the seasonal variation in the ability of NE and clonidine to stimulate GtH release, as well as the observation that the NE-stimulated elevation in serum GtH levels was reversed by concurrent applications of phentolamine (Chang *et al.*, 1984c), indicates that NE acts through α -adrenergic receptors to directly stimulate GtH secretion.

Although NE can directly stimulate pituitary GtH secretion, a central site of action for NE in stimulating GtH release is also possible. Chang and Peter (1984) reported that injection of a low dose of NE into the third cranial ventricle increased goldfish serum GtH concentrations. Interestingly, this stimulatory effect of centrally administered NE on serum GtH levels was also demonstrable in female goldfish at early, but not at later stages of ovarian recrudescence. The significance of the seasonality of NE to stimulate GtH release is not known.

In a recent experiment using female goldfish at late stages of ovarian recrudescence, it was demonstrated that intraperitoneal injection of E

FIG. 13. Changes in gonadotropin (GtH) released from perfused (A) dispersed pituitary cells, and (B) pituitary fragments prepared from sexually regressed female goldfish during and following exposure to norepinephrine (NE). Reprinted with permission from *Life Sciences* 35, 2027-2033, Chang *et al.* (1984c); copyright Pergamon Press, Ltd.

increased serum GtH levels (J. P. Chang and R. E. Peter, unpublished results). Therefore, it remains possible that E may also participate in the neuroendocrine regulation of GtH release in goldfish.

C. DA AS GRIF IN OTHER TELEOSTS

Few studies have been performed to systematically investigate the role of neurotransmitters in the neuroendocrine regulation of GtH release in other teleost species. However, information available in the literature suggests that an inhibitory DA influence on spontaneous and/or GnRH-stimulated GtH release exists in teleost species other than the goldfish.

Lin *et al.* (1985a,c) reported that in the Chinese loach (*Paramisgurnus dabryanus*), injection of pimozide or reserpine potentiated the action of LHRH-A in stimulating increases in serum GtH as well as the frequency of induced ovulation. Injection of α -methyl-*p*-tyrosine and carbidopa, but not diethyl-dithiocarbamate, also potentiated the action of LHRH-A in stimulating GtH release and in inducing ovulation (Lin *et al.*, 1985c). Similarly, in common carp (*Cyprinus carpio*) (Billard *et al.*, 1983; Lin *et al.*, 1985c) and bream (*Parabramis pekinensis*) (Lin *et al.*, 1985c) injection of pimozide potentiated the ability of superactive analogs of LHRH in stimulating GtH release, and in inducing oocyte maturation and ovulation. Lin *et al.* (1985c) also reported that reserpine treatment enhanced the ability of LHRH-A to cause increased circulating levels of GtH and induce ovulation in the common carp. These results demonstrate that in the Chinese loach and common carp, depletion of DA by blockage of synthesis or blockage of DA receptors removes the inhibitory influence of DA on GtH release, resulting in enhancement of the GtH release-response to LHRH-A.

De Leeuw *et al.* (1984) reported that apomorphine inhibits spontaneous GtH release from cultured pituitaries of the African catfish (*Clarias lazera*). In the catfish injection of pimozide was found to increase plasma GtH levels in juvenile fish, and to potentiate the ability of LHRH-A to stimulate GtH release in both juvenile and mature fish (de Leeuw *et al.*, 1985a,b). In sexually immature European eel (*Anguilla anguilla*) treatment with estradiol stimulates the synthesis of GtH and increases pituitary GtH content; injection of LHRH-A together with pimozide, but not LHRH-A alone, stimulates release of GtH in the estradiol-treated eels (Dufour *et al.*, 1984). In the white sucker (*Catostomus commersoni*), injection of pimozide increased, whereas apomorphine decreased, basal serum GtH levels (Chang, 1983).

Although GtH measurements were not available, results from studies on the effects of pimozide on the response to injection of LHRH agonists

in the walleye (*Stizostedion vitreum*) are consistent with the existence of an inhibitory DA influence on GtH release. Injection of pimozide alone was found to be effective in advancing germinal vesicle migration and oocyte maturation in walleye (Pankhurst *et al.*, 1985).

In salmonids, it is well established that injection of LHRH superactive analogs alone stimulates a marked increase in blood levels of GtH and ovulation (Donaldson *et al.*, 1981; Sower *et al.*, 1982; Crim *et al.*, 1983a,b; Van Der Kraak, *et al.*, 1983, 1985). However, in spite of this greater sensitivity to LHRH and superactive analogs than is apparent in other teleosts, an inhibitory DA influence on spontaneous, as well as GnRH-stimulated GtH release is also present. Billard *et al.* (1985) reported that pimozide increased the GtH release-response to injections of an LHRH agonist in rainbow trout (*Salmo gairdneri*) and brown trout (*S. trutta*). In rainbow trout, injection of apomorphine was reported to inhibit the post-ovulatory GtH release (Gielen, unpublished results, cited by de Leeuw *et al.*, 1985b), and DA was found to inhibit the release of GtH from pituitaries incubated *in vitro* (Crim, 1981). In the coho salmon (*Oncorhynchus kisutch*), pimozide injection also increased the basal as well as the LHRH analog-stimulated GtH secretion *in vivo* (Van Der Kraak, 1984).

It is clear from these studies that the GRIF action of DA is found in a wide phylogenetic range of teleosts, perhaps in all teleosts. The apparent difference between salmonids and other teleosts in the degree of GRIF activity exerted by DA suggests that the relative importance of both DA and GnRH in regulating GtH secretion in teleosts may be altered through evolution.

VI. DA as GRIF in Mammals?

Several excellent and extensive reviews on the involvement of catecholamines and other neurotransmitters in the regulation of LH secretion in mammals are available (Krulich, 1979; Sawyer, 1979; Barraclough and Wise, 1982; Kalra and Kalra, 1983, 1985; Barraclough *et al.*, 1984) and it would be inappropriate for us to attempt another review. In brief, although the influence of DA on LH secretion is still controversial, catecholamines have been shown to be involved in the modulation of pulsatile LH release and the preovulatory LH surge in mammals. Based on studies in rats, it is generally believed that, in mammals, neurotransmitters influence LH release by altering LHRH release from the median eminence. This is in contrast with the situation in fish where DA and NE exert direct inhibitory and stimulatory influences, respectively, on GtH release.

Although it has been demonstrated, *in vitro*, that DA does not directly

influence pituitary LH release in rats (e.g., Kamberi *et al.*, 1969, 1970, 1971; Deneff and Andries, 1983), similar information on other mammalian species is not available. Results from a number of reports are not at variance with a possible pituitary level inhibitory action of DA on LH release in mammals. Metoclopramide has been reported to increase circulating LH levels in patients with hypothalamic amenorrhea and prolactin-secreting microadenomas (Quigley *et al.*, 1979, 1980). Metoclopramide treatment has also been reported to be effective in increasing plasma LH concentrations in patients with normoprolactinemic amenorrhea (Hagen *et al.*, 1983, 1984). These stimulatory effects of metoclopramide on LH release in women with various pathological conditions have always been attributed to the blockage, by metoclopramide, of a central inhibitory DA tone on LHRH secretion since metoclopramide readily crosses the blood-brain barrier and the infusion of DA, which does not cross the blood-brain barrier, has been reported not to alter LH secretion in normal, as well as hyperprolactinemic female subjects (Connell *et al.*, 1984; Ho *et al.*, 1984). However, Esquifino *et al.* (1984) reported that in rats in which hyperprolactinemia was experimentally induced by ectopic pituitary transplants, acute as well as chronic treatment with metoclopramide enhanced the LH response to LHRH administration. These data clearly suggest that metoclopramide acts at an extrahypothalamic site to potentiate LHRH effects.

Recently, DA infusion has been shown to significantly decrease circulating LH levels in normal men, castrated men, and normal men treated with clomiphene citrate (Fig. 14; Foresta *et al.*, 1984; Delitala *et al.*, 1983). Similarly, DA infusion causes a decrease in circulating LH levels in women with polycystic ovarian disease (Paradisi *et al.*, 1985), and in normal as well as hyperprolactinemic women pretreated with α -methyl-*p*-tyrosine (Nicholetti *et al.*, 1984). Although Pehrson *et al.* (1983) reported that infusion of DA did not alter the LH response to simultaneous infusion of LHRH in postmenopausal women, Judd *et al.* (1978) reported that DA infusion attenuated the LH release response to a bolus of LHRH in women at the early follicular phase. DA infusion also attenuates the LH release-response to LHRH in normal men (Leebaw *et al.*, 1978; Huseman *et al.*, 1980; Fig. 15). These observations suggest that under certain pathological conditions, and possibly also in the normal human, a direct inhibitory effect of DA on LH release may be present. Steroid condition of the test subjects seems to be of importance; the depression of circulating LH levels by DA infusion was more pronounced in castrated than in clomiphene citrate-treated men, which in turn showed a greater response than normal men (Foresta *et al.*, 1984). Also, the metoclopramide-stimu-

FIG. 14. Net decrease (Δ) in plasma luteinizing hormone (LH) levels during dopamine infusion ($0.4 \mu\text{g/kg/minute}$) in normal men ($\bullet$), normal men after clomiphene citrate treatment ($\times$), and castrated subjects ($\circ$). Values are mean $\pm$ SD ($n = 4$). From C. Foresta, G. Scanelli, S. Marra, and C. Scandellari: The influence of gonadal steroids on the dopamine inhibitory effect on gonadotropin release in man. *Fertil. Steril.* **42**, 942 (1984). Reproduced with permission of the publisher, The American Fertility Society

lated increase in serum LH levels was only observed in normal menstruating women during the mid-luteal phase, but not in the early or late follicular phases (Ropert *et al.*, 1984).

The possible existence of a direct DA inhibitory influence on LH release in mammals is not restricted to humans. Dailey *et al.* (1981) reported that DA directly inhibits the actions of LHRH on LH release in normal and stalk-sectioned rabbits (Fig. 16). DA infusion has also been shown to reduce plasma LH concentrations in a dose-dependent manner in ovariec-

FIG. 15. Mean serum luteinizing hormone (LH) concentrations in five normal men during a 4 hour constant intravenous infusions of luteinizing hormone-releasing hormone (GnRH), dopamine (DA) plus GnRH, oral haloperidol (HA), and DA and after saline infusion. Values are mean $\pm$ SD ($n = 5$). Reproduced from Huseman *et al.* (1984). Copyright © 1984 The Endocrine Society.

FIG. 16. The effects of a dopamine infusion followed by a saline infusion on LHRH-induced LH release in an acutely pituitary stalk-sectioned rabbit. Reprinted with permission from *Life Sciences* 22, 1491–1498, Dailey *et al.* (1981); copyright 1981, Pergamon Press, Ltd.

tomized (Deaver and Dailey, 1982) and cycling ewes during luteal regression (Deaver and Dailey, 1983), but not in anestrus ewes (Domanski *et al.*, 1975; Deaver and Dailey, 1982).

Further studies are required to systemically prove or disprove the existence of direct influences of DA and/or other neurotransmitters on gonadotropin release in mammals. It is possible that direct DA inhibition on gonadotropin secretion is an ancient evolutionary component of the neuroendocrine regulation of release of reproductive (gonadotropic) hormones; however, the intensity of this inhibition may differ between classes of vertebrates, as well as within a smaller phylogenetic unit as the Subclass Teleostei. During the evolution of higher vertebrates such as mammals, the importance of a direct inhibitory influence of DA on gonadotropin release may have been diminished so that it is now of minor significance or nonfunctional under normal conditions. On the other hand, this action of DA on gonadotropin release may be manifested only under special hormonal or pathological conditions.

VII. Conclusions

Available information on the neuroendocrine regulation of gonadotropin in teleost fish reviewed in this article indicates that GtH release is under the stimulatory influence of GnRH and the inhibitory control of DA. DA acts as a GRIF to directly inhibit spontaneous GtH release and modulate GnRH action (Fig. 17). The intensity of the DA inhibitory tone

FIG. 17. Summary diagram of the influences of dopamine (DA) and norepinephrine (NE) on gonadotropin (GtH) release in goldfish.

differs between species and the intensity may also change along with sexual recrudescence or maturation within the same species. In species such as the goldfish, the inhibitory DA influence is intense and the removal of this inhibitory tone is part of the mechanism regulating the ovulatory GtH increase. Although DA acts as a GRIF in a number of teleost species, other GRIFs may also exist. In the goldfish, although NE can directly stimulate GtH release, it may also act centrally to increase GtH secretion (Fig. 17). The stimulatory influence of NE on GtH secretion is seasonal, dependent on the stage of gonadal recrudescence. Information on the possible participation of other neurotransmitters in the neuroendocrine regulation of GtH release in teleosts is not available. Although it is generally believed that neurotransmitters do not exert a direct influence on the pituitary to modify gonadotropin release in mammals, results from some studies on humans, rabbits, and ewes are strongly suggestive of a direct inhibitory DA influence on LH release.

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DISCUSSION

G. B. Cutler. I was interested in the increases in gonadotropin secretion you found with pituitary transplants since in mammals the loss of LHRH support usually results in low levels. Do you think that the unusual anatomy in the goldfish with the projection of the median eminence into the pituitary results in your transplanting LHRH activity along with the pituitary?

R. E. Peter. I think that in mammals it depends on when you look at the LH levels following pituitary transplantation, because if LH levels are measured very shortly after transplantation the LH levels are in fact quite high. In other words a situation may occur in mammals similar to what we find in goldfish, that, over the short term following pituitary transplantation, there is quite a lot of gonadotropin release.

G. Aurbach. I enjoyed your presentation very much. Your results with dopamine inhibitors suggests a D-2 receptor-mediated phenomenon. I presume that cyclic AMP mediates release of gonadotropin and that a cyclic AMP rise in response to some signal would be inhibited by the D-2 mechanism. Do you think this might be a calcium-mediated event? Have you looked into this?

R. E. Peter. No, we have not yet looked at postreceptor mechanisms. We hope to do some studies on GnRH receptors, but we haven't yet gotten to the stage of looking at the kinds of things that you suggest. It would be very interesting and I think most appropriate.

M. V. Nekola. Do the gonads have any effect on modulating gonadotropin release from the pituitary?

R. E. Peter. Of course, there are steroid actions on the brain and the pituitary in teleost fish. There is evidence for positive as well as negative feedback actions of sex steroids. We found in recent experiments that when we treat goldfish with steroids and test their responsiveness to the combination of LHRH analog plus pimozone at 72 and 48 hours after steroid treatment, in fact the response to the combination is potentiated by steroid treatment.

M. V. Nekola. Have you tried reducing the level of LHRH that you give to see if you could get a dose-response? The gonadotropin response may have been maximal with the doses you gave.

R. E. Peter. Yes, the dosages we administered in those experiments were relatively high. However, where we had a dose dependency of LHRH analog in the presence of pimozone. With LHRH alone at a low dose without pimozone we get no response.

M. V. Nekola. Then have you tried sampling at an earlier time frame? You were looking at 1.5 hours and in the rat by that time the effect of LHRH has already disappeared.

R. E. Peter. Yes, we have looked at earlier times as well. If the experiments are done with intravenous infusion of low dosages of LHRH or LHRH analog a release response occurs within a few minutes. However, the experiments I showed you all involved intraperitoneal injections which gives a longer time frame of response. Also, the LHRH analog apparently stays in circulation in the goldfish for a very long period of time.

G. Anogianakis. I would like to go back over the pituitary transplants that you described. I noticed that when you transplanted the pituitary in the preoptic area you did not have as great an effect as when you transplanted it to other areas. If I recall correctly the existence of the blood-brain barrier in the preoptic area is disputed and it may not exist. Does this mean that whatever inhibitory factor you have coming from the anterior hypothalamus and from the preoptic area, gets to your transplant more readily?

R. E. Peter. I think the reason why the pituitary transplants in the brain ventricles release less gonadotropin is because of the high levels of dopamine in the cerebrospinal fluid. If the cerebrospinal fluid is perfusing the transplanted pituitary, the dopamine in the CSF will suppress gonadotropin release.

G. Anogianakis. Then this means that when you refer to inhibition you are not really referring to direct inhibitory innervation, but to a release factor. I was not clear on that.

S. J. McConnell. You said that there was a seasonality in the response to GnRH. I may have missed this, but what photoperiod conditions did you keep the fish under?

R. E. Peter. We have mimicked the seasonal photoperiod changes throughout the year during our experiments.

S. J. McConnell. You demonstrated that a photoperiod shift alters the period of ovulation during the day. Do you have any idea which factor provides this photic information?

R. E. Peter. Via the eyes, not the pineal.

N. B. Schwartz. I was curious about what the gonadotropin you are measuring is doing in the ovary. You did tantalize us with the observation that there is a second factor which you are not measuring. What kinds of bioassays have you used for the immunoassayable gonadotropin and what kind of effects do these gonadotropins have on the ovaries of these animals?

R. E. Peter. The gonadotropin that we are measuring by radioimmunoassay, we have bioassayed by *in vitro* steroidogenesis. It has been bioassayed in a number of laboratories for both cyclic AMP and steroidogenic actions on the gonads.

N. B. Schwartz. What action does it have on follicular maturation? I assume there has to be precedent follicular maturation in the ovary before the rather broad LH surge occurs.

R. E. Peter. Yes, the migration of the germinal vesicle to the periphery of the oocyte and break down of the germinal vesicle are the first steps in the process of oocyte maturation. At 5 hours following a preoptic lesion the germinal vesicle has already migrated to the periphery and is starting to break down.

N. B. Schwartz. Are all of those events occurring from the time in which the goldfish are put back into an aquarium which is stimulatory?

R. E. Peter. The time frame is similar to the sequence of events that we find during spontaneous ovulation. In other words, the timing of the maturation events during spontaneous ovulation seems to be on the same time course as we find following injections of pimozide and LHRH analog, or following brain lesions in the preoptic region.

R. M. Carey. Have you had an opportunity to study the effects of dopamine on GnRH binding in the pituitary gonadotrope?

R. E. Peter. No, we are just starting that work now.

S. Y. Ying. Did you look at other types of hormones in your lesioned animals? Such as GH.

R. E. Peter. There is a major somatostatin area in the preoptic region of the brain, and if

we do large preoptic lesions we will also cause increases in growth hormone levels. The time course, however, of the increases in growth hormone and gonadotropin is quite different.

G. T. Campbell. I have a comment as it relates to the first question by Dr. Cutler. We have been studying the cellular composition and secretory activity of long-term pituitary allografts beneath the renal capsule in the hamster. Depending upon the age of the donor tissue, cells which contain LH actually end up being the numerically dominant cell in the allograft. They don't release very much LH but that may be a consequence of other intraallograft factors. I think the question of what happens to the pituitary cell types in malregulated adenohypophyseal tissue in mammals may need to be reinvestigated. Second, although I cannot quote the year in which this was published in abstract form, the late Dr. E. M. Bogdanove once published some results having to do with seminal vesicle hypertrophy following lesioning of various parts of the hypothalamus of the rat. One of many interpretations of that experiment is that he was lesioning out neurons which contain an LH inhibiting factor.

R. E. Peter. Thank you very much. I can add that a colleague in France, Dr. Olivier Kah, has found a dopaminergic nucleus in the anterior preoptic region of goldfish, in the area that I interpreted as being the origin of GRIF. A pathway comes from that nucleus through to the pituitary stalk. Also, in recent work that Dr. Kah and I did in my laboratory we found that if we lesioned the preoptic brain area, tyrosine hydroxylase activity disappeared from the area of the pituitary containing the gonadotropin cells.

I. M. Spitz. Thank you for your elegant presentation. I wondered if prolactin and TSH are also under dopamine control in the goldfish?

R. E. Peter. Dopamine does inhibit prolactin release in teleost fish, but at this point we have no evidence that dopamine inhibits TSH release in goldfish.

I. M. Spitz. Can you down-regulate the gonadotropin receptor *in vivo* with repeated or continuous administration of LHRH or dopamine antagonists? If you can down-regulate with one of these stimuli is the response to the second maintained?

R. E. Peter. In short, I can't tell you about down-regulation of dopamine receptors. However, regarding the response to LHRH analog, we see that this response goes on for literally days in the pimozide-blocked animal. We know that there is a protein in plasma that binds the GnRH and seems to result in a reservoir of circulating GnRH in the plasma. GnRH does seem to be very stable in the fish. Likewise the GnRH receptors do not down-regulate very readily, which is quite different from the situation occurring in mammals.

T. Horton. You made a couple of interesting allusions to some evolutionary biology and a statement about the median eminence moving into the pituitary. I wondered if you could comment on the embryology of the median eminence over a phylogenetic scale. Does the median eminence in fact move into the pituitary in teleosts or out of the pituitary in higher vertebrates?

R. E. Peter. The situation in teleosts is a derived condition. In more primitive bony fishes a true median eminence is present, but the teleosts are the oddballs in this situation by having direct innervation of the pituitary. The standard condition in vertebrates is the median eminence with a hypothalamo-hypophyseal portal system.

J. D. Veldhuis. Do you have any information in this relatively small animal as to whether the short-term release of dopamine is periodic, i.e., episodic or nonuniform over time?

R. E. Peter. No, although I wish we did, we don't have such information.

J. D. Veldhuis. What central mechanisms do you envision in this species as being linked to the inhibitory dopaminergic system? For example, in a number of other species, the opiate system is inhibitory of gonadotropin release. Have you had occasion to investigate that in the goldfish?

R. E. Peter. The opiate system seems, if anything, to be stimulatory to gonadotropin release. As to what may regulate the activity of the dopaminergic input to the gonadotropin cells in the pituitary, I really don't know. We are hoping to try and address that question subsequently, but it is going to be a difficult problem to investigate.

H. Kulin. One experiment of nature that may bear on the possible dual control, i.e., inhibitory and facilitory, of gonadotropins in man is the recent observation in a small group of children who received head and neck irradiation that hypopituitarism developed in the setting of precocious puberty. This paradox could be explained by the radiation-induced destruction of facilitory centers for hGH, TSH, and ACTH but an inhibitory center for gonadotropin control. This is admittedly speculative but of interest with respect to your own data in the fish.

R. E. Peter. Thank you very much.

J. M. Hershman. I noted in the data showing that pimozide potentiated the action of GnRH that many of the species listed are grown commercially in culture. I wonder if there has been application of either pimozide or other dopamine blockers in commercial culture of fish. Could you elaborate on that?

R. E. Peter. At this point it is under development. I have collaborative work going on in China and in Poland on cultured fish to test the system to be sure that the combination is appropriate for inducing ovulation, and also that the treatment of the animals with pimozide doesn't cause some undesirable secondary side effects.

G. Anagnostakis. I will go back to what you mentioned about the dopaminergic nucleus in the anterior hypothalamic area. Now in higher mammals this nucleus is under certain serotonergic control from the raphe nuclei and also projects down the CNS axis through serotonergic neurons. Did you try any experiments with serotonin?

R. E. Peter. We haven't yet investigated serotonin actions. I know the work you refer to, but I should also add that recently a dopaminergic pathway has been described from the anterior preoptic region in mammals to the median eminence.

B. M. Dohyns. Quite some years ago we were particularly interested in exophthalmos in fish using goldfish and *Fundulus*. The exophthalmos response was produced by pituitary extract. In the course of experiments we found that the response was much better using pituitary extracts that were rich in the LH. I wonder if by any chance you've observed exophthalmos in any of your studies in these goldfish. Variations in exophthalmos responses were particularly true with respect to seasonality and to salinity of the environment.

R. E. Peter. No, we haven't found any cases of exophthalmos. We measured thyroid hormone levels in some of the preoptic lesioned animals and the thyroid hormone levels don't change.

M. O. Thorner. I would like to come back to the whole thesis that you are proposing and ask you whether there are dopamine receptors on the gonadotrophs. I wonder if you would speculate about that. I know that in some fish the pituitary is divided up into neat lobes. In the fish that you are working with are there only gonadotropes in that area or are there lactotropes too? Have you considered the possibility of an interaction between the lactotrope and the gonadotrope to produce this effect?

R. E. Peter. In the model we presented which shows dopamine acting directly on the gonadotropin cells, we infer that there are dopamine receptors on the gonadotropin cells. We believe the action is direct and not indirect. The answer to your second question is that this particular region of the pituitary contains gonadotropin cells and growth hormone cells. Dopamine does not have a similar action on growth hormone and gonadotropin release. The prolactin cells are located in a different region of the pituitary, so there isn't the opportunity, so far as we can envisage, of interaction of prolactin and gonadotropin cells.

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